

Equine Septic Arthritis and Serum Amyloid A

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in  
partial fulfillment of the requirements for the degree of

Master of Science  
In  
Biomedical and Veterinary Sciences

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June 2, 2016

Blacksburg, VA

Keywords: equine, septic arthritis, synovitis, acute phase protein, serum amyloid A

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## **Academic Abstract**

Bacterial infection within a joint, septic arthritis, is a serious condition in horses that can lead to long-term joint disease if the infection is not resolved quickly. Equine septic arthritis is diagnosed primarily based on clinical signs and synovial fluid cytology. Septic synovial fluid is characterized by significant elevations in total protein (TP) and total nucleated cell count (TNCC). However, in some cases it can be difficult to distinguish between septic arthritis and non-septic joint inflammation (synovitis) based on clinical signs and synovial fluid cytology alone. A rapid assay to help confirm septic arthritis would be advantageous. A new assay to quantify the major equine acute phase protein, serum amyloid A (SAA) may fulfill this need. Serum amyloid A increases in the body in response to injury, infection, and inflammation and shows promise as a useful tool in confirming a diagnosis of sepsis, as inflammation causes mild increases in SAA and infection causes marked elevations.

In our study, serial serum and synovial fluid samples were collected from horses with experimental models of synovitis and septic arthritis, synovial fluid cytology was performed, and serum and synovial fluid SAA were quantified. Synovial fluid TNCC and TP concentrations increased significantly following induction of both models. Serum and synovial fluid SAA concentrations remained normal in synovitis horses and increased significantly in septic arthritis horses. Any elevation in serum or synovial fluid SAA above normal values may be supportive of synovial sepsis since synovial inflammation alone did not result in SAA elevations in our model.

## Equine Septic Arthritis and Serum Amyloid A

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### **Public Abstract**

Horses are both pets and athletes and unsoundness due to inflammatory joint disease or joint infection can be career limiting or life ending for horses. Bacterial infection within a joint is a serious condition that can lead to long-term joint disease if the infection is not diagnosed and treated quickly. Joint infections in horses are diagnosed primarily based on clinical signs and specific changes in the joint fluid. However, in some cases it can be difficult to distinguish between joint infection and joint inflammation based on clinical signs and joint fluid changes alone. If joint infection is not identified and treated due to ambiguous clinical signs and joint fluid changes, significant damage to the joint can result. A rapid test that could be used to help confirm septic arthritis would be advantageous, especially for veterinarians trying to determine if a horse needs to be referred to a hospital for treatment. Measurement of the protein serum amyloid A (SAA) may be useful as a rapid test. Serum amyloid A increases in the body in response to injury, infection, and inflammation and may be a useful tool in confirming a diagnosis of infection, as inflammation causes mild increases in SAA while infection causes substantial increases. Experimentally-induced joint inflammation in horses did not cause increases in SAA concentration in blood or joint fluid, while experimentally-induced joint infection caused significant increases in SAA. Elevations in blood or joint fluid SAA, along with clinical signs and joint fluid changes may indicate joint infection in horses.

## Acknowledgements

I would like to acknowledge and thank the members of my committee, Dr. Linda Dahlgren, Dr. Julie Settlage, and Dr. Chris Byron, for the intellect and support they have provided to me throughout the duration of my project. Most importantly, I want to express my sincerest gratitude to Dr. Dahlgren. Without her knowledge, devotion, and unshakable encouragement, this research project and thesis would not exist. Dr. Dahlgren has been the vital component of my surgical residency; the support and guidance she has given me is invaluable.

I would also like to thank Dr. Christina Petersson-Wolfe for all her incredible insight and contributions regarding *Staphylococcus aureus*, as well as Dr. Isis Kanvesky-Mullarky for kindly allowing me to use her laboratory and equipment. I owe a very special thanks to Wendy Wark for her generous donation of time, expertise, and incredible patience in the laboratory. Without Wendy, the septic arthritis model and induction of joint infection would have never come to fruition. I am also thankful for the countless hours of work and valuable assistance of Dr. Stephen Werre. Additionally, I would like to thank Brandon Wiese, Megan Graham, and Amelia Tyler for their help with the horses and sample processing. Special thanks to the Veterinary Memorial Fund of the Virginia-Maryland College of Veterinary Medicine for the funding of my research project. Finally, I must thank my family for their love and support throughout my residency and graduate experience.

### **Attributions**

Several colleagues aided in the writing and research behind Chapter 3: Serum and Synovial Fluid Serum Amyloid A Response in Equine Models of Synovitis and Septic Arthritis. All colleagues listed are co-authors of the manuscript and brief descriptions of their contributions are below.

Linda A. Dahlgren, DVM, PhD, DACVS, is an Associate Professor of Large Animal Clinical Sciences at the Virginia-Maryland College of Veterinary Medicine. Dr. Dahlgren helped design and perform the study, supervised data analysis and interpretation, and contributed to manuscript preparation.

Julie M. Settlage, DVM, MSc, DACVS, is a Lab Animal Program Veterinarian at Virginia Tech. Dr. Settlage helped design the study and assisted with sample preparation.

Stephen R. Werre, PhD, is a Research Assistant Professor of Veterinary Medicine Experimental Statistics at the Virginia-Maryland College of Veterinary Medicine. Dr. Werre helped design the study, performed statistical analysis on all study data, and was critical to data interpretation.

Christina S. Petersson-Wolfe, PhD, an Associate Professor of Dairy Sciences at Virginia Tech, and Isis Kanevsky-Mullarky, PhD, an Associate Professor of Mucosal Immunology in Dairy Sciences at Virginia Tech, both and helped design and execute the bacterial septic arthritis model.

R. Brandon Wiese, Megan R. Graham, MS, and Amelia J. Tyler are veterinary students at the Virginia-Maryland College of Veterinary Medicine and assisted with the care of the experimental horses and sample processing.

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## **Chapter 1: Introduction**

### **Thesis Organization**

This thesis is presented in a format that contains a journal publication as the central portion of the document. The publication is entitled “Serum and Synovial Fluid Serum Amyloid A Response in Equine Models of Synovitis and Septic Arthritis” and contains its own introduction, materials and methods, results, discussion, and references. The following provides a brief overview of the research topic. The literature review is an expansion of the introduction to the manuscript and provides a summary of pertinent literature background information.

### **Introduction**

In veterinary medicine, horses are considered to be both pets and athletes, and unsoundness due to inflammatory joint disease or joint infection can be career limiting or life ending for a horse. Thus, an area of clinical significance for veterinarians is the diagnosis and treatment of joint infections. Bacterial infection within a joint, also called septic arthritis or synovial sepsis, is a serious condition that requires emergency treatment and can lead to long-term joint disease if the infection is not brought under control quickly. In adult horses, septic arthritis is commonly associated with traumatic wounds that communicate with the adjacent joint, seeding the joint with bacteria and organic material that develop into active infection. Due to difficulties clearing established joint infections and the degenerative joint changes that result, septic arthritis is considered an emergency that can become life threatening. The combination of significant joint inflammation, joint fluid (synovial fluid) changes, fibrin accumulation, organism

proliferation, and pain due to an established joint infection requires multimodal therapies for successful control and resolution. Diagnosis of septic arthritis early in the disease process allows for immediate treatment, controlling bacterial proliferation and thus preventing or diminishing detrimental changes within the joint.

Following treatment of septic arthritis, 56 to 81% of horses return to their original function,<sup>1,2</sup> therefore rapid diagnosis is critical for early treatment and a better prognosis.<sup>3</sup> Successful treatment of septic arthritis involves several goals: prompt and accurate recognition of the condition, thorough diagnostic examinations, complete elimination of infection, timely resolution of inflammation and pain, and a speedy return to function.<sup>4</sup>

Synovial sepsis is diagnosed primarily based on clinical signs and synovial fluid analysis. Additional diagnostic imaging can be used to identify concurrent injuries such as fractures or soft tissue damage. Septic synovial fluid is classically characterized by marked elevations in total protein (TP) and total nucleated cell count (TNCC). However, in some cases it can be difficult to distinguish between synovial infection and acute non-septic synovial inflammation based on clinical signs and synovial fluid cytology alone.<sup>4,5</sup> There can be substantial overlap in the synovial fluid TP and TNCC of septic arthritis and synovitis<sup>5</sup> and thus sepsis can be mistaken for inflammation and therefore missed.

A variety of other diagnostics can be used to help distinguish between septic arthritis and non-septic synovitis such as synovial fluid gram stain, bacterial culture, pH, lactate, glucose, and biomarkers.<sup>5</sup> While these diagnostics can help confirm septic arthritis, these diagnostic assays may be expensive, timely to perform, or their values can be affected by other variables such as the systemic status of the horse, joint size, and the cause of the joint inflammation or infection. A rapid assay that could be used in

conjunction with traditional diagnostic tools to confirm septic arthritis as early as possible would be clinically advantageous, especially for a veterinarian in the field trying to determine whether a horse needs to be referred to a hospital for treatment. An example of such an assay is one that quantifies the acute phase protein serum amyloid A (SAA) in serum and synovial fluid. Serum amyloid A is the major acute phase protein in horses and increases in response to injury, infection, and inflammation.<sup>6-8</sup> Acute phase proteins function to resolve inflammation and infection and restore normal physiologic function. Serum amyloid A is produced primarily in the liver, but is also synthesized locally within joints.<sup>8,9</sup> Serum amyloid A is increasingly used as a diagnostic tool in the horse for the detection of active inflammation,<sup>7,8</sup> and SAA concentrations may be higher with bacterial infection than inflammation.<sup>6,8</sup> Thus, SAA could be useful in differentiating between synovitis and septic arthritis.

Currently, equine SAA concentrations are quantified primarily by immunoturbidometric assay using an automated chemistry analyzer and monoclonal anti-human SAA antibodies.<sup>10</sup> Recently, a handheld lateral flow immunoassay (handheld test) has become available to measure SAA in equine whole blood or serum. The handheld assay has not yet been used for quantification of SAA in equine synovial fluid. It is unknown if the viscosity of the synovial fluid will affect the handheld assay's function, or if bacterial infection in the joint will incite a change in SAA concentration in synovial fluid that can be detected by the handheld assay. The aim of this Master of Science research project was to investigate the potential for using serum and synovial fluid SAA concentrations to aid in the diagnosis of septic arthritis in the horse and to compare SAA

results from a handheld test with those from a validated immunoturbidometric assay using equine models of acute synovitis and septic arthritis.

## **Chapter 2: Literature Review**

The equine joint is an encapsulated, sterile environment with unique metabolic processes designed to maintain homeostasis and proper synovial function. Introduction of foreign organisms into the joint can result in synovial inflammation and infection, metabolic changes, and disrupted homeostasis, which can lead to degenerative joint disease and osteoarthritis.

Bacterial infection of a joint, or septic arthritis, is a common equine orthopedic problem that occurs secondary to bacterial contamination of the joint by wounds, hematogenous spread, or iatrogenic induction.<sup>1</sup> Septic arthritis in adult horses is most often due to wounds or iatrogenic induction of bacteria.<sup>4</sup> In the horse, the distal limbs have minimal soft tissue protection and are exposed to potential threats on a daily basis. Therefore, traumatic injury can frequently result in synovial infection. Intra-articular injections are used commonly in equine practice for the delivery of local anesthetics during lameness evaluation and for the administration of medications for the treatment of joint disease. Although the frequency of iatrogenic synovial sepsis is low (less than 0.08% of joints injected become infected),<sup>11</sup> when it does occur it can be very difficult to distinguish between aseptic inflammation in response to the medication or the early stages of sepsis. The degree of inflammation and immunologic response within an infected joint depends on a multitude of factors, such as the horse's age and immune status, the virulence of the microorganism and the number of colonies inoculated in the joint, the duration of infection, and the presence of pre-existing joint pathology.<sup>12,13</sup>

It is critical that the distinction between joint inflammation and joint infection is made in order to select the most appropriate treatment plan. Without the ability to

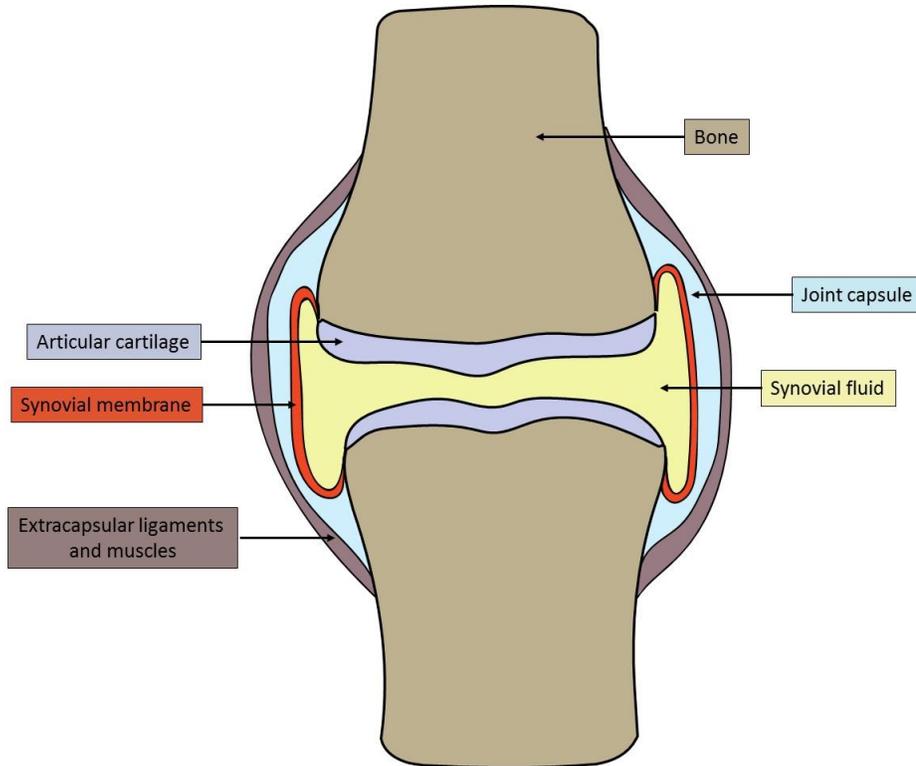
distinguish between synovitis and septic arthritis, horses may be subjected to unnecessary surgery and anesthesia in order to err on the side of an abundance of caution, or may not be treated aggressively enough and develop degenerative joint changes.

### **Joint Anatomy and Physiology**

The appendicular skeleton is composed of a variety of different types of joints with different functions. Diarthrodial, or freely moveable, joints are located commonly in the limbs of the horse and function to transfer load and enable locomotion.

Diarthrodial joints have a unique structure specifically adapted to achieve these highly specialized functions. These joints are formed by the articulation of the ends of two bones covered by a thick layer of articular cartilage and are defined by the joint capsule, which surrounds the joint, and the periarticular ligaments that support it structurally (Figure 2.1).<sup>14,15</sup>

The joint capsule is composed of a fibrous component that provides structure and a synovial membrane that lines the inside of the fibrous joint capsule and is characterized by a series of synovial villi that function to increase the synovial membrane surface area.<sup>14,15</sup> The synovial membrane, or synovium, consists of two layers, the subintima and the intima. The subintimal layer is made of connective tissue and with a blood supply and innervation.<sup>14-16</sup> The intimal layer is one to four synoviocytes (synovial lining cells) thick and lacks a basement membrane.<sup>14,15</sup> There are two primary synoviocyte cell types with distinct functions that occupy the intimal layer.<sup>14,15</sup> Type A synoviocytes can phagocytose (engulf and break down whole particles) or pinocytose (engulf and break down partial particles) foreign material or organisms, while type B synoviocytes secrete



**Figure 2.1** Diagram of representative diarthrodial joint anatomy. Articular surface of bones are covered by articular cartilage, surrounded by synovial membrane, joint capsule, and extracapsular ligaments and muscles.

protein.<sup>15</sup> The secretion of proteins by type B cells is an important function of the synoviocytes, as the secreted proteins contribute to the synovial membrane, are a component of the synovial fluid, and are involved in the metabolic processes within the joint.<sup>15</sup> These proteins include collagen, interleukins, hyaluronan, promatrix metalloproteinases, and eicosanoids.<sup>14,15</sup> Hyaluronan and lubricin are molecules produced by synoviocytes that are involved in boundary lubrication of the joint and cartilage surfaces.<sup>14-16</sup> These molecules are an important part of healthy joint function, as boundary lubrication decreases friction between soft tissue and bone.<sup>15,16</sup> A third

synovial cell type, type C, is described to be a cell transitioning between type A and type B.<sup>15,16</sup>

The synovial membrane also functions to regulate the composition of synovial fluid, which is an ultrafiltrate of plasma.<sup>15,16</sup> Hydrostatic pressure differences and colloid osmotic pressure differences drive an exchange between plasma and the synovial cavity.<sup>14-16</sup> Plasma components of less than 10 kDa in size are able to pass through the abundant capillary vasculature of the subintimal layer and the passage of components is further facilitated by the lack of a basement membrane.<sup>14-16</sup> This exchange supplies nutrients to the joint cavity while allowing for the removal of waste products.<sup>14,16</sup> The cellular composition of synovial fluid is 90% mononuclear cells (synovial lining cells, macrophages, large mononuclear cells, lymphocytes, and small mononuclear cells), with the remaining 10% of cells being neutrophils.<sup>5,14</sup> Normal synovial fluid is transparent to pale yellow in color with a high viscosity due to the high hyaluronan (hyaluronic acid) content, and the nucleated cell content is very low (less than 500 cells/ $\mu$ L).<sup>15,16</sup> Normal synovial fluid has a total protein concentration of less than 2.0 g/dL and does not clot because it does not contain clotting factors or fibrinogen.<sup>5</sup>

The articular, or hyaline, cartilage that covers the articular surfaces of the bones within the joint plays an important role in both the response to compressive forces and providing a low friction environment for joint movement.<sup>15</sup> The viscoelastic property of articular cartilage is the response of cartilage to compressive forces and when an external force, or load, is applied to cartilage, water moves out of the cartilage matrix and the cartilage compresses.<sup>15,16</sup> When the load is removed from the cartilage, water is drawn back into the cartilage matrix.<sup>16</sup> Additionally, when water is expressed from the cartilage

during load, the water is then located between the cartilage and compressing structure, separating their surfaces and reducing friction.<sup>15</sup>

In general, the state of the articular cartilage in the joint determines the health of the joint.<sup>15</sup> Cartilage lacks lymphatic, neural, and vascular supplies and thus relies on diffusion from the synovial fluid for nutrition and waste removal.<sup>15</sup> The regular efflux and influx of fluid into the cartilage matrix during loading and unloading facilitates nutrient and waste transport.<sup>16</sup> Articular cartilage is primarily composed of extracellular matrix that contains sparse chondrocytes that make up 1 to 12% of cartilage volume.<sup>14,15</sup> The extracellular matrix is mainly composed of water (approximately 70-80% of the wet weight), collagen, and proteoglycans.<sup>15,16</sup>

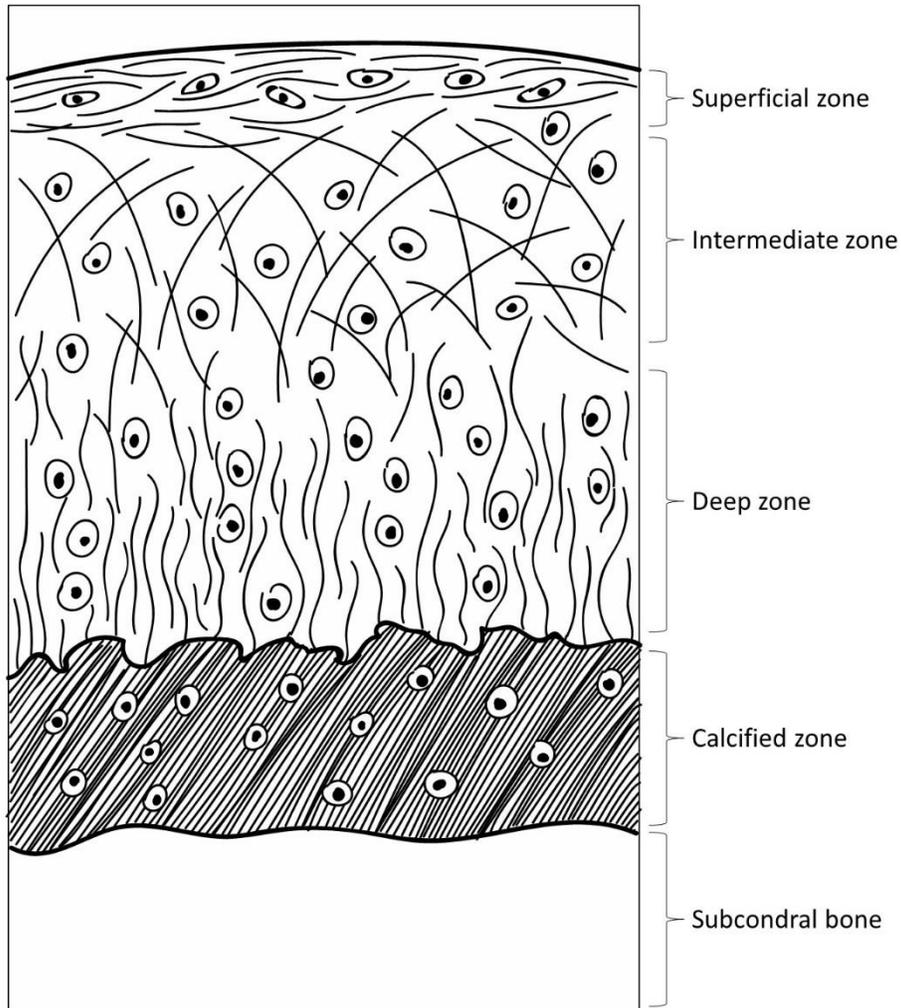
Collagen is produced by chondrocytes, provides the framework for articular cartilage, and also acts to counteract tensile stresses at the joint surface.<sup>15</sup> Sixteen different collagen types have been described in animals, with the majority (85 to 95%) of the total collagen content in equine articular cartilage being type II.<sup>14-16</sup> Type II collagen provides tensile strength to cartilage and is the principle fibrillary collagen in cartilage.<sup>14</sup> Other types of collagen found in small amounts in the extracellular matrix of articular cartilage function in association with fibrillar collagen or other matrix components to provide organization and mechanical stability to the fibrillar network.<sup>15,16</sup>

Proteoglycan is another important component of the articular cartilage extracellular matrix and is composed of glycosaminoglycan chains attached to a protein core.<sup>14-16</sup> Aggrecan is a proteoglycan molecule that comprises about 85% of the proteoglycans in the cartilage extracellular matrix and provides resistance to compressive forces within the articular cartilage.<sup>15</sup> In the cartilage extracellular matrix, aggrecan

forms large aggregates composed of aggrecan monomers attached to hyaluronan.<sup>14,15</sup> The highly charged hyaluronan and the high molecular weight aggrecan together in aggregates are hydrophilic and results in the large osmotic pressure within the cartilage, drawing water in and expanding the collagen matrix.<sup>17</sup>

While chondrocytes constitute only a small percentage of the articular cartilage volume, they are critically involved in maintaining and repairing the extracellular matrix.<sup>14</sup> Chondrocytes have cytoplasmic processes that sense changes in the biomechanical and biochemical environment and using these cytoplasmic processes chondrocytes are able to respond to articular cartilage loading.<sup>15,16</sup> Chondrocyte metabolism is affected by biochemical changes and mechanical stress.<sup>15</sup> At normal physiologic levels of mechanical stress, chondrocyte metabolism favors anabolic processes.<sup>15</sup>

Structurally, cartilage is divided into four different zones: superficial, intermediate, deep, and the calcified zone (Figure 2.2).<sup>15,16</sup> The superficial zone has a high density of chondrocytes and type II collagen fibrils oriented parallel to the joint surface, a small amount of proteoglycans, and a high water content.<sup>15,16</sup> The intermediate zone lies below the superficial, and contains irregularly dispersed round chondrocytes and collagen fibrils, more proteoglycans, and a lower content of water and collagen.<sup>15,16</sup> The deep zone of the cartilage contains the largest chondrocytes, the highest proteoglycan content, lowest collagen content, and lowest water content of the four zones.<sup>15,16</sup> In the deep zone, the chondrocytes and collagen fibrils are oriented perpendicular to the joint surface.<sup>15,16</sup> The calcified zone is composed of mineralized cells and matrix and sits atop the subchondral bone.<sup>15,16</sup>



**Figure 2.2** Diagram of the zones of articular cartilage.

The subchondral bone functions to stabilize the articular cartilage, which is attached to the subchondral bone by the calcified layer of cartilage.<sup>15,16</sup> While the articular cartilage of the joint is avascular and aneural, subchondral bone is extensively vascularized and innervated.<sup>16</sup> If there is loss of articular cartilage and subchondral bone is exposed in the joint, the vascularity of the bone results in a tissue response, including sclerosis, osteophyte formation, or fibrocartilaginous repair tissue formation.<sup>16</sup>

A balance of anabolic and catabolic processes within the joint provides a stable homeostatic environment to support proper joint function.<sup>16</sup> The structures of the joint (i.e., synovial membrane, articular cartilage, subchondral bone) have significant differences in their metabolic processes and turnover rate.<sup>16</sup> This, along with the lack of cartilage vascular and neural supply, affects how the damaged joint tissues undergo healing in order to return to their normal composition.<sup>16</sup> If joint homeostasis cannot be maintained, tissue damage can result in improper joint function, which can lead to further structural damage and the development of disorders such as osteoarthritis.<sup>16</sup> Damage resulting in the loss of the osmotic pressure within cartilage, and thus the lack of water influx, leads to loss of cartilage strength, making it susceptible to further damage.<sup>16</sup> Septic arthritis results in the loss of joint homeostasis, as infectious organisms within joints release toxins and enzymes that stimulate an intrasynovial inflammatory response.<sup>18</sup> Both the infecting organism's virulence factors and the joint's inflammatory reaction alter the metabolism and function of the joint, leading to degeneration.<sup>18</sup>

### **Equine Septic Arthritis**

The intra-articular environment is a privileged environment. Synovial homeostasis and proper function are compromised by infection, which can result in degenerative changes within the joint. Equine septic arthritis, or the infection of a joint by either bacteria or fungi, can result from hematologic spread or direct introduction of microorganisms.<sup>18</sup> Septic arthritis can affect horses of any age, as joint trauma or the iatrogenic introduction of intrasynovial organisms can occur in both foals and adult horses. Immunodeficiency can result in a systemic infection causing septic arthritis, but

immunodeficiency is quite rare in adult horses. In foals, however, septic arthritis commonly occurs secondary to failure of passive transfer of maternal antibodies and bacteremia from systemic disease such as diarrhea or respiratory infection and can affect the joint itself, as well as the adjacent epiphysis and/or physis.<sup>4,18,19</sup> Common organisms causing septic arthritis and osteomyelitis in foals include *Escherichia coli*, *Streptococcus spp.*, *Rhodococcus equi*, *Salmonella spp.*, *Pseudomonas spp.*, *Enterobacter spp.*, and *Actinobacillus spp.*<sup>4,19</sup>

In contrast to the pathophysiology in foals, septic arthritis in adult horses is more commonly due to introduction of microorganisms into the joint in association with a puncture wound, an open wound adjacent to a joint, or following an intra-articular injection for diagnostic or therapeutic purposes.<sup>4,19</sup> The organisms involved are generally those present as commensals on the skin or within the environment. *Staphylococcus aureus*, the most common commensal on equine skin, is often associated with iatrogenic septic arthritis following arthrocentesis or arthroscopy, but is also commonly isolated from wounds.<sup>4,19</sup> Lacerations near joints can result in infection from a large variety of microorganisms, with *Staphylococcus aureus*, *Pseudomonas spp.*, other staphylococci species, and Enterobacteriaceae being commonly isolated bacteria.<sup>4,19</sup> Other bacterial joint contaminants include *Escherichia coli*, *Salmonella spp.*, *Corynebacterium pseudotuberculosis*, and anaerobes.<sup>18</sup> The focus of this thesis is septic arthritis in adult horses.

#### *Incidence of Joint Infections*

The most common cause of septic arthritis in adult horses is due to a penetrating wound, affecting 24% of horses with septic synovial structures.<sup>1</sup> Additional causes of

septic arthritis include intra-articular injection in 22% of cases, post-surgical infection affecting 13% of cases, and 6% of cases having septic arthritis due to no known etiology.<sup>1</sup> Septic arthritis can result from the iatrogenic introduction of organisms into joints through injection of local anesthetics for lameness evaluations, therapeutic administration of medications, synoviocentesis, or arthroscopy. One large retrospective study reported that the joints most commonly affected with septic arthritis in adult horses are the tibiotarsal joint (34% of joints), fetlock joint (24%), carpus (20%), pastern (6%), and stifle (3%).<sup>1</sup>

The skin of horses is colonized by a group of normal bacteria, called commensal organisms, which can protect skin and mucosal surfaces. However, these organisms can contaminate a wound or injection site and can lead to infection. Microorganisms that colonize the skin and hair of the horse can be introduced into the joint by the arthrocentesis needle, along with skin and hair fragments.<sup>20</sup> Clipping the hair of the arthrocentesis site, use of large gauge needles, and the reuse of needles significantly increased the risk of intrasynovial contamination with hair and tissue.<sup>20</sup> Intra-articular medications, such as polysulfated glycosaminoglycans and certain corticosteroids, may increase the risk of septic arthritis due to their actions that decrease the intra-articular inflammatory response, resulting in alteration of the joint's normal defense mechanisms.<sup>4,11,12,21</sup> Intra-articular polysulfated glycosaminoglycans may inhibit white blood cell chemotaxis and phagocytosis and alter synoviocyte enzyme functions.<sup>21</sup> Corticosteroids inhibit white blood cell migration, interfere with phagocytic actions of synoviocytes and white blood cells, and may delay the clinical onset of septic arthritis.<sup>22</sup>

Elective arthroscopy is considered a minimally invasive, “clean” surgery (non-contaminated), and carries a relatively low risk of post-operative joint infection, with approximately 0.9% of joints developing septic arthritis following arthroscopy.<sup>23</sup> Horse breed and the joint undergoing arthroscopic surgery are associated with increased risk factors for septic arthritis, with draft breeds and the tibiotarsal joint being more likely to be affected.<sup>23</sup> Draft breeds have an unknown reason for increased risk of developing septic arthritis post-arthroscopy.<sup>23</sup> The arthroscopic portal skin incisions for the tibiotarsal joint are in close proximity to the underlying synovial incisions, and fluid leaking from the synovial incisions can easily contact skin bacteria, leading to synovial infection.<sup>23</sup> As elective arthroscopy carries a low risk for post-operative septic arthritis, the use of perioperative antimicrobials is controversial. The joint infection rate in horses undergoing elective arthroscopy that did not receive perioperative antimicrobials is 0.5% of joints.<sup>24</sup> This rate is comparable to the incidence of infection reported in other elective arthroscopy studies.<sup>23,24</sup>

Synovial infection associated with a laceration or puncture wound can involve any joint, tendon sheath, or bursa. Abrasions and contused wounds near joints can result in joint infection that develops within a few days of injury, as microorganisms from the infected soft tissue move through the damaged joint capsule and into the joint.<sup>13</sup> The structures distal to and including the carpus and the tarsus are most commonly affected. Fetlock joints are the most frequently affected joint with septic arthritis resulting from a wound (33% of joints), followed by the tarsus (17%), and the pastern (13%).<sup>1</sup>

### *Pathophysiology of Joint Infection*

Joint infection is defined as the introduction of microorganisms into the synovial membrane or synovial fluid, resulting in an inflammatory reaction, microorganism proliferation and attachment, and establishment of active infection within the joint.<sup>12</sup> The lack of a basement membrane in the synovial membrane allows bacteria to easily cross into the joint, and once in the joint, relatively low synovial fluid movement allows for bacterial adherence and infection.<sup>25,26</sup> Additionally, the synovial membrane villi provide a good environment for bacteria to adhere and proliferate. The synovial membrane can normally control introduced bacteria, preventing bacterial proliferation and joint infection through the phagocytic properties of Type A synoviocytes and the actions of cytokines and inflammatory mediators produced by all synovial cell types.<sup>4,12,16</sup> Synovial damage, an organism's virulence, and inoculum amount all play a role in whether an organism can overcome the joint's defense mechanisms and cause infection.<sup>4,12</sup> The pathogenicity and virulence of an organism is defined as its ability to release extracellular toxins and enzymes, binding to joint tissue and establishing growth.<sup>12</sup>

Following bacterial colonization, the synovium responds by releasing inflammatory mediators, enzymes, and free radicals.<sup>4,12</sup> The synovial membrane becomes hyperemic, with increased vascular permeability, resulting in intrasynovial hemorrhage, extravasation of fibrin, macrophages, and neutrophils from the synovial membrane, and release of inflammatory mediators into the joint.<sup>4,18</sup>

Neutrophils kill bacteria by intracellular phagocytosis and by releasing extracellular enzymes such as lysozyme, gelatinase, collagenase, elastase, or cathepsin G.<sup>12</sup> Furthermore, neutrophils and macrophages release oxygen-derived free radicals,

which can cleave proteoglycans and collagen.<sup>27</sup> Free radicals can cleave hyaluronic acid, which can result in lower synovial fluid viscosity, reducing its biomechanical protection and boundary lubrication.<sup>12,18,27</sup>

Intrasynovial inflammatory mediators activate the plasmin, kinin, coagulation, and fibrinolytic pathways, leading to amplification of inflammation and activation of synoviocytes and chondrocytes.<sup>4,12</sup> Activation of both synoviocyte cell types upregulates production of key inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ).<sup>18</sup> The concentrations of these cytokines are elevated in the synovial fluid of horses affected with synovitis and septic arthritis.<sup>12,28</sup> Interleukin-1 and TNF- $\alpha$  increase the synthesis of matrix metalloproteinases (MMP) by chondrocytes and synoviocytes, induce the production of cartilage matrix proteinases, and induce the degradation of cartilage matrix by chondrocytes.<sup>27</sup> Joint infections usually result in the highest concentrations of cytokines such as IL-1, IL-6, TNF- $\alpha$ , and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>).<sup>12,18</sup>

In response to increased inflammatory mediators in the joint, MMP are produced by activated chondrocytes, synoviocytes, macrophages, fibroblasts, osteoblasts, and endothelial cells.<sup>15,27</sup> These MMP have a net catabolic effect on cartilage, resulting in degradation of cartilage matrix.<sup>4,12</sup> The main classes of MMP are collagenases, gelatinases, and stromelysins, with collagenases and stromelysins primarily contributing to cartilage degradation.<sup>15,27</sup> Collagenases (MMP-1, MMP-8, and MMP-13) cleave collagen types II, IV, IX, and X; stromelysins (MMP-3 and MMP-10) can break down proteoglycans and collagen types IV, IX, X, and XI; and gelatinases (MMP-2 and MMP-9) can degrade collagens and elastins.<sup>15,27</sup> The actions of these MMP result in cartilage

degradation, which manifests as cartilage fibrillation, chondrocyte necrosis, and softer and weaker articular cartilage and can further perpetuate the inflammatory process within the joint.<sup>27</sup> Activated chondrocytes also produce prostaglandins (primarily PGE<sub>2</sub>), which can decrease the proteoglycan content in cartilage by degradation and reduced synthesis.<sup>27</sup> The reduction of proteoglycan content in articular cartilage along with proteoglycan structural alterations result in changed degree of aggregation and an increase in cartilage water content, making the cartilage less resistant to loading forces.<sup>27</sup>

Joint effusion and fibrin accumulation also contribute to the disease process. Infection induces joint effusion, and excessive joint effusion causes increased intra-articular pressure and pain, reduced blood flow to the synovium, and ischemia of subchondral bone and articular structures.<sup>4,12</sup> Extravasation of fibrin from the synovial membrane results in fibrin accumulation on synovial membrane surfaces and free fibrin in the synovial fluid.<sup>4,12</sup> Fibrin accumulation can prevent nutritional exchange through the synovial fluid and can contribute to pannus formation, an intrasynovial fibrinocellular conglomerate that collects tissue, foreign material, and bacteria.<sup>4,12</sup> Organisms hidden within the pannus are protected from antimicrobials and phagocytic white blood cells within the synovial fluid.<sup>18</sup> Joint lavage is required to remove the fibrin conglomerates.<sup>18</sup>

If untreated, septic arthritis can result in significant joint damage. Proteoglycan loss and collagen damage reduce biomechanical resistance of the cartilage, which can lead to significant damage to articular cartilage.<sup>18</sup> Chondrocyte cellular activity and proteoglycan synthesis is increased in order to repair damaged cartilage, but eventually, cartilage degradation exceeds repair and results in osteoarthritis.<sup>27</sup> Timely diagnosis and treatment of suspected septic arthritis is critical, as degenerative joint changes can

prevent a horse from returning to its previous level of work, or, if changes are severe enough, may result in euthanasia.

### **Clinical Signs and Diagnosis of Septic Arthritis**

Early diagnosis and treatment of joint infections is critical for obtaining a successful outcome in affected horses. According to one clinical report, horses with open wounds involving a synovial structure that were treated medically or surgically within 24 hours of injury were less likely to develop septic arthritis, more likely to survive, and more likely to return to function than horses that were treated more than 24 hours after joint injury occurred.<sup>3</sup> In this report, 53% of horses with open joint wounds that were treated within 24 hours of injury developed septic arthritis, with 65% of these horses surviving.<sup>3</sup> Additionally in this same report, 92% of horses that were treated 2 to 7 days following open joint injury developed septic arthritis, with a 39% survival rate.<sup>3</sup>

When presented with a case of possible septic arthritis, the veterinarian should obtain a patient history and perform a complete physical examination on the horse. History of the horse having a recent wound, joint injection, arthroscopic surgery, surgery near a joint, systemic illness or immunocompromise is often reported and can aid in determining the duration of infection and possible infecting microorganisms.<sup>4</sup> Horses with septic arthritis are usually significantly lame (non-weight bearing lame, AAEP grade 5) due to increased pressure within the infected joint, hypersensitivity of the joint capsule, and inflammation of surrounding tissues.<sup>4,29</sup> If the affected joint is open and draining, if the joint was recently injected with a corticosteroid, or if analgesic medications were recently administered, the horse may present with less severe lameness

or may not appear lame at all, complicating the diagnosis.<sup>4</sup> Additionally, if the joint infection occurred shortly before the horse's evaluation, lameness may be less severe.<sup>12</sup> The horse's vital parameters may be within normal limits, but heart rate and respiratory rate may be elevated depending on the level of pain.<sup>4</sup> Joint effusion, periarticular soft tissue heat and swelling, and sensitivity to palpation and manipulation of the joint are clinical findings associated with joint infection.<sup>4,12</sup> A careful physical examination should focus on evidence of trauma or wounds of any kind, such as blood or exudate on the skin. Small puncture wounds that have sealed over can be difficult to identify and may require the hair be clipped to see. Peripheral blood analysis should include evaluation of a complete blood count. Usually adult horses do not show significant changes to their peripheral blood analysis. The average peripheral white blood cell count of 192 horses in one retrospective study was mildly elevated at  $12.53 \times 10^9$  cells/L and ranged from  $6.0\text{-}21.4 \times 10^9$  cells/L.<sup>1,4</sup> When complete blood count values are abnormal, a high normal white blood cell count, mild neutrophilia, and mild hyperfibrinogenemia are the most common changes noted in laboratory data.<sup>4,12</sup>

### *Diagnostic Imaging*

Radiography of the affected joint, ultrasonography of the joint and surrounding tissues, nuclear scintigraphy, computed tomography (CT), or magnetic resonance imaging (MRI) can aid in the diagnosis of horses with suspected septic arthritis. A full radiographic series of the affected joint is indicated to evaluate for bone involvement, such as osteoarthritis, osteomyelitis, osteitis, physitis, or fracture.<sup>4,12</sup> Fistulograms or

intra-articular injection of radiographic contrast solution can be used to determine communication of a wound with a joint, or to highlight articular cartilage defects.<sup>4,12</sup>

Ultrasonography can be used to evaluate joint effusion and inflammation of the synovium, to identify foreign bodies within the joint or surrounding tissues, or to determine communication of a wound with an adjacent joint.<sup>4,12,30</sup> Common ultrasonographic findings of equine joints with septic arthritis include marked synovial effusion in 81% of cases, moderate to severe synovial thickening in 69%, echogenic synovial fluid in 55%, focal hyperechogenic areas in 33%, and the presence of intrasynovial fibrin in 64% of cases.<sup>30</sup> These ultrasonographic findings, in combination with the clinical signs of septic arthritis, can be useful in confirming a diagnosis of septic arthritis.<sup>30</sup>

Nuclear scintigraphy can be used to localize inflammation or infection. <sup>99m</sup>Tc-methylene diphosphonate (<sup>99m</sup>Tc-MDP) binds to hydroxyapatite in the bone and is the most commonly used radiopharmaceutical in horses.<sup>31,32</sup> The uptake of <sup>99m</sup>Tc-MDP is related to bone metabolism (osteoblastic activity) and/or blood flow to the bone.<sup>32</sup> <sup>99m</sup>Tc-MDP scintigraphy results in identification of non-specific inflammation.<sup>4,33</sup> Additionally, <sup>99m</sup>Tc-MDP scintigraphy can detect areas of low radioactivity, “cold” spots, that result from ischemia, a possible sequela of infection.<sup>12,18</sup> Leukocytes labeled with <sup>99m</sup>Tc-hexamethylpropyleneamine oxine (<sup>99m</sup>Tc-HMPAO) may be more accurate in detecting areas of inflammation and infection, as leukocytes accumulate at sites of active inflammation.<sup>4,33,34</sup> The use of <sup>99m</sup>Tc-HMPAO scintigraphy for orthopedic infections is limited in the horse; however, its use may aid in the diagnosis

of equine septic arthritis, especially in cases that are not easily confirmed by routine diagnostics.<sup>4,33</sup>

CT and MRI offer excellent detail of bones and soft tissues; however, the added expense and potential for general anesthesia can be limiting. These diagnostic imaging techniques may be useful to help determine if there is ongoing infection, a nidus of infection, or if lameness is due to chronic synovitis and osteoarthritis.<sup>35</sup> CT can reveal osteomyelitis and bone destruction, but lacks soft tissue detail. MRI can reveal soft tissue injury, fluid proliferation, bone edema, and osteochondral bone damage in horses with suspected septic arthritis.<sup>35</sup> MRI may aid in earlier diagnosis of equine septic arthritis, especially when the clinical diagnosis is challenging, such as if synovial fluid cannot be obtained or synovial fluid analysis is not conclusive.<sup>35</sup>

### *Synovial Fluid Analysis*

The most important diagnostic tool for cases of suspected septic arthritis is synovial fluid collection and analysis. Synovial fluid analysis provides an indication of the degree of inflammation within the joint and helps to distinguish between septic and non-septic synovitis.<sup>5</sup> This differentiation can be difficult due to the large amount of variability in clinical symptoms and changes in synovial fluid characteristics and parameters. In conjunction with clinical signs, it is generally considered that synovial fluid with a total protein (TP) of greater than 4.0 g/dL, a total nucleated cell count (TNCC) of greater than 30,000 cells/ $\mu$ L, and a cellularity of greater than 80% neutrophils is considered septic.<sup>5,36,37</sup>

A significant concern regarding the diagnosis of equine septic arthritis is the clinical similarities between joint infection and the less insidious joint inflammation. Horses with synovitis, or joint inflammation, can have clinical signs very similar to septic arthritis, and thus synovial fluid analysis is a very important aid in the proper diagnosis. Horses early in the disease process, sepsis resulting from corticosteroid injection, non-septic inflammation, or infection with an organism of low virulence may have synovial fluid TNCC below 30,000 cells/ $\mu$ L.<sup>5</sup> Non-septic inflammation may be due to joint trauma, repeated arthrocentesis, and intra-articular medications such as corticosteroids, antimicrobials, or local anesthetics.<sup>5</sup> Additionally, non-septic inflammation can result in synovial fluid TP concentration, TNCC, and cytological findings similar to the classic septic synovial fluid parameters.<sup>5</sup>

To prevent the introduction of skin fragments or microorganisms during arthrocentesis, synovial fluid collection should be performed aseptically (Figure 2.3).<sup>5</sup> Once collected, synovial fluid should be aliquoted into samples for cytology, culture and sensitivity, and other analyses. Routine synovial fluid analysis includes evaluation of the gross appearance of the fluid, quantification of TP and TNCC, and fluid cytology.<sup>5</sup> Iatrogenic trauma from arthrocentesis may cause the synovial fluid to be bloody, but the blood may not be present in the whole sample of synovial fluid, while hemarthrosis caused by joint sepsis or synovitis results in the synovial fluid appearing uniformly bloody, due to hemorrhage from the inflamed synovium.<sup>5</sup> Septic synovial fluid may be orange or red in color (Figure 2.4), cloudy, turbid, and nonviscous.<sup>5</sup> Synovial fluid turbidity is due to synovial inflammation, resulting in increased fluid cellularity and the loss of viscosity is due to the enzymatic breakdown of hyaluronic acid.<sup>5</sup> Synovial



**Figure 2.3** Arthrocentesis. Aseptic collection of joint fluid from a fetlock joint. Photo credit: Dr. Chris Byron.

inflammation causes protein to leak from damaged vessels, increasing synovial fluid TP concentration, which can vary depending on the duration and severity of the disease process.<sup>5,12</sup> Septic synovial fluid usually has a TP greater than 4.0 g/dL, while non-septic synovitis has lower TP concentrations.<sup>5,37</sup> However, TP concentrations of less than 2.5 g/dL have been reported in cases with positive synovial fluid bacterial cultures.<sup>38</sup>



**Figure 2.4** Abnormal synovial fluid. Red, cloudy synovial fluid from a horse with septic arthritis.

Synovial fluid infection may take 12 to 24 hours to cause changes in TNCC, with counts greater than 30,000 cells/ $\mu$ L suggestive of sepsis.<sup>12,18,37</sup> Septic synovial fluid predominantly contains neutrophils, with neutrophil counts of greater than 80% of the nucleated cells a common finding.<sup>1,4,12,37</sup> The neutrophils present in septic synovial fluid may be normal in appearance, or rarely have degenerative changes.<sup>36</sup>

Although a positive bacterial culture from synovial fluid may be considered by some to be the gold standard for the diagnosis of equine septic arthritis, isolation of bacteria from infected synovial fluid can be challenging using standard culture techniques. Cultures from infected joints are negative in almost 50% of clinical cases, as bacteria can be hidden in the pannus or synovial membrane.<sup>5,38,39</sup> One study reported that synovial fluid samples that had no bacterial growth on initial culture, re-culture of samples that were incubated in blood culture medium for 24 hours resulted in a positive

bacterial culture for all samples.<sup>40</sup> Bacterial culture is recommended in all cases of suspected septic arthritis because it determines the cause of infection and is important for the determination of antimicrobial sensitivity and susceptibility testing that can help determine antimicrobial selection.<sup>18</sup> Ideally, synovial fluid samples should be collected for bacterial culture before the administration of systemic and local antibiotics, as antibiotics can interfere with bacterial growth in the culture medium. Gram stain of synovial fluid is positive for microorganisms in about 25% of clinical cases.<sup>1,5</sup> Despite this low rate of detection, if bacteria are identified on Gram stain, this can greatly aid in antimicrobial selection.<sup>1,5</sup>

#### *Additional Synovial Fluid Diagnostics*

There are a variety of alternative diagnostics and assays used to help differentiate between non-septic synovitis and septic arthritis. Synovial fluid pH, lactate concentration, and glucose concentration may be useful in diagnosing septic arthritis. Synovial fluid pH decreases with septic arthritis.<sup>41</sup> Normal synovial fluid pH is approximately 7.3, while septic synovial fluid pH is often less than 6.9.<sup>41,42</sup> The correlation between increased total nucleated cell count and decreased pH in septic synovial fluid suggests that white blood cell metabolism is responsible for synovial fluid acidosis.<sup>43</sup> Lactate increases in septic synovial fluid due to anaerobic glycolysis.<sup>41</sup> Synovial fluid lactate concentration in healthy joints is less than 3.9 mmol/L and a lactate concentration of greater than 4.9 mmol/L is suggestive of septic arthritis.<sup>5,41,42</sup> In healthy horses, synovial fluid glucose concentrations are similar to those of peripheral blood.<sup>41</sup> In the acute stages of synovial infection, the increased glycolytic activity of synoviocytes

and neutrophils result in a decreased synovial fluid glucose concentration and a serum and synovial fluid glucose difference of greater than 2.2 mmol/L.<sup>41,42</sup> While synovial fluid pH, lactate, and glucose are useful diagnostics to help confirm septic arthritis, their values can be affected by other variables. Synovial fluid pH can be affected by the size of the joint and the temperature of the synovial fluid sample.<sup>41</sup> The lactate concentration in synovial fluid increases with inflammation and infection, and some non-septic inflammatory conditions can result in elevated lactate values.<sup>41</sup> The difference in serum and synovial fluid glucose can be affected by the animal's level of pain, nutrition, synovial fluid white blood cell count, and synovial necrosis.<sup>41</sup> As a result, these assays are used as an additional diagnostic for determining septic arthritis in horses and are not relied on solely for diagnosis.

Recently, equine synovial fluid biomarkers have become an area of interest to aid in the diagnosis of equine septic arthritis. Biomarkers include biochemical molecules such as enzymes or proteins and the results of their measurement can be used to monitor biologic and disease processes.<sup>44</sup> Biomarkers commonly used to help diagnose equine septic arthritis include the activity of the latent and bioactive form of MMP, the activity of myeloperoxidase, and the concentration of serum amyloid A (SAA).<sup>5,45-47</sup>

Synoviocytes and chondrocytes express a family of enzymes, MMP, that are normally present in a latent, or inactive, form in synovial fluid.<sup>44</sup> Once activated by inflammation, MMP have the potential to destroy the matrix components of cartilage.<sup>44,45</sup> Analysis of synovial fluid MMP-2 and MMP-9 can be used to help diagnose equine joint sepsis.<sup>5,45</sup> The proenzyme (latent) forms of MMP-2 and MMP-9 are significantly elevated in septic joints and inflamed joints.<sup>45</sup> Conversely, only the active form of MMP-

9 is significantly elevated in purely septic joints.<sup>45</sup> Additionally, there is a correlation between the white blood cell concentration of synovial fluid and the concentration of MMP-9.<sup>45</sup> MMP-9 is mainly produced by neutrophils and the increased concentration of MMP-9 in septic joints may be due to release from the increased numbers of synovial fluid neutrophils.<sup>45</sup>

Myeloperoxidase (MPO) is an antibacterial enzyme produced by neutrophils and is involved in the production of reactive oxygen species.<sup>47</sup> MPO is usually released into phagolysosomes to assist bacterial killing, but has been shown to cause tissue damage in severe inflammatory conditions.<sup>47</sup> Synovial fluid MPO concentration is elevated in inflamed and septic joints; however, septic synovial fluid contains significantly higher concentrations of MPO than inflamed synovial fluid.<sup>47</sup>

As mentioned earlier, SAA is an acute phase protein that increases in response to inflammation or infection in the horse.<sup>46</sup> Some SAA serum isoforms are produced by the liver and other specific isoforms are produced in joints, which can be useful in the diagnosis of septic arthritis.<sup>46</sup> Both serum and synovial fluid SAA concentrations increase with non-septic synovitis and septic arthritis, with septic arthritis resulting in the highest concentrations of SAA.<sup>46</sup> As the focus of this manuscript is the response of serum and synovial fluid SAA to synovitis and septic arthritis in the horse, a detailed discussion of SAA appears later.

### **Treatment of Septic Arthritis**

Once a diagnosis of septic arthritis is made, appropriate and aggressive treatment should be initiated immediately to resolve infection, reduce inflammation, provide

analgesia, and restore the normal physiologic conditions to the joint as rapidly as possible.<sup>13,48</sup> A combination of treatment modalities for septic arthritis such as systemic and local antibiotics, joint lavage, surgery, anti-inflammatory medications, and intra-articular medications is often necessary to achieve the best outcome.

#### *Antimicrobial Therapy for Septic Arthritis*

Frequently a combination of systemic and local antibiotic administration is used in horses to achieve the best outcome. Immediately following the collection of synovial fluid for diagnostics, broad-spectrum antibiotics should be initiated.<sup>4</sup> Antibiotic treatments can be adjusted based on the results of the synovial fluid culture and sensitivity.<sup>4</sup> Systemic antibiotics can be administered intravenously, intramuscularly, or orally. In the acute stage of septic arthritis, intravenous antibiotic administration is usually the most efficacious route of administration for horses. Intravenous antimicrobial administration requires either repeated venipuncture or venous access using an intravenous catheter. Both administration techniques carry the risk of inducing thrombophlebitis. Therefore, intravenous medications are generally administered during acute disease states in horses and oral medications are administered when antibiotics need to be administered for long durations. Antimicrobial treatment should be continued for two to four weeks after clinical signs decline, and if no improvement in clinical signs are noted after 72 hours of treatment, diagnostics may need to be repeated and treatments may need to be changed.<sup>4,13</sup> If a positive synovial fluid bacterial culture is not obtained, broad-spectrum antimicrobial therapy should be continued two to four weeks after the

clinical signs of septic arthritis have resolved and the synovial fluid parameters have normalized.<sup>4</sup>

The addition of local antibiotic therapy allows for the direct penetration of antibiotics into the joint and enables the use of high doses of concentration-dependent antibiotics, such as aminoglycosides, to augment systemic antibiotic administration.<sup>18</sup> Local administration techniques include intra-articular antibiotics, regional limb perfusions, continuous rate infusions into the joint, or antibiotic-impregnated delivery systems. Intra-articular administration results in higher concentrations of antibiotics locally when compared to systemic antibiotic administration, but intra-articular administration also induces a mild synovitis; however, this synovitis causes less synovial damage than septic arthritis.<sup>4,49</sup> Intra-articular medication can be administered by arthrocentesis, which must be repeated during the course of local antimicrobial therapy. Alternatively, specialized infusion systems can be attached to catheters and placed within affected joints to provide constant rate infusion of antimicrobials or to allow antimicrobials to be administered multiple times per day, negating the need for repeated arthrocentesis.<sup>4,50</sup> Additionally, an intrasynovial catheter can be used for repeated synovial lavage performed on the standing, sedated horse.<sup>50</sup> Continuous intrasynovial antimicrobial infusion can be performed using an intrasynovial catheter and an attached “balloon” continuous rate infusion system.<sup>51</sup> Continuous rate infusion of antimicrobials results in maintenance of the synovial fluid minimal inhibitory concentration of the administered antimicrobial over a longer period of time than systemic antimicrobial administration alone.<sup>4,51</sup> While the use of these intrasynovial catheter systems does require intensive management to prevent catheter site complications such as kinking or

leakage and strict aseptic handling of the catheter to avoid further contamination of the joint, these systems should be considered for septic arthritis cases that require repeated synovial lavage or intra-articular medications.<sup>50</sup>

Other possibilities for regional antimicrobial application are intravenous and intraosseous perfusions. Both routes of administration result in synovial fluid antimicrobial concentrations much higher than the minimal inhibitory concentration during the 24 hours after administration.<sup>13,52</sup> Broad spectrum, concentration dependent antimicrobials are desired for the treatment of orthopedic infections, therefore aminoglycosides are frequently administered for the treatment of septic arthritis.<sup>12,53,54</sup> It is important to note that drug interactions can occur when different antimicrobials are administered together, which can result in decreased efficacy of the medications. The combination of amikacin, an aminoglycoside, with ticarcillin/clavulanate, a beta-lactam, resulted in lower synovial fluid amikacin concentrations and antimicrobial activity than amikacin administered alone.<sup>53</sup> Ultimately, synovial fluid bacterial culture and sensitivity provides the best guidance regarding antimicrobial choice.<sup>54</sup> The use of intravenous regional limb perfusions is common practice for treatment of infections in the distal limb of horses. Regional limb perfusion is performed by administering an antimicrobial solution into peripheral vein of a selected portion of the limb that has been isolated from the systemic circulation by the application of a tourniquet.<sup>54</sup> After the tourniquet has been applied, the antimicrobial solution is injected slowly into the isolated vein, and the tourniquet is left in place for 20 to 30 minutes to allow for the medication to remain within the isolated area.<sup>13</sup> Regional limb perfusions can be performed on anesthetized or standing horses. Movement during a standing procedure may result in failure of vascular

occlusion by the tourniquet, causing leakage of the perfusate into the systemic circulation, and decreased synovial fluid antimicrobial concentration.<sup>55</sup> While general anesthesia prevents horses from moving, the increased risk of general anesthesia to the horse and the increased expense associated with general anesthesia precludes its routine use for regional limb perfusions. Alternatively, sedation and perineural anesthesia can be used to decrease sensation to the limb being perfused, thus reducing horse movement during standing regional limb perfusions.<sup>55</sup>

Tourniquet type and efficacy can also play a role in synovial fluid antimicrobial concentrations. Wider tourniquets, such as pneumatic tourniquets and wide rubber Esmarch bandages, should be used for regional limb perfusions, as both provide appropriate vascular occlusion to maintain antimicrobial concentration within the synovial fluid above minimal inhibitory concentration for causal bacteria.<sup>56</sup> In addition to the use of an appropriate tourniquet, the perfusate volume used for regional limb perfusions affects the concentration of antimicrobial in the synovial fluid.<sup>57</sup> The selected antimicrobial is diluted in a solution to create the perfusate, with a final perfusate volume ranging from 10 mL to 60 mL depending on the volume of tissue being perfused.<sup>54,57</sup> Perfusate volume influences the intravascular pressure and how well the antimicrobial diffuses into the surrounding tissue.<sup>54,57</sup> The ideal perfusate volume is unknown.<sup>54,57</sup> While a large perfusate volume may result in high intravascular pressure and good tissue diffusion of the medication, too large a perfusate volume may exceed the tourniquet pressure and the perfusate may leak into the systemic vasculature.<sup>57</sup> Synovial fluid concentrations of the antibiotic gentamicin were not significantly different when compared between low (10 mL), medium (30 mL), and high (60 mL) perfusate

volumes.<sup>57</sup> This suggests that antimicrobial diffusion down a concentration gradient may be equally important as intravascular pressure in the dissemination of an antimicrobial during intravenous regional limb perfusion and that low perfusate volumes can achieve appropriate synovial fluid antimicrobial concentrations.<sup>57</sup> Regional limb perfusions are commonly repeated every 24 to 48 hours, based on the nature of the infection and clinician preference.<sup>13</sup> Often three sequential treatments are performed; however, longer duration of treatment may be used if necessary.<sup>13</sup>

Intraosseous regional limb perfusion is an alternative to the intravenous technique, eliminating the need for repeated venipuncture.<sup>13</sup> To facilitate intraosseous perfusion, a unicortical hole is drilled in the bone proximal or distal to the affected joint and temporary tubing or an intraosseous bone port is placed.<sup>4,58</sup> Antimicrobials are then administered directly into the medullary cavity of the bone.<sup>4,58</sup> Similar to intravenous perfusion, a tourniquet is placed proximal to the site of administration to confine the antimicrobial to a localized area. Intraosseous and intravenous regional limb perfusions result in similar synovial fluid concentrations of antimicrobial.<sup>58</sup> While intraosseous perfusions require special equipment and are more difficult to perform than intravenous perfusions, the intraosseous technique can be used when cellulitis, soft tissue trauma, or vascular damage around the affected joint preclude intravenous regional perfusion.<sup>13</sup>

Antimicrobial-impregnated delivery systems, or implants, are another method to reach elevated levels of antimicrobials at the site of application. These implants can be categorized as bioabsorbable or non-absorbable.<sup>13,59</sup> Polymethylmethacrylate (PMMA) is a commonly used non-absorbable, high density polymer to which antimicrobials can be added prior to formation of beads or cylinders.<sup>13,60</sup> Antibiotic elution from PMMA

occurs in a bimodal manner, with 5% of the antibiotic released within the first 24 to 48 hours, followed by a slow release that can occur for years.<sup>60</sup> The local concentration of antibiotic released from PMMA implants is up to 200 times the systemic antibiotic concentration, resulting in extremely high local antibiotic concentrations at the site of infection.<sup>60</sup> PMMA implants should not be placed intra-articularly, as they induce synovitis and superficial cartilage damage.<sup>60,61</sup> PMMA implants can be placed peri-articularly and are often removed 2 to 4 weeks later, as the implant can cause localized inflammation or can cause bacterial resistance.<sup>4,13,59,60</sup>

Bioabsorbable materials, including microspheres, chitosan, plaster of paris, collagen-based systems, and hydroxyapatite.<sup>4,13,59</sup> These have advantages over PMMA implants because they have a faster and more constant release of antibiotics, better biocompatibility, and biodegradability.<sup>4,62</sup> The use of gentamicin-impregnated collagen sponges following arthroscopic lavage in horses with synovial sepsis has been reported to have an excellent clinical outcome.<sup>59,63</sup> In addition to their local antibiotic effect, gentamicin-impregnated collagen sponges may also stimulate wound healing.<sup>59,63</sup> Bioabsorbable implants do not cause as much inflammation as PMMA implants and do not require a second surgery to be removed.<sup>4</sup>

### *Joint Lavage and Debridement*

Wounds adjacent to or involving joints should undergo surgical debridement to decrease contamination.<sup>13</sup> Possibly the most important component in the treatment of septic arthritis is the removal of inflammatory products, debris, devitalized tissue, fibrin, and bacteria from the infected joint.<sup>4</sup> As previously stated, the presence of these

materials and products affect the joint's function and metabolism and can ultimately lead to irreversible joint damage and osteoarthritis.<sup>18</sup> Methods of synovial lavage or drainage include through-and-through lavage, arthroscopy, and arthrotomy.<sup>4,12,13,18</sup> Through-and-through lavage is easy to perform and inexpensive. Typically used when arthroscopic lavage is not possible, through-and-through lavage can be performed under general anesthesia or standing sedation.<sup>19</sup> Large (usually 18 to 14 gauge) ingress and egress needles are aseptically placed into the infected joint and 3 to 5 liters of sterile, isotonic fluids are flushed through the joint.<sup>18,19,64</sup> A stab incision can be an alternative to the egress needle, as needles can become obstructed with fibrin.<sup>18,19</sup> Through-and-through lavage is best used for acute infections, which may not have significant fibrin deposition as this technique of joint lavage cannot effectively remove fibrin. Additional disadvantages of through-and-through lavage include the inability to remove large foreign material, to assess the articular cartilage, and to debride bone lesions.<sup>64,65</sup>

Accordingly, chronic or severe synovial infections should be treated by arthroscopy or arthrotomy. Arthroscopic lavage and debridement is the preferred approach for horses with septic arthritis.<sup>18</sup> Arthroscopy allows for visualization of the synovial structures, directed removal of fibrin or foreign material, and debridement of bony or tendinous lesions.<sup>2,4,64</sup> Direct visualization of the articular cartilage and surrounding structures helps determine prognosis.<sup>64</sup> Arthroscopic findings of osteochondral lesions, osteomyelitis, and the presence of marked deposits of pannus are associated with non-survival.<sup>2</sup> Arthroscopic treatment of equine septic arthritis has also been reported to decrease the duration of systemic antimicrobial administration and decrease the length of hospitalization.<sup>2,4</sup>

Arthrotomy may be used for chronic cases of septic arthritis and involves creating an incision into the joint to provide drainage at the ventral aspect of the joint.<sup>13,64</sup> Combined with systemic and local antibiotics and joint lavage, open drainage of the affected joint by arthrotomy can successfully eliminate infection and is useful for infections that have been unresponsive to other treatment methods.<sup>48</sup> Arthrotomy incisions require care to prevent environmental contamination of the open joint and can be surgically closed or left to heal by second intention.<sup>48</sup> Complications of arthrotomy include secondary joint infection, delayed healing of the arthrotomy incision, joint capsule fibrosis, or decreased range of motion; however, these complications are rare.<sup>48</sup> Ultimately, the need for arthrotomy is low, due to the effectiveness of arthroscopic lavage and systemic and local antimicrobials.<sup>64,65</sup>

#### *Additional Therapies for Septic Arthritis*

While surgical and antimicrobial treatment does improve comfort in horses affected with septic arthritis, the condition is very painful and necessitates pain management.<sup>4,18</sup> Nonsteroidal anti-inflammatory (NSAID) medications provide analgesia and reduce inflammation by decreasing prostaglandin synthesis, with flunixin meglumine, phenylbutazone, or firocoxib being common NSAIDs used in the horse.<sup>4,12,17</sup> Nonsteroidal anti-inflammatory medications have variable effects on the joint, ranging from no effect to inhibition of chondrocyte metabolism to chondroprotective effects, depending on the type of NSAID.<sup>17,18</sup> In general, reduction of pain results in improved ambulation and joint motion, reducing the risk of support limb laminitis, preventing fibrous adhesion formation, and improving articular cartilage nutrition.<sup>12,64</sup> Other

methods of analgesia include the use of epidural analgesia for severe hind limb pain, constant rate intravenous infusions of lidocaine, opioids, or ketamine, or topically applied anti-inflammatory medications such as diclofenac.<sup>4</sup>

Other medications that may be useful adjuncts to treatment include polysulfated glycosaminoglycan, corticosteroids, and hyaluronan. Intra-articular polysulfated glycosaminoglycan and corticosteroids can increase the risk of synovial infection and their administration is not advised.<sup>12,21,64</sup> Intra-articular hyaluronan has anti-inflammatory effects on septic joints, but the synovial inflammation may degrade the hyaluronan before it can produce an effect.<sup>64</sup> Additionally, intra-articular hyaluronan may help preserve synovial membrane integrity in models of equine septic arthritis<sup>66</sup> and has been reported to reduce intra-articular adhesions in rabbit models of knee injury.<sup>67</sup> Administration of these medications intra-articularly should be performed at least two weeks after joint infection has resolved, to reduce the risk of secondary inflammation or infection.<sup>64</sup> In general, systemic administration of hyaluronan (intravenously) or polysulfated glycosaminoglycan (intramuscularly) may provide the most benefit for septic joints.<sup>12,64</sup>

### *Further Case Management*

To prevent further trauma to the infected joint, horses affected with septic arthritis should be confined to a stall during initial treatment, with the duration of stall rest dependent on the degree of infection.<sup>4,64</sup> Stall rest limits the horse's movement, improving wound healing, preventing wound dehiscence, and reducing damage to the articular cartilage of the infected joint.<sup>64</sup> Bandaging of the limb protects the area from

contamination, reduces soft tissue swelling, and immobilizes the limb.<sup>12,64</sup> Bandaging should be performed daily using sterile bandage material during the initial treatment period.<sup>12</sup> Once the septic process has resolved, the wound or incisions have healed, and the limb is no longer swollen, bandaging may be discontinued.<sup>12</sup>

Upon resolution of joint infection, hand walking should be implemented to help prevent the formation of synovial adhesions.<sup>4,64</sup> Joint passive range of motion exercises may also be performed, possibly reducing the risk of adhesion formation and capsulitis (inflammation of the fibrous layer of the joint capsule resulting in joint stiffness and pain).<sup>12,64</sup>

### **Prognosis for Horses with Septic Arthritis**

The prognosis for adult horses with septic arthritis depends on the causative agent, duration of infection, degree of inflammation, presence of additional lesions, and the treatment regimen employed.<sup>13,64</sup> Prognosis improves with early recognition of synovial involvement and initiation of treatment.<sup>4,64</sup> Treatments that include arthroscopic debridement and lavage, systemic and local antimicrobials, and appropriate pain management provide the best opportunity for successful resolution of septic arthritis.<sup>64</sup> Overall, approximately 54-85% of horses affected with septic synovial structures survive and 33-77% of these horses return to athletic function.<sup>1-3,64,68</sup> Even with successful treatment of septic arthritis, horses may suffer from degenerative joint changes. Therefore research into new ways to help diagnose and treat equine septic arthritis is important to the advancement of veterinary medicine.

## The Acute Phase Response

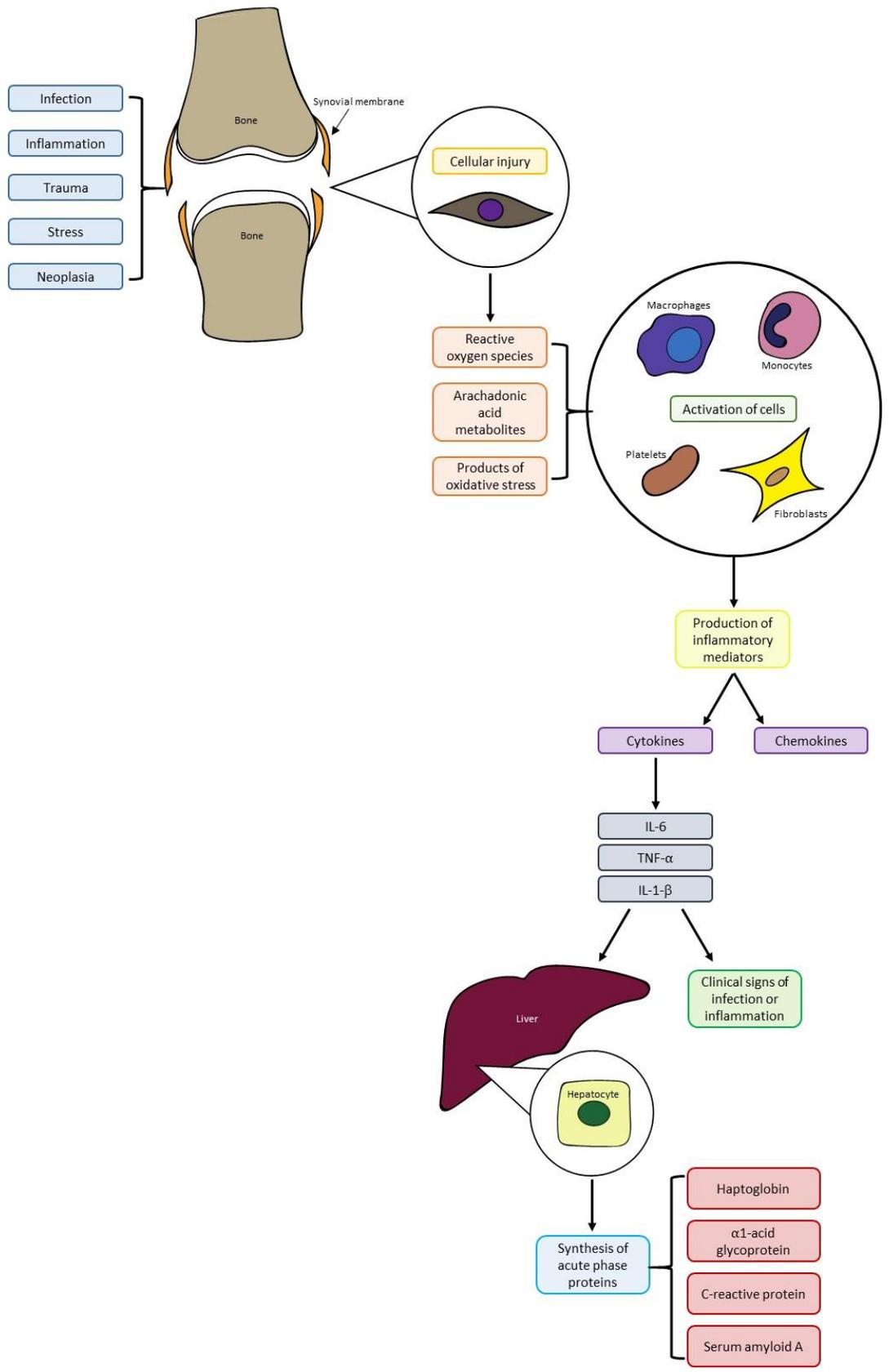
The acute phase response (APR) is a critical part of the innate immune system and is induced by infection, inflammation, trauma, stress, and neoplasia.<sup>8,69</sup> The APR functions to remove the inciting cause of inflammation, promote healing, and restore normal physiological function.<sup>8,69-71</sup> The diverse innate immune system functions to eliminate pathogens, prevent infection, initiate the inflammatory response, and activate the acquired immune response.<sup>69</sup> The system is activated when injured cells release alarm molecules, such as reactive oxygen species, arachadonic acid metabolites, and products of oxidative stress.<sup>8,70</sup> These alarm molecules lead to the activation of cells such as macrophages, monocytes, fibroblasts, and platelets that produce inflammatory mediators such as cytokines and chemokines.<sup>8,69,70</sup> Cytokines play an important role in the APR, producing the clinical signs associated with inflammation or infection, as well as stimulating other cells in the APR cascade.<sup>8,70</sup> The major cytokines involved in the APR include IL-6, TNF- $\alpha$ , and IL-1- $\beta$ .<sup>69,72</sup>

Interleukin-6 is considered the primary cytokine stimulator for the production of acute phase proteins (APP), while other cytokines have effects on subgroups of acute phase proteins.<sup>73</sup> Interleukin-6 knock-out mice, when stimulated with turpentine (an inflammatory agent), were unable to mount a normal inflammatory response, with acute phase protein production significantly reduced.<sup>74</sup> These same IL-6 knock-out mice, when stimulated with lipopolysaccharide (LPS), increased their production of TNF- $\alpha$ , and were able to produce a normal inflammatory response.<sup>74</sup> Interestingly, the IL-6 knock-out mice stimulated with turpentine did not increase their production of TNF- $\alpha$ , and normal mice stimulated with LPS produced a significant amount of IL-6.<sup>74</sup> The IL-6 knock-out

mice stimulated with LPS compensated for the lack of IL-6 by increasing production of TNF- $\alpha$ , while the turpentine-stimulated IL-6 knock-out mice were unable to increase production of TNF- $\alpha$ .<sup>74</sup> These findings suggest that cytokine production and the APR vary, depending on the inflammatory condition.<sup>73</sup> The hepatic APR is stimulated by IL-6, TNF- $\alpha$ , and IL-1- $\beta$ , which leads to the synthesis of APP, blood proteins synthesized by the liver in response to acute inflammation (Figure 2.5).<sup>8,69-71</sup>

### *Acute Phase Proteins*

During homeostasis, liver hepatocytes produce proteins at steady state concentrations.<sup>75</sup> The APR results in increased hepatocyte synthesis of APP and therefore increased plasma APP concentrations.<sup>8,75</sup> Acute phase proteins change their plasma concentrations by at least 25% following an inflammatory stimulus and are grouped as either positive APP or negative APP.<sup>73</sup> Positive APP plasma concentrations increase during the APR, while negative APP plasma concentrations decrease.<sup>8,73</sup> In most species, albumin is the major negative APP, decreasing in plasma during the APR due to either loss of the albumin through the renal or gastrointestinal systems or due to decreased hepatic synthesis.<sup>8,69</sup> During the APR there is increased demand for the amino acids used for APP synthesis decreasing hepatic albumin synthesis to utilize amino acids for increased production of positive APP.<sup>8,69,70</sup> Positive APP are classified as major, moderate, or minor, depending on their response to stimulation. Major APP increase 10- to 1,000-fold, moderate APP increase 2- to 10-fold, and minor APP increase only slightly.<sup>8,69</sup> Major APP tend to significantly increase 24 to 48 hours after an inflammatory stimulus and decline rapidly due to their short half-lives, while moderate



**Figure 2.5** Diagram of the acute phase response. Infection, inflammation, trauma, stress, or neoplasia result in cellular injury. Injured cells release alarm molecules, including reactive oxygen species, arachadonic acid metabolites, and products of oxidative stress. These alarm molecules activate cells such as macrophages, monocytes, fibroblasts, and platelets, which results in the production of inflammatory mediators (cytokines and chemokines). Cytokines (IL-6, TNF- $\alpha$ , and IL-1- $\beta$ ) produce clinical signs of infection and inflammation as well as acting on hepatocytes to induce the synthesis of acute phase proteins (e.g., haptoglobin,  $\alpha$ 1-glycoprotein, C-reactive protein, and SAA).

and minor APP may increase less rapidly and remain elevated for a longer duration.<sup>8,69,70</sup> In horses, SAA is the only major positive APP. Moderate and minor positive APP include haptoglobin, fibrinogen,  $\alpha$ 1-acid glycoprotein, C-reactive protein, and ceruloplasmin.<sup>8,70</sup> Overall, APP functions include immune system modulation, complement activation, protein transport, and tissue protection and healing, with the individual APP each participating differently in the APR.<sup>69,70,73,75</sup> The quantification of APP can be a useful diagnostic in medicine, providing insight into the degree and duration of inflammation, infection, or disease.<sup>69,70</sup>

### *Serum Amyloid A*

Serum amyloid A is the major acute phase protein of horses and many other mammalian species and is the precursor of the amyloid A protein.<sup>76</sup> Amyloid A protein is the main component of amyloid plaques or fibrils that can develop on tissues and organs due to chronic inflammatory disease.<sup>71,76</sup> Synthesized primarily in the liver, SAA is an apolipoprotein with two classes, the “acute-phase” SAA and the “constitutive” SAA.<sup>71</sup> The “acute-phase” SAA is the major acute-phase reactant, which can increase more than 1,000-fold following an inflammatory stimulus and is associated with the third fraction of high-density lipoprotein (HDL) in plasma.<sup>71,76,77</sup> “Constitutive” SAA is minimally induced during the APR, and has not been identified in horses, only in humans and mice.<sup>71</sup>

All SAA genes have a four-exon, three-intron organization and human SAA proteins ranges in size from 104 to 112 amino acids and weigh 11.5 to 19 kDa, depending on whether the SAA protein is “acute-phase” or “constitutive”.<sup>71,78-80</sup> In comparison, the

equine SAA protein consists of 110 amino acids and has a molecular weight of between 9 and 14 kDa.<sup>8,76,81</sup> There is limited information available regarding human or equine SAA protein structure, but it is suggested that the “acute phase” SAA protein contains two  $\alpha$ -helix regions and  $\beta$ -sheet regions.<sup>71</sup> In people, both the “acute phase” SAA and “constitutive” SAA proteins can associate with HDL, with the HDL-associated “acute phase” SAA being present during the APR, and the HDL-associated “constitutive” SAA being present under normal conditions.<sup>71,80</sup>

In people, there are three SAA genes (SAA1, SAA2, and SAA4) that code for SAA proteins and one SAA pseudogene (SAA3).<sup>71,82</sup> The SAA1 and SAA2 genes encode the “acute phase” SAA proteins, while SAA4 encodes the “constitutive” SAA proteins.<sup>71,80,82</sup> SAA1 and SAA2 genes in people can produce extrahepatic synthesis of SAA from a wide variety of tissues and cells (e.g., endothelial cells, macrophages, adipocytes, smooth muscle cells, brain, pituitary gland, mammary gland, kidney, pancreas, placenta, prostate, skin, spleen, thyroid, tonsil, esophagus, stomach, small intestine, large intestine, synovial tissue, and chondrocytes).<sup>71,82</sup> These extrahepatic locations of SAA synthesis may provide immediate, local immunologic defense against inflammatory stimuli while a systemic response is being synthesized hepatically.<sup>71</sup> In people SAA3 is a pseudogene and does not produce an intact SAA molecule.<sup>71</sup> However, in non-human species the SAA3 gene (or genes homologous to SAA3) encodes SAA proteins, which are synthesized extrahepatically. Horses are one of the species in which the SAA3 gene results in extrahepatic synthesis of SAA.<sup>8,9,71</sup>

In horses, SAA is produced primarily by hepatocytes and three major equine SAA isoforms are found in serum.<sup>8,76,83</sup> However, the equine SAA3 gene induces extrahepatic

SAA synthesis, and SAA mRNA expression has been detected in the lung, mammary gland, pancreas, synovial membrane, thymus, thyroid gland, and uterus.<sup>9,84</sup> One additional major and one additional minor SAA isoform are found in equine synovial fluid, which are not found systemically, indicative of local SAA production in the joint.<sup>8</sup> Synoviocytes and chondrocytes are sources of synovial fluid SAA in people and rabbits and are also the likely sources in horses.<sup>8,46,82,85-87</sup>

Following the induction of the APR by an inflammatory stimulus, cytokines (mainly IL-6, TNF- $\alpha$ , and IL-1- $\beta$ ) stimulate SAA protein synthesis, while glucocorticoids released during inflammation enhance cytokine-induced SAA production.<sup>71,77</sup> Serum amyloid A is considered a type-1 acute phase protein, meaning SAA is induced by IL-1- $\beta$  or TNF- $\alpha$ , and IL-6 can synergize with IL-1- $\beta$  and TNF- $\alpha$  to increase SAA production.<sup>71,77</sup> Type-2 acute phase proteins are those that are induced by IL-6-type cytokines, and IL-6 alone has very minimal effects on the induction of SAA.<sup>71</sup>

Transcriptional regulation plays an important role in the expression of SAA genes.<sup>71,73</sup> Cytokine response and cell specificity of expression on genes are regulated by the NF- $\kappa$ B and C/EBP transcription factor recognition sequences, with the interactions of these transcription factors involved in the induction of SAA gene transcription.<sup>71,77</sup> Post-transcriptional mechanisms, such as increased mRNA stability, also regulate SAA.<sup>71,73,77</sup> An increase in SAA mRNA synthesis does not result in an equal increase in SAA protein synthesis, as SAA mRNA translational efficiency is decreased due to a mechanism that slows ribosome migration.<sup>71,75</sup> Additionally, cytokines may induce intracellular changes that control the post-transcription of SAA mRNA.<sup>71</sup>

The functions of SAA are not fully understood, but can be broken down into immune-related, lipid-related, and anti-inflammatory functions.<sup>8,71</sup> Hepatic SAA enters the circulation, acting systemically, while extrahepatic SAA remains local, interacting with surrounding cells.<sup>88</sup> Serum amyloid A can act as a chemoattractant, recruiting monocytes, mast cells, leukocytes, neutrophils, and lymphocytes to areas of inflammation.<sup>71,80,89</sup> Recombinant SAA has been reported to stimulate and activate neutrophils, up-regulating neutrophil antimicrobial activities.<sup>90</sup> Serum amyloid A can induce extracellular matrix degrading enzymes that are involved in the repair of damaged tissues, such as MMP-2 and -3, collagenase, and stromelysins.<sup>71,91</sup> As discussed earlier, these enzymes can also result in tissue destruction and degenerative joint disease.<sup>71</sup> Recombinant SAA induces the transcription and expression of IL-1 $\beta$ , IL-1-receptor agonist, IL-6, IL-8, TNF- $\alpha$ , and TNF-receptor 2, which can have pro-inflammatory functions.<sup>71,80,88</sup> Recombinant SAA may induce the expression of pro-inflammatory cytokines, but also induces the expression of cytokines that have anti-inflammatory properties, such as IL-10 and IL-1rn.<sup>80,88</sup> In people, circulating SAA bound to HDL during inflammation may affect HDL metabolism and increase the delivery of cholesterol to cells at inflammatory sites to aid in tissue regeneration.<sup>71</sup> Additionally, SAA may aid in the removal of cholesterol that is present at sites of tissue damage, recycling cholesterol.<sup>71,92</sup> T cell-macrophage interactions and helper T lymphocyte functions are affected by SAA, suppressing immune responses.<sup>71,80</sup> Serum amyloid A also inhibits platelet aggregation, which may ultimately down-regulate pro-inflammatory events during the APR, as platelets and their mediators are involved in inflammatory and thrombic processes.<sup>71</sup> Chronic inflammation or recurrent acute inflammation can lead to

the development of amyloid deposits, also called amyloidosis in which SAA protein is partially degraded, resulting in intermediate SAA peptides and fibrils that accumulate to form the amyloid deposits.<sup>71</sup>

Serum amyloid A is both synthesized and degraded by the liver, with a half-life of 24 hours.<sup>71</sup> When there is increased SAA synthesis, the capacity of the liver to degrade SAA is decreased in order to maintain elevated systemic SAA concentrations.<sup>71</sup> Serum SAA concentrations begin to increase 6 to 8 hours following an inflammatory stimulus, peak within approximately 36 to 48 hours, and return to normal concentrations within 1 to 2 weeks following resolution of the inflammatory stimulus.<sup>93</sup> Serum amyloid A is degraded in the liver 30 to 120 minutes after it is synthesized, making it useful to monitor SAA concentrations to determine a patient's status and response to treatments.<sup>70,93</sup>

#### *Serum Amyloid A in Human Medicine*

In people, normal serum SAA concentrations are 1-5 µg/mL.<sup>94,95</sup> Measurement of serum SAA concentrations is useful in diagnosing and monitoring of a multitude of diseases.<sup>94,95</sup> Serum amyloid A concentrations have been evaluated in patients with bacterial and viral infections, trauma, neoplasia, inflammatory disorders, arthrogenesis, transplant rejections, rheumatoid arthritis, and osteoarthritis.<sup>94,95</sup> In general, the SAA response in people to bacterial infection is usually more significant than the response to viral infection with acute bacterial infections resulting in increased SAA values up to 10 times greater those that result from viral infections.<sup>95-98</sup> One study measured serum SAA concentrations of human patients admitted to infectious disease clinics and compared the values between patients affected with viral infections and patients affected with bacterial

infections, finding the median SAA concentration of all patients with bacterial infections was significantly higher than the median SAA concentration of all patients with viral infections (1,179.5 vs. 164.5  $\mu\text{g/mL}$ ).<sup>98</sup> While overall SAA concentrations in that study could differentiate between bacterial and viral infections, the accuracy varied depending on the infecting organism, as certain viral organisms induced higher SAA concentrations than certain bacterial organisms.<sup>98</sup> These results are comparable to a separate study that similarly evaluated SAA concentrations of admitted patients with bacterial and viral infections, finding SAA concentrations were higher in patients with bacterial infections than viral infections.<sup>96</sup>

People affected with cystic fibrosis carry a high risk of respiratory tract infection that often leads to death, and although these respiratory tract infections can be successfully treated, they often rapidly recur, making it difficult to evaluate treatment efficacy.<sup>99</sup> Serum amyloid A measurements in cystic fibrosis patients revealed that SAA concentration was correlated to the level of lung impairment, the presence of bacteria, and the response to antimicrobial treatment, making SAA concentration a useful aid in determining the need for antimicrobials and the effectiveness of treatment.<sup>99</sup> Additionally, in human leukemia patients with fevers, SAA concentrations may aid in differentiating between fevers due to infection and fevers that are non-infectious in origin, as infection causes significantly higher SAA concentrations.<sup>100</sup>

Serum amyloid A can also aid in diagnosing neonatal early-onset sepsis in infants that have risk factors and/or are suspected of having sepsis.<sup>101</sup> Early-onset sepsis is diagnosed based on the clinical and biochemical signs of sepsis, as well as a positive blood bacterial culture.<sup>101</sup> Similar to equine bacterial culture sensitivity, human blood

bacterial cultures for early-onset sepsis have a sensitivity of 50-80%, thus a negative blood bacterial culture does not rule out infection.<sup>101,102</sup> Serum amyloid A has been reported to be a useful additional diagnostic for neonates with criteria matching early-onset sepsis, with SAA concentrations increasing significantly in neonates with sepsis.<sup>101</sup>

Serum amyloid A may also be used as an aid in diagnosing organ transplant failure.<sup>94,103</sup> Results of daily monitoring of SAA concentrations in people with kidney allografts indicated that SAA concentrations were elevated approximately 1 to 3 days prior to allograft rejection.<sup>104,105</sup> The organ transplant operation itself causes an increase in SAA concentrations, with reported mean values of 97 µg/mL in liver allograft patients.<sup>106</sup> In liver allograft patients, significantly increased SAA concentrations (mean of 169 µg/mL) was suggestive of rejection.<sup>106</sup>

Serum amyloid A increases in response to inflammation caused by neoplasia and while increased SAA concentrations do not have specificity for neoplastic disease or tumor type, metastatic tumors generally cause higher SAA concentrations.<sup>107</sup> Lower SAA concentrations are present in people with lower neoplastic stages (stages 1 and 2) and SAA concentrations increase concurrently with the neoplastic stage.<sup>108</sup> Stages 3 and 4 have the highest SAA concentrations.<sup>108</sup> At the time of diagnosis of neoplasia, lower SAA concentrations are correlated with an increased prognosis for survival.<sup>108</sup> Additionally, SAA concentrations can be used as an aid in monitoring neoplastic disease progression and response to treatments.<sup>108</sup>

Rheumatoid arthritis is a systemic, chronic inflammatory disease that significantly affects joints.<sup>109,110</sup> The systemic inflammation of rheumatoid arthritis results in a significantly larger APR than osteoarthritis.<sup>109</sup> Serum amyloid A concentrations in

people with rheumatoid arthritis aid in determining the patient's clinical status and changes in SAA concentrations are correlated to changes in the disease process.<sup>86</sup> Overall, serum and synovial fluid SAA concentrations are higher in people affected with rheumatoid arthritis (median 333  $\mu\text{g/mL}$  and 31.1  $\mu\text{g/mL}$ , respectively), versus patients with osteoarthritis (38  $\mu\text{g/mL}$  and 3.5  $\mu\text{g/mL}$ , respectively).<sup>109,111,112</sup> In both osteoarthritis and rheumatoid arthritis, serum levels of SAA are higher than synovial fluid, suggesting that synovial fluid SAA was mainly due to passive diffusion of SAA from the systemic circulation into the synovial structure.<sup>111</sup> However, SAA has been reported to be expressed in the synovial tissues and cells of people affected with rheumatoid arthritis and SAA is expressed in osteoarthritic cartilage.<sup>85,86</sup> Serum amyloid A concentrations can be used to differentiate rheumatoid arthritis from other inflammatory arthropathies, because rheumatoid arthritis affects multiple joints and causes a systemic inflammatory response, while other causes of arthritis usually affect fewer joints and do not tend to cause systemic inflammation.<sup>87,113,114</sup>

### *Serum Amyloid A in Equine Medicine*

In horses, normal serum SAA concentrations are between 0.5 and 20  $\mu\text{g/mL}$ , with the majority of healthy horses having SAA concentrations of less than 7  $\mu\text{g/mL}$ .<sup>8,70,115</sup> Sex does not seem to have an effect on baseline SAA concentrations, although age may have an effect.<sup>8,93,116</sup> In foals, SAA concentrations may be slightly elevated for approximately one week following parturition, after which SAA concentrations decrease to low values by one month of age.<sup>116</sup> The elevation of SAA concentrations during the first week of age may be due to the trauma and inflammation of parturition, maternal

cytokines released during parturition, and/or the ingestion of immunoglobulins from colostrum.<sup>93,116,117</sup> However, this increase in SAA concentration in young foals is not always present, making age-related SAA concentrations unlikely to be significant.<sup>8</sup> Perinatal mares have normal concentrations of SAA for the majority of their pregnancy, although some fluctuations in SAA concentration can occur during the last 4 months of pregnancy.<sup>8,93,116</sup> Following parturition, SAA concentrations increase, peaking around 3 days postpartum, and returning to normal concentrations by 1 month postpartum.<sup>116</sup> The passage of the fetus through the vaginal canal results in tissue damage and local inflammation that may result in elevations of SAA.<sup>8,93</sup>

The magnitude of change in SAA concentration can vary depending on the inciting cause of inflammation.<sup>6,8</sup> In horses, a variety of causes can result in increased concentrations of SAA, including bacterial infection, viral infection, joint disease, gastrointestinal disease, reproductive disease, neonatal disease, surgery, and local inflammation.<sup>7,8,93,116</sup> In an experimental study, local inflammation was induced in horses by intramuscular injections of turpentine, which resulted in SAA concentrations 15-fold higher than normal.<sup>116</sup> Equine surgical procedures can result in 4- to 20-fold increases in SAA concentrations and the level of surgical invasiveness relates to the degree of SAA concentration increase, with more invasive, more traumatic surgical procedures resulting in the highest SAA values.<sup>93,115,116,118,119</sup>

In horses, bacterial infections may incite a significantly elevated SAA response, while viral infections usually result in moderate elevations in SAA concentrations.<sup>6,7</sup> A study evaluating the SAA response of sick foals at the time of admission to a hospital found that foals affected with verified (culture-positive) bacterial infections had higher

SAA concentrations (median 65 µg/mL) than foals affected with nonbacterial or uncertain diagnoses (median, 1.6 µg/mL).<sup>120</sup> This SAA response is not limited to horses. A study comparing the APR of calves infected with bovine viral diarrhea virus to calves infected with *Mannheimia haemolytica* found that bovine viral diarrhea virus calves had increased SAA values ranging from 78-375 µg/mL, while all of the *Mannheimia haemolytica* calves had SAA values greater than 375 µg/mL.<sup>121</sup> In animals, infectious agents result in the highest increase in SAA concentrations, which may be a useful diagnostic aid to distinguish between infection and non-infectious inflammation.<sup>6,8,117,120</sup>

Research on equine SAA responses to different pathologies or diseases include bacterial infections, viral infections, gastrointestinal diseases, respiratory diseases, reproductive diseases, neonatal diseases, surgical response, and other inflammatory disease states.<sup>8,93</sup> In a 2007 study by Hobo et al. where horses were experimentally infected with pneumonia using *Streptococcus equi zooepidemicus*, plasma SAA concentrations peaked 3 days following *S. zooepidemicus* inoculation and when the infection was treated with antimicrobials administered 24 hours post-infection SAA values returned to baseline 15 days following inoculation, at the same time as resolution of the clinical signs.<sup>122</sup> In the Hobo et al. study, when the SAA response was compared to another APP, fibrinogen, SAA was peaked 1 to 2 days faster than fibrinogen and to returned to baseline 7 days faster.<sup>122</sup> Hobo et al. concluded that measurement of plasma SAA is useful for monitoring the clinical status of horses affected with bacterial pneumonia.<sup>122</sup>

In an assessment of clinical cases affected with a variety of bacterial infections such as pneumonia, *Streptococcus equi equi* infection, bacterial cholangiohepatitis,

enterocolitis, and meningitis, SAA concentrations at the time of admission to the hospital ranged from 800.8-3,628.0 µg/mL, suggesting that bacterial infections induce a particularly high SAA response, and that SAA concentrations can be useful in distinguishing between infectious and non-infectious disease.<sup>7</sup> In a study evaluating sick foals, foals affected with septicemia and focal infections such as septic joints were reported to have median SAA concentrations of 279.9 and 195.0 µg/mL, respectively, while foals affected with non-inflammatory conditions or failure of passive transfer had median SAA concentrations of 5.1 and 3.1 µg/mL, respectively.<sup>117</sup> Sick foals with SAA concentrations of greater than 100 µg/mL may be suggestive of infection, while foals with SAA concentrations between 27 and 100 µg/mL may be affected by inflammation or a changing SAA concentration (actively increasing or decreasing).<sup>117</sup>

While bacterial infections may induce significant production of APPs, viral infections result in a more variable response, usually with mild increases in APP production.<sup>123</sup> The SAA response in horses affected with equine influenza was assessed in a study, finding that SAA serum concentrations were higher in the acute stage of the viral infection than the convalescent stage, with serum SAA concentrations increasing by 48 hours following the onset of clinical signs.<sup>123</sup> The median serum SAA concentration of horses with positive immunofluorescence detection for equine influenza virus on the day of inclusion in the study was 69 µg/mL.<sup>123</sup> The SAA concentrations of the horses affected with equine influenza virus returned to baseline within 11 to 22 days in uncomplicated cases, and horses in this study with SAA concentrations that remained persistently elevated exhibited clinical signs of secondary bacterial infections.<sup>123</sup>

Several studies have been performed evaluating the response of SAA to equine colic.<sup>124-126</sup> Horses affected with colic have been reported to have mean SAA concentrations of 249.3 µg/mL in the serum and 97.0 µg/mL in the peritoneal fluid, both significantly higher than SAA concentrations in healthy horses.<sup>126</sup> A study that quantified serum SAA concentration from horses at the time of admission that presented to a hospital for colic evaluated the relationship between SAA concentrations and survival, disease process, and affected portion of gastrointestinal tract.<sup>124</sup> This study found that colicking horses with serum SAA concentrations greater than or equal to 50 µg/mL are more likely to have inflammatory lesions causing the colic and have a lower prognosis for survival.<sup>124</sup> Additionally, horses affected with equine grass sickness have been reported to have serum SAA concentrations significantly greater than healthy horses and horses affected with non-inflammatory causes of colic.<sup>125</sup>

In addition to the SAA response of horses with bacterial causes of respiratory diseases, the SAA response has been evaluated in horses affected with other airway diseases.<sup>127,128</sup> Serum SAA concentrations were increased in horses affected with heaves that were subjected to antigen challenge compared to control horses subjected to the same challenge.<sup>128</sup> In contrast, race horses affected with exercise intolerant inflammatory airway disease did not have differing serum SAA concentrations from horses with exercise intolerant non-inflammatory airway disease.<sup>127</sup>

Serum SAA concentrations can be used to monitor endurance horse training and condition prior to competition.<sup>129,130</sup> One study found that experienced endurance horses had serum SAA concentrations that were very low both before, and after, a 50 kilometer ride with no change in SAA concentrations before and after the ride.<sup>129</sup> The same study

reported that following a 50 kilometer ride, serum SAA concentrations of inexperienced endurance horses increased 4-fold.<sup>129</sup> This difference in SAA concentrations in inexperienced endurance horses may be due to an adaption to increased workload or may signify systemic inflammation caused by too strenuous a workload.<sup>129</sup> Serum SAA concentrations measured pre- and post-competition for experienced endurance horses participating in 120 and 160 kilometer rides were evaluated to assess the status of the horses.<sup>130</sup> The mean pre-competition SAA concentration in eliminated horses was significantly higher than in horses that finished (5.89 vs. 0.41 µg/mL).<sup>130</sup> Horses that completed the competition had pre-competition SAA concentrations of less than 1.0 µg/mL and none of the horses with pre-competition SAA concentrations of greater than 1.0 µg/mL finished.<sup>130</sup> Serum SAA concentrations measured before a competition may serve as a useful aid in detecting subclinical disorders and the status of the horse.<sup>130</sup> Additionally, serum SAA concentrations may be a useful aid in diagnosing equine rhabdomyolysis, as horses that presented to a hospital for rhabdomyolysis were found to have significantly elevated mean SAA concentrations (162.6 µg/mL) when compared to healthy horses.<sup>131</sup>

Serum SAA concentrations have been evaluated in mares affected with endometritis, placentitis, and early embryonic death.<sup>132-135</sup> In one study mares were infected by intrauterine infusions of *Escherichia coli* to induce endometritis and were found to have significantly increased expression of endometrial SAA mRNA peaking at 3 hours post-infusion, as well as significantly increased mean serum SAA concentrations (greater than 100 µg/mL) peaking at 48 hours post-infusion, when compared to baseline values.<sup>132</sup> Placentitis induced in mares by intracervical inoculation of *Streptococcus equi*

*zooepidemicus* resulted in significant elevations of serum SAA concentrations, ranging from 274.2 to 4,385.9 µg/mL at the time of abortion.<sup>133,134</sup> Mares with induced placentitis that underwent treatment either had no increase in serum SAA concentrations, or had mild elevations in SAA concentration that returned to baseline in response to treatment.<sup>133</sup> Clinical cases of mares suffering early embryonic death at 15-21 days post-ovulation found that mean serum SAA concentrations were either significantly elevated at the time of ovulation and remained elevated until early embryonic death (68.6 µg/mL) or were normal at the time of ovulation and became elevated prior to early embryonic death (29.7 µg/mL).<sup>135</sup> Monitoring serum SAA concentrations during equine pregnancy may be useful in identifying genital inflammation, which may lead to infection or abortion.<sup>135</sup>

Recently, equine serum and synovial fluid SAA concentrations have been evaluated to determine their use in the differentiation between septic and aseptic arthritis.<sup>5</sup> Experimentally-induced aseptic equine arthritis using intrasynovial injection of amphotericin B has been reported to result in significant increases in mean peak serum SAA concentrations (163 µg/mL) and experimental intrasynovial injection of LPS induced significant increases in both serum and synovial fluid SAA concentrations.<sup>83,136</sup> These experimentally-induced aseptic arthropathies resulted in significant systemic inflammation, with the horses becoming febrile, tachycardic, tachypnea, and severely lame.<sup>83,136</sup>

Repeated arthrocentesis or repeated intra-articular administration of medications can lead to increased synovial fluid TP and TNCC, which can make the diagnosis of septic arthritis difficult.<sup>49</sup> However, SAA concentrations in the serum and synovial fluid

remained below reference limits in experiments with repeated arthrocentesis or intra-articular administration of amikacin.<sup>46,49,83</sup>

Horses with septic arthritis have significantly higher serum and synovial fluid SAA concentrations than horses with non-inflammatory joint diseases, such as osteoarthritis and osteochondrosis.<sup>46</sup> While systemic bacterial or viral infections appear to induce the largest SAA concentration increase in horses, there is limited literature available regarding the differences between aseptic and septic arthritis SAA concentrations in serum and synovial fluid.<sup>6,8,49</sup> Experimental studies evaluating the differences in serum and synovial fluid SAA concentrations between horses affected with septic and aseptic arthritis will be useful in determining whether SAA can aid in the diagnosis of septic arthritis.

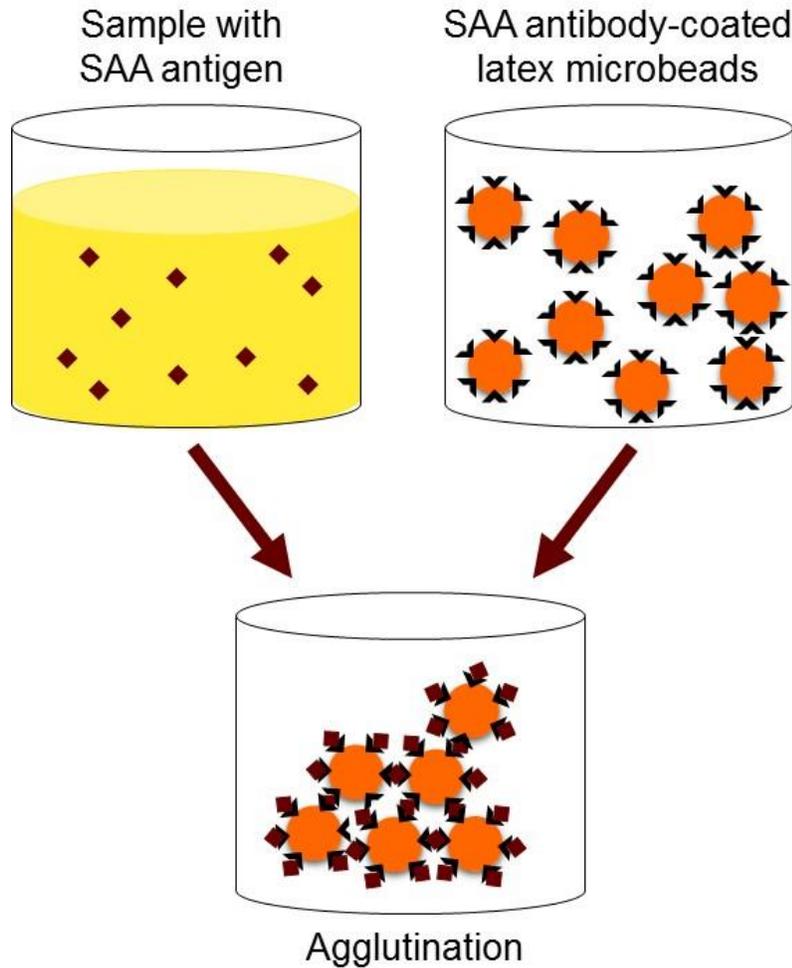
#### *Assays for Serum Amyloid A*

A variety of assays are available to quantify SAA in serum, such as enzyme-linked immunosorbent assay (ELISA),<sup>115,137</sup> single radial immunodiffusion,<sup>116</sup> electroimmunoassay,<sup>6</sup> latex agglutination immunoturbidometric assay,<sup>10,117</sup> and lateral flow immunoassay. The non-competitive chemiluminescence ELISA described by Hulten et al. can be performed in 3 hours and can measure serum SAA concentrations ranging from 3-1,210 µg/mL.<sup>115</sup> The single radial immunodiffusion assay and the electroimmunoassay both take greater than 24 hours to be performed and thus are not practical for clinical use.<sup>115,117</sup> In contrast, latex agglutination immunoturbidometric assays for serum SAA are commercially available, can be performed by common automated analyzers, and can be rapidly completed in less than 10 minutes.<sup>10,97,117,138</sup>

Latex agglutination immunoturbidometric assays use anti-human SAA antibodies, as equine SAA has not been purified to date.<sup>10</sup>

Latex agglutination immunoturbidometric assays detect the presence of a targeted antigen in a sample, such as the SAA protein. The sample is mixed with latex microbeads coated with SAA antibodies. If the SAA antigen is present in the sample, antigen-antibody binding results in agglutination, or clumping, of the microbeads (Figure 2.6). The amount of agglutination is proportional to the SAA concentration in the sample and is measured optically by a photometer, which is known as immunoturbidometry.

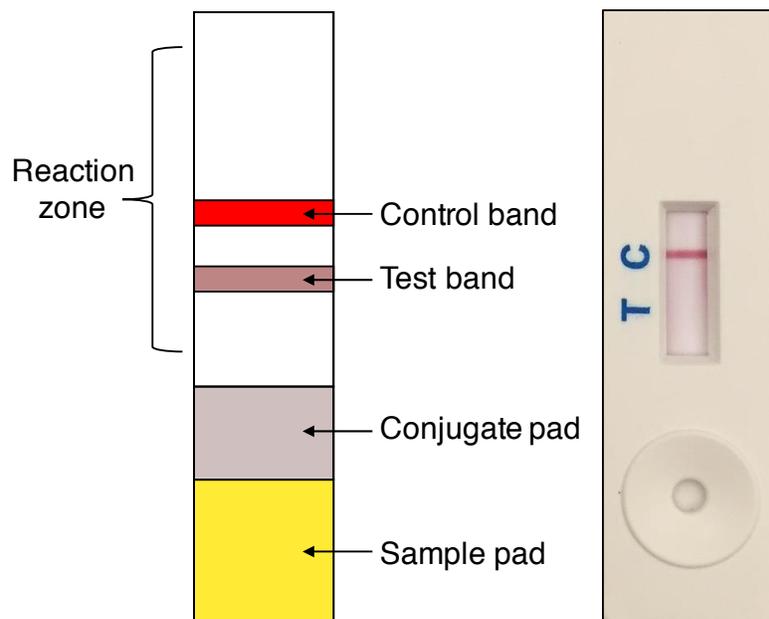
The “Eiken” SAA latex agglutination immunoturbidometric assay was developed for measuring human serum SAA concentrations, has been validated for equine use, and has a test range of 5-500  $\mu\text{g}/\text{mL}$ .<sup>10,139</sup> The “Eiken” SAA latex agglutination immunoturbidometric assay is the validated commercial assay used in the experimental study that serves as the basis for this thesis. “Equinostic EVA1” is another commercially available immunoturbidometric assay, with a manufacturer reported assay range of 10-300  $\mu\text{g}/\text{mL}$ .<sup>140,141</sup> Dilution of samples is required to measure serum SAA concentrations of greater than 270  $\mu\text{g}/\text{mL}$ .<sup>140,141</sup> The dilutions of samples for the “Equinostic EVA1” assay leads to variability in reported SAA concentrations and inaccurate results.<sup>140</sup> While the “Eiken” assay has been most commonly studied for equine veterinary use, the assay has limitations.<sup>142</sup> The “Eiken” assay is not available at all veterinary practices and thus SAA analysis requires that samples be shipped to a laboratory facility, increasing expense and delaying results.



**Figure 2.6** Diagram of latex agglutination immunoturbidometric assay. When SAA antibody-coated latex microbeads are combined with a sample containing SAA antigen, antigen-antibody binding results in agglutination.

Several handheld, lateral flow immunoassays have recently been developed and that can be performed immediately following sample collection. Lateral flow immunoassays detect the presence or absence of a specific antigen in a sample. The lateral flow test is composed of capillary beds, such as porous paper, that can transport fluid. The test kit consists of several zones (Figure 2.7). The sample pad is the first zone,

and is where the diluted sample is applied. Once the sample pad is soaked, the fluid travels to the second zone, which is the conjugate pad. The conjugate pad contains SAA antibody conjugated to a colored particle (usually colloid gold or a latex particle). As the sample travels across the conjugate pad, the SAA antigen of the sample and the conjugate mix, and this reagent migrates to the next zone of the pad, the reaction zone. The reaction zone has bands where more antibodies are bound, which serves to capture the



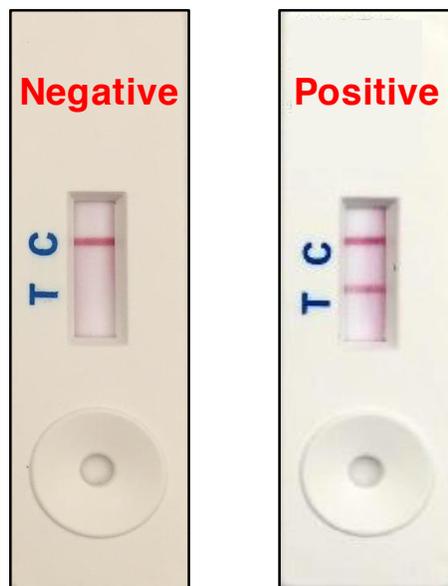
**Figure 2.7** Diagram of lateral flow immunoassay. The test kit consists of the sample pad, conjugate pad, and reaction zone. The reaction zone contains two bands, the control band and the test band.

reagent. As the sample flows by the test band, most of the reagent becomes entrapped in the band. The remaining reagent continues to flow to the control band, which contains an antibody specific for the colored particle in the conjugate. The test has worked properly if the control band color appears (Figure 2.7), and the sample is positive for the antigen if

the test band color is present (Figure 2.8). Visualization of the presence of the test band provides a qualitative result, while a quantitative result can be obtained using a colorimetric reader that correlates the intensity of the test band with a numeric SAA concentration.

The StableLab<sup>®</sup> (StableLab, Epona Biotech Limited, Sligo, Ireland) handheld test measures serum SAA qualitatively by visual assessment or quantitatively using a colorimetric reader (Figure 2.9). Limited data are available regarding the effectiveness and reliability of these lateral flow immunoassays.<sup>142</sup>

In addition to serum, SAA concentrations have been measured in a variety of other samples, such as urine, cerebrospinal fluid, and cultured cells.<sup>97</sup> The “Eiken” assay has been used in several studies for the quantification of equine SAA in serum and



**Figure 2.8** Negative and positive lateral flow immunoassays. The test is negative for the presence of SAA antigen when only the control line, “C”, is present, as seen on the left. The test is positive for SAA antigen when the test line, “T”, is present in addition to the control line, as seen on the right.



**Figure 2.9** StableLab handheld test and colorimetric reader. Application of diluted sample to lateral flow immunoassay for quantification of SAA concentration.

synovial fluid.<sup>46,83</sup> The “StableLab” handheld lateral flow immunoassay was used for immediate serum and synovial fluid equine SAA analysis in the experimental study of this thesis.

## **Conclusions**

Equine septic arthritis can be difficult to diagnose in a subset of cases. History, clinical signs, and synovial fluid analysis are commonly used to diagnose septic arthritis in horses. Synovial fluid analysis includes quantifying TP, TNCC, differential cell count, and bacterial culture. While a positive synovial fluid bacterial culture is considered the gold standard of septic arthritis diagnostics, false negative cultures are common. Synovial fluid TP, TNCC, and differential cell count are altered by a multitude of different joint pathologies, and overlap in synovial fluid parameters between nonseptic

synovitis and septic arthritis can occur. The ambiguity that can result from synovial fluid analysis may prevent a horse from timely diagnosis and treatment. Additional diagnostics for septic arthritis are currently being investigated, including the acute phase protein, SAA.

Serum amyloid A is an acute phase protein produced in response to an inflammatory stimulus and in general SAA increases significantly in response to bacterial or viral infection, while local inflammation usually results in a mild to moderate increase in SAA concentrations. No data have are available to determine reliable cut-offs for SAA concentration between causes of inflammation. Measurement of SAA in human medicine has been used to diagnose and monitor different disease states, such as infection, inflammation, neoplasia, transplant rejections, and various arthropathies. While the use of SAA quantifications in veterinary medicine is currently limited, SAA has the potential to be a useful biomarker for infection, severity of disease processes, and response of diseases to treatment.

Research on equine SAA is relatively recent, with the majority of studies focusing on the systemic SAA response in response to various inflammatory stimuli. Following an inflammatory stimulus certain tissues can locally express SAA, including joints, and a small number of equine studies have evaluated the response of SAA in serum and synovial fluid. These clinical studies have shown that synovial SAA appears to increase significantly in concentration due to joint infection. To date, no studies have been performed evaluating the use of equine SAA as an aid in diagnosing septic arthritis. As a result, an experimental study was designed to evaluate the equine SAA response in serum and synovial fluid using models of synovitis and septic arthritis. The study design and

details are outlined in Chapter 3 of this thesis. The results of this study may provide data that will be useful for studies evaluating the equine SAA response to clinical cases of septic *arthritis and synovitis*.

## References

1. Schneider R, Bramlage L, Moore R, et al: A retrospective study of 192 horses affected with septic arthritis/tenosynovitis. *Equine Vet J* 1992;24:436-442
2. Wright I, Smith M, Humphrey D, et al: Endoscopic surgery in the treatment of contaminated and infected synovial cavities. *Equine Vet J* 2003;35:613-619
3. Gibson K, McIlwraith C, Turner A, et al: Open joint injuries in horses: 58 cases (1980-1986). *J Am Vet Med Assoc* 1989;194:398-404
4. Morton AJ: Diagnosis and treatment of septic arthritis. *Vet Clin North Am Equine Pract* 2005;21:627-649
5. Steel CM: Equine synovial fluid analysis. *Vet Clin North Am Equine Pract* 2008;24:437-454
6. Pepys M, Baltz ML, Tennent GA, et al: Serum amyloid A protein (SAA) in horses: objective measurement of the acute phase response. *Equine Vet J* 1989;21:106-109
7. Belgrave RL, Dickey MM, Arheart KL, et al: Assessment of serum amyloid A testing of horses and its clinical application in a specialized equine practice. *J Am Vet Med Assoc* 2013;243:113-119
8. Jacobsen S, Andersen P: The acute phase protein serum amyloid A (SAA) as a marker of inflammation in horses. *Equine Vet Educ* 2007;19:38-46
9. McDonald TL, Larson MA, Mack DR, et al: Elevated extrahepatic expression and secretion of mammary-associated serum amyloid A 3 (M-SAA3) into colostrum. *Vet Immunol Immunopathol* 2001;83:203-211

10. Jacobsen S, Kjølgaard-Hansen M, Hagbard Petersen H, et al: Evaluation of a commercially available human serum amyloid A (SAA) turbidometric immunoassay for determination of equine SAA concentrations. *Vet J* 2006;172:315-319
11. Steel C, Pannirselvam R, Anderson G: Risk of septic arthritis after intra-articular medication: a study of 16,624 injections in Thoroughbred racehorses. *Aust Vet J* 2013;91:268-273
12. Bertone AL: Infectious Arthritis, in McIlwraith CW, Trotter GW (eds): *Joint Disease in the Horse* (ed 1st). Vol. Philadelphia, WB Saunders, 1996, pp 397-409
13. Carstanjen B, Boehart S, Cislakova M: Septic arthritis in adult horses. *Pol J Vet Sci* 2010;13:201
14. Todhunter RJ: General Principles of Joint Pathobiology, in McIlwraith CW, Trotter GW (eds): *Joint Disease in the Horse* (ed 1st). Vol. Philadelphia, WB Saunders, 1996, pp 1-28
15. Frisbie DD: Synovial Joint Biology and Pathobiology, in Auer JA, Stick JA (eds): *Equine Surgery* (ed 4th). Vol. St. Louis, Missouri, Elsevier, 2012, pp 1096-1113
16. van Weeren PR: General Anatomy and Physiology of Joints, in McIlwraith CW, Frisbie DD, Kawcak CE, et al (eds): *Joint Disease in the Horse* (ed 2nd). Vol. St. Louis, Missouri, Elsevier, 2016, pp 1-24
17. Palmer JL, Bertone AL: Joint structure, biochemistry and biochemical disequilibrium in synovitis and equine joint disease. *Equine Vet J* 1994;26:263-277
18. van Weeren PR: Septic Arthritis, in McIlwraith CW, Frisbie DD, Kawcak CE, et al (eds): *Joint Disease in the Horse* (ed 2nd). Vol. St. Louis, Missouri, Elsevier, 2016, pp 91-104

19. Richardson DW, Ahern BJ: Synovial and Osseous Infection, in Auer JA, Stick JA (eds): *Equine Surgery* (ed 4th). Vol. St. Louis, Missouri, Elsevier, 2012, pp 1189-1200
20. Adams SB, Moore GE, Elrashidy M, et al: Effect of needle size and type, reuse of needles, insertion speed, and removal of hair on contamination of joints with tissue debris and hair after arthrocentesis. *Vet Surg* 2010;39:667-673
21. Gustafson S, McIlwraith C, Jones R: Comparison of the effect of polysulfated glycosaminoglycan, corticosteroids, and sodium hyaluronate in the potentiation of a subinfective dose of *Staphylococcus aureus* in the midcarpal joint of horses. *Am J Vet Res* 1989;50:2014-2017
22. Tulamo R, Bramlage L, Gabel A: The influence of corticosteroids on sequential clinical and synovial fluid parameters in joints with acute infectious arthritis in the horse. *Equine Vet J* 1989;21:332-337
23. Olds AM, Stewart AA, Freeman DE, et al: Evaluation of the rate of development of septic arthritis after elective arthroscopy in horses: 7 cases (1994–2003). *J Am Vet Med Assoc* 2006;229:1949-1954
24. Borg H, Carmalt JL: Postoperative septic arthritis after elective equine arthroscopy without antimicrobial prophylaxis. *Vet Surg* 2013;42:262-266
25. Garcia-Arias M, Balsa A, Mola EM: Septic arthritis. *Best Prac Res Clin Rheumatol* 2011;25:407-421
26. Shirliff ME, Mader JT: Acute septic arthritis. *Clin Microbiol Rev* 2002;15:527-544

27. McIlwraith CW: General Pathobiology of the Joint and Response to Injury, in McIlwraith CW, Trotter GW (eds): *Joint Disease in the Horse* (ed 1st). Vol. Philadelphia, WB Saunders, 1996, pp 40-70
28. Wang G, Li X, Jiang R, et al: Changes in synovial fluid inflammatory mediators and cartilage biomarkers after experimental acute equine synovitis. *Bull Vet Instit Pulawy* 2015;59:129-134
29. American Association of Equine Practitioners A: *Guide for veterinary service and judging of equestrian events* (ed 4th). Lexington, KY, AAEP, 1991
30. Beccati F, Gialletti R, Passamonti F, et al: Ultrasonographic findings in 38 horses with septic arthritis/tenosynovitis. *Vet Radiol Ultrasound* 2015;56:68-76
31. Dyson S: Musculoskeletal scintigraphy of the equine athlete, Proceedings, Seminars in Nuclear Medicine, 2014 (available from Elsevier)
32. Driver AJ: Basic Principles of Equine Scintigraphy, in Dyson SJ, Pilsworth RC, Twardock AR, et al (eds): *Equine Scintigraphy* (ed 1st). Vol. Suffolk, UK, Equine Veterinary Journal, 2003, pp 15-22
33. Long CD, Galuppo LD, Waters NK, et al: Scintigraphic Detection of Equine Orthopedic Infection Using Tc - HMPAO Labeled Leukocytes in 14 Horses. *Vet Radiol Ultrasound* 2000;41:354-359
34. Malton RJ: Nonorthopaedic Scintigraphy, in Dyson SJ, Pilsworth RC, Twardock AR, et al (eds): *Equine Scintigraphy* (ed 1st). Vol. Suffolk, UK, Equine Veterinary Journal, 2003, pp 237-259
35. Easley JT, Brokken MT, Zubrod CJ, et al: Magnetic resonance imaging findings in horses with septic arthritis. *Vet Radiol Ultrasound* 2011;52:402-408

36. Bertone AL: Update on infectious arthritis in horses. *Equine Vet Educ* 1999;11:143-152
37. Van Pelt R: Interpretation of synovial fluid findings in the horse. *J Am Vet Med Assoc* 1974;165:91-95
38. Madison J, Sommer M, Spencer P: Relations among synovial membrane histopathologic findings, synovial fluid cytologic findings, and bacterial culture results in horses with suspected infectious arthritis: 64 cases (1979-1987). *J Am Vet Med Assoc* 1991;198:1655-1661
39. Taylor AH, Mair TS, Smith LJ, et al: Bacterial culture of septic synovial structures of horses: Does a positive bacterial culture influence prognosis? *Equine Vet J* 2010;42:213-218
40. Montgomery R, Long I, Milton J, et al: Comparison of aerobic culturette, synovial membrane biopsy, and blood culture medium in detection of canine bacterial arthritis. *Vet Surg* 1989;18:300-303
41. Tulamo R, Bramlage L, Gabel A: Sequential clinical and synovial fluid changes associated with acute infectious arthritis in the horse. *Equine Vet J* 1989;21:325-331
42. Dechant JE, Symm WA, Nieto JE: Comparison of pH, Lactate, and Glucose Analysis of Equine Synovial Fluid using a Portable Clinical Analyzer with a Bench-Top Blood Gas Analyzer. *Vet Surg* 2011;40:811-816
43. Ward TT, Steigbigel RT: Acidosis of synovial fluid correlates with synovial fluid leukocytosis. *Am J Med* 1978;64:933-936

44. Frisbie DD, McIlwraith CW, de Grauw JC: Synovial Fluid and Serum Biomarkers, in McIlwraith CW, Frisbie DD, Kawcak CE, et al (eds): *Joint Disease in the Horse* (ed 2nd). Vol. St. Louis, Missouri, Elsevier, 2016, pp 179-191
45. Trumble TN, Trotter GW, Oxford JRT, et al: Synovial fluid gelatinase concentrations and matrix metalloproteinase and cytokine expression in naturally occurring joint disease in horses. *Am J Vet Res* 2001;62:1467-1477
46. Jacobsen S, Thomsen MH, Nanni S: Concentrations of serum amyloid A in serum and synovial fluid from healthy horses and horses with joint disease. *Am J Vet Res* 2006;67:1738-1742
47. Wauters J, Pille F, Martens A, et al: Equine myeloperoxidase: A novel biomarker in synovial fluid for the diagnosis of infection. *Equine Vet J* 2013;45:278-283
48. Schneider R, Bramlage L, Mecklenburg LM, et al: Open drainage, intra-articular and systemic antibiotics in the treatment of septic arthritis/tenosynovitis in horses. *Equine Vet J* 1992;24:443-449
49. Sanchez Teran A, Rubio-Martinez LM, Villarino N, et al: Effects of repeated intra-articular administration of amikacin on serum amyloid A, total protein and nucleated cell count in synovial fluid from healthy horses. *Equine Vet J* 2012;44:12-16
50. Stewart A, Goodrich L, Byron C, et al: Antimicrobial delivery by intrasynovial catheterisation with systemic administration for equine synovial trauma and sepsis. *Aust Vet J* 2010;88:115-123
51. Lescun TB, Vasey JR, Ward MP, et al: Treatment with continuous intrasynovial antimicrobial infusion for septic synovitis in horses: 31 cases (2000–2003). *J Am Vet Med Assoc* 2006;228:1922-1929

52. Werner LA, Hardy J, Bertone AL: Bone gentamicin concentration after intra-articular injection or regional intravenous perfusion in the horse. *Vet Surg* 2003;32:559-565
53. Zantingh AJ, Schwark WS, Fubini SL, et al: Accumulation of amikacin in synovial fluid after regional limb perfusion of amikacin sulfate alone and in combination with ticarcillin/clavulanate in horses. *Vet Surg* 2014;43:282-288
54. Rubio-Martínez LM, Cruz AM: Antimicrobial regional limb perfusion in horses. *J Am Vet Med Assoc* 2006;228:706-712, 655
55. Mahne AT, Rioja E, Marais HJ, et al: Clinical and pharmacokinetic effects of regional or general anaesthesia on intravenous regional limb perfusion with amikacin in horses. *Equine Vet J* 2014;46:375-379
56. Levine DG, Epstein KL, Ahern BJ, et al: Efficacy of three tourniquet types for intravenous antimicrobial regional limb perfusion in standing horses. *Vet Surg* 2010;39:1021-1024
57. Hyde RM, Lynch TM, Clark CK, et al: The influence of perfusate volume on antimicrobial concentration in synovial fluid following intravenous regional limb perfusion in the standing horse. *Can Vet J* 2013;54:363
58. Butt TD, Bailey JV, Dowling PM, et al: Comparison of 2 techniques for regional antibiotic delivery to the equine forelimb: intraosseous perfusion vs. intravenous perfusion. *Can Vet J* 2001;42:617
59. Haerdi-Landerer MC, Habermacher J, Wenger B, et al: Slow release antibiotics for treatment of septic arthritis in large animals. *Vet J* 2010;184:14-20

60. Sayegh AI, Moore RM: Polymethylmethacrylate beads for treating orthopedic infections. *Compendium on Continuing Education for the Practicing Veterinarian* 2003;25:788-795
61. Farnsworth KD, White NA, Robertson J: The Effect of Implanting Gentamicin - Impregnated Polymethylmethacrylate Beads in the Tarsocrural Joint of the Horse. *Vet Surg* 2001;30:126-131
62. Miclau T, Dahners LE, Lindsey RW: In vitro pharmacokinetics of antibiotic release from locally implantable materials. *J Orthop Res* 1993;11:627-632
63. Summerhays G: Treatment of traumatically induced synovial sepsis in horses with gentamicin-impregnated collagen sponges. *Vet Rec* 2000;147:184-188
64. Baxter GM: Diagnosis and Management of Wounds Involving Synovial Structures, in Stashak TS, Theoret C (eds): *Equine Wound Management* (ed 2nd). Vol. Ames, Iowa, Blackwell, 2008, pp 463-488
65. Baxter GM: Management of wounds involving synovial structures in horses. *Clin Tech Eq Prac* 2004;3:204-214
66. Brusie R, Sullins K, II NW, et al: Evaluation of sodium hyaluronate therapy in induced septic arthritis in the horse. *Equine Vet J* 1992;24:18-23
67. Wang M, Liu C, Xiao W: Intra-articular injection of hyaluronic acid for the reduction in joint adhesion formation in a rabbit model of knee injury. *Knee Surg Sports Traumatol Arthrosc* 2014;22:1536-1540
68. Walmsley E, Anderson G, Muurlink M, et al: Retrospective investigation of prognostic indicators for adult horses with infection of a synovial structure. *Aust Vet J* 2011;89:226-231

69. Cray C, Zaias J, Altman NH: Acute phase response in animals: a review. *Comp Med* 2009;59:517
70. Crisman MV, Scarratt WK, Zimmerman KL: Blood proteins and inflammation in the horse. *Vet Clin North Am Equine Pract* 2008;24:285-297
71. Uhlar CM, Whitehead AS: Serum amyloid A, the major vertebrate acute-phase reactant. *Eur J Biochem* 1999;265:501-523
72. Murata H, Shimada N, Yoshioka M: Current research on acute phase proteins in veterinary diagnosis: an overview. *Vet J* 2004;168:28-40
73. Epstein FH, Gabay C, Kushner I: Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999;340:448-454
74. Fattori E, Cappelletti M, Costa P, et al: Defective inflammatory response in interleukin 6-deficient mice. *J Exp Med* 1994;180:1243-1250
75. Steel DM, Whitehead AS: The major acute phase reactants: C-reactive protein, serum amyloid P component and serum amyloid A protein. *Immunol Today* 1994;15:81-88
76. Hulten C, Sletten K, Foyn Bruun C, et al: The acute phase serum amyloid A protein (SAA) in the horse: isolation and characterization of three isoforms. *Vet Immunol Immunopathol* 1997;57:215-227
77. Jensen L, Whitehead A: Regulation of serum amyloid A protein expression during the acute-phase response. *Biochem J* 1998;334:489-503
78. Whitehead A, De Beer M, Steel D, et al: Identification of novel members of the serum amyloid A protein superfamily as constitutive apolipoproteins of high density lipoprotein. *J Biol Chem* 1992;267:3862-3867

79. Malle E, Steinmetz A, Raynes JG: Serum amyloid A (SAA): an acute phase protein and apolipoprotein. *Atherosclerosis* 1993;102:131-146
80. Eklund KK, Niemi K, Kovanen P: Immune functions of serum amyloid A. *Crit Rev Immunol* 2012;32
81. Sletten K, Husebekk A, Husby G: The primary structure of equine serum amyloid A (SAA) protein. *Scand J Immunol* 1989;30:117-122
82. Upragarin N, Landman W, Gastra W, et al: Extrahepatic production of acute phase serum amyloid A. *Histol Histopathol* 2005;20:1295-1307
83. Jacobsen S, Niewold TA, Halling-Thomsen M, et al: Serum amyloid A isoforms in serum and synovial fluid in horses with lipopolysaccharide-induced arthritis. *Vet Immunol Immunopathol* 2006;110:325-330
84. Berg LC, Thomsen PD, Andersen PH, et al: Serum amyloid A is expressed in histologically normal tissues from horses and cattle. *Vet Immunol Immunopathol* 2011;144:155-159
85. Vallon R, Freuler F, Desta-Tsedu N, et al: Serum amyloid A (apoSAA) expression is up-regulated in rheumatoid arthritis and induces transcription of matrix metalloproteinases. *J Immunol* 2001;166:2801-2807
86. O'Hara R, Murphy EP, Whitehead AS, et al: Acute-phase serum amyloid A production by rheumatoid arthritis synovial tissue. *Arthritis Res* 2000;2:142-144
87. O'Hara R, Murphy EP, Whitehead AS, et al: Local expression of the serum amyloid A and formyl peptide receptor-like 1 genes in synovial tissue is associated with matrix metalloproteinase production in patients with inflammatory arthritis. *Arthritis Rheum* 2004;50:1788-1799

88. Richard DY, Sun L: Emerging functions of serum amyloid A in inflammation. *J Leukoc Biol* 2015;98:923-929
89. Badolato R, Wang JM, Murphy WJ, et al: Serum amyloid A is a chemoattractant: induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. *J Exp Med* 1994;180:203-209
90. Badolato R, Wang JM, Stornello S, et al: Serum amyloid A is an activator of PMN antimicrobial functions: induction of degranulation, phagocytosis, and enhancement of anti-Candida activity. *J Leukoc Biol* 2000;67:381-386
91. Migita K, Kawabe Y, Tominaga M, et al: Serum amyloid A protein induces production of matrix metalloproteinases by human synovial fibroblasts. *Lab Invest* 1998;78:535-539
92. Kisilevsky R, Manley PN: Acute-phase serum amyloid A: perspectives on its physiological and pathological roles. *Amyloid* 2012;19:5-14
93. Satué K, Calvo A, Gardón JC: Factors Influencing Serum Amyloid Type A (SAA) Concentrations in Horses. *Open J Vet Med* 2013;3:58-66
94. Pizzini C, Mussap M, Plebani M, et al: C-reactive protein and serum amyloid A protein in neonatal infections. *Scand J Infect Dis* 2000;32:229-235
95. Malle E, De Beer F: Human serum amyloid A (SAA) protein: a prominent acute - phase reactant for clinical practice. *Eur J Clin Invest* 1996;26:427-435
96. Nakayama T, Sonoda S, Urano T, et al: Monitoring both serum amyloid protein A and C-reactive protein as inflammatory markers in infectious diseases. *Clin Chem* 1993;39:293-297

97. Yamada T: Serum amyloid A (SAA): a concise review of biology, assay methods and clinical usefulness. *Clin Chem Lab Med* 1999;37:381-388
98. Lannergård A, Larsson A, Kragstjerg P, et al: Correlations between serum amyloid A protein and C-reactive protein in infectious diseases. *Scand J Clin Lab Invest* 2003;63:267-272
99. Marhaug G, Permin H, Husby G: Amyloid-related serum protein (SAA) as an indicator of lung infection in cystic fibrosis. *Acta Paediatr* 1983;72:861-866
100. Časl MT, Rogina B, Glojnarčić-Spasić I, et al: The differential diagnostic capacity of serum amyloid A protein between infectious and non-infectious febrile episodes of neutropenic patients with acute leukemia. *Leuk Res* 1994;18:665-670
101. Arnon S, Litmanovitz I, Regev R, et al: Serum amyloid A: an early and accurate marker of neonatal early-onset sepsis. *J Perinatol* 2007;27:297-302
102. Gerdes JS: Diagnosis and management of bacterial infections in the neonate. *Pediatr Clin North Am* 2004;51:939-959
103. Maury C, Teppo A: Comparative study of serum amyloid-related protein SAA, C-reactive protein, and beta 2-microglobulin as markers of renal allograft rejection. *Clin Nephrol* 1984;22:284-292
104. Časl M, Bulatovic G, Orlić P, et al: The diagnostic capacity of serum amyloid A protein for early recognition of kidney allograft rejection. *Nephrol Dial Transplant* 1995;10:1901-1904
105. Hartmann A, Eide T, Fauchald P, et al: Serum amyloid A protein is a clinically useful indicator of acute renal allograft rejection. *Nephrol Dial Transplant* 1997;12:161-166

106. Feussner G, Stech C, Dobmeyer J, et al: Serum amyloid A protein (SAA): a marker for liver allograft rejection in humans. *Clin Investig* 1994;72:1007-1011
107. Weinstein P, Skinner M, Sipe J, et al: Acute-Phase Proteins or Tumour Markers: The Role of SAA, SAP, CRP and CEA as Indicators of Metastasis in a Broad Spectrum of Neoplastic Diseases. *Scand J Immunol* 1984;19:193-198
108. Biran H, Friedman N, Neumann L, et al: Serum amyloid A (SAA) variations in patients with cancer: correlation with disease activity, stage, primary site, and prognosis. *J Clin Pathol* 1986;39:794-797
109. Sipe JD: Acute-phase proteins in osteoarthritis, Proceedings, Seminars in Arthritis and Rheumatism, 1995
110. Van Leeuwen MA, Van Rijswijk MH: Acute phase proteins in the monitoring of inflammatory disorders. *Baillieres Clin Rheumatol* 1994;8:531-552
111. de Seny D, Cobraiville G, Charlier E, et al: Acute-phase serum amyloid a in osteoarthritis: regulatory mechanism and proinflammatory properties. *PLoS One* 2013;8:e66769
112. de Seny D, Cobraiville G, Charlier E, et al: Apolipoprotein-A1 as a Damage-Associated Molecular Patterns Protein in Osteoarthritis: Ex Vivo and In Vitro Pro-Inflammatory Properties. *PLoS One* 2015;10:e0122904
113. Cunnane G, Grehan S, Geoghegan S, et al: Serum amyloid A in the assessment of early inflammatory arthritis. *J Rheumatol* 2000;27:58-63
114. Cunnane G: Amyloid precursors and amyloidosis in inflammatory arthritis. *Curr Opin Rheumatol* 2001;13:67-73

115. Hulten C, Tulamo R, Suominen M, et al: A non-competitive chemiluminescence enzyme immunoassay for the equine acute phase protein serum amyloid A (SAA)-a clinically useful inflammatory marker in the horse. *Vet Immunol Immunopathol* 1999;68:267-281
116. Nunokawa Y, Fujinaga T, Taira T, et al: Evaluation of serum amyloid A protein as an acute-phase reactive protein in horses. *J Vet Med Sci/Jap Soc Vet Sci* 1993;55:1011
117. Stoneham S, Palmer L, Cash R, et al: Measurement of serum amyloid A in the neonatal foal using a latex agglutination immunoturbidimetric assay: determination of the normal range, variation with age and response to disease. *Equine Vet J* 2001;33:599-603
118. Jacobsen S, Nielsen JV, Kjelgaard-Hansen M, et al: Acute phase response to surgery of varying intensity in horses: a preliminary study. *Vet Surg* 2009;38:762-769
119. Pollock PJ, Prendergast M, Schumacher J, et al: Effects of surgery on the acute phase response in clinically normal and diseased horses. *Vet Rec* 2005;156:538-542
120. Hulten C, Demmers S: Serum amyloid A (SAA) as an aid in the management of infectious disease in the foal: comparison with total leucocyte count, neutrophil count and fibrinogen. *Equine Vet J* 2002;34:693-698
121. Gånheim C, Hulten C, Carlsson U, et al: The acute phase response in calves experimentally infected with bovine viral diarrhoea virus and/or Mannheimia haemolytica. *J Vet Med, Series B* 2003;50:183-190

122. Hobo S, Niwa H, Anzai T: Evaluation of serum amyloid A and surfactant protein D in sera for identification of the clinical condition of horses with bacterial pneumonia. *J Vet Med Sci* 2007;69:827-830
123. Hulten C, Sandgren B, Skiöldebrand E, et al: The acute phase protein serum amyloid A (SAA) as an inflammatory marker in equine influenza virus infection. *Acta Vet Scand* 1998;40:323-333
124. Vandenplas ML, Moore JN, Barton MH, et al: Concentrations of serum amyloid A and lipopolysaccharide-binding protein in horses with colic. *Am J Vet Res* 2005;66:1509-1516
125. Copas V, Durham A, Stratford C, et al: In equine grass sickness, serum amyloid A and fibrinogen are elevated, and can aid differential diagnosis from non-inflammatory causes of colic. *Vet Rec* 2013;172:395-395
126. Pihl TH, Andersen PH, Kjelgaard-Hansen M, et al: Serum amyloid A and haptoglobin concentrations in serum and peritoneal fluid of healthy horses and horses with acute abdominal pain. *Vet Clin Pathol* 2013;42:177-183
127. Leclere M, Lavoie-Lamoureux A, Lavoie JP: Acute Phase Proteins in Racehorses with Inflammatory Airway Disease. *J Vet Intern Med* 2015;29:940-945
128. Lavoie-Lamoureux A, Leclere M, Lemos K, et al: Markers of systemic inflammation in horses with heaves. *J Vet Intern Med* 2012;26:1419-1426
129. Cywinska A, Witkowski L, Szarska E, et al: Serum amyloid A (SAA) concentration after training sessions in Arabian race and endurance horses. *BMC Vet Res* 2013;9:91
130. Cywinska A, Gorecka R, Szarska E, et al: Serum amyloid A level as a potential indicator of the status of endurance horses. *Equine Vet J* 2010;42:23-27

131. El-Deeb WM, El-Bahr SM: Selected Biochemical Indicators of Equine Rhabdomyolysis in Arabian Horses: Acute Phase Proteins and Trace Elements. *J Equine Vet Sci* 2014;34:484-488
132. Mette C, Dooleweerd BC, Stine J, et al: Evaluation of the systemic acute phase response and endometrial gene expression of serum amyloid A and pro-and anti-inflammatory cytokines in mares with experimentally induced endometritis. *Vet Immunol Immunopathol* 2010;138:95-105
133. Coutinho da Silva M, Canisso I, MacPherson M, et al: Serum amyloid A concentration in healthy periparturient mares and mares with ascending placentitis. *Equine Vet J* 2013;45:619-624
134. Canisso IF, Ball BA, Cray C, et al: Serum amyloid A and haptoglobin concentrations are increased in plasma of mares with ascending placentitis in the absence of changes in peripheral leukocyte counts or fibrinogen concentration. *Am J Reprod Immunol* 2014;72:376-385
135. Krakowski L, Krawczyk C, Kostro K, et al: Serum levels of acute phase proteins: SAA, Hp and progesterone (P4) in mares with early embryonic death. *Reprod Domest Anim* 2011;46:624-629
136. Hulten C, Grönlund U, Hirvonen J, et al: Dynamics in serum of the inflammatory markers serum amyloid A (SAA), haptoglobin, fibrinogen and  $\alpha$ 2-globulins during induced noninfectious arthritis in the horse. *Equine Vet J* 2002;34:699-704
137. Sipe JD, Gonnerman WA, Loose LD, et al: Direct binding enzyme-linked immunosorbent assay (ELISA) for serum amyloid A (SAA). *J Immunol Methods* 1989;125:125-135

138. Wada A, Yamada T, Kubota N: Measurement of serum amyloid A by commonly used automated analysers. *Ann Clin Biochem* 1997;34:569
139. Anhold H, Candon R, Chan D-S, et al: A Comparison of Elevated Blood Parameter Values in a Population of Thoroughbred Racehorses. *J Equine Vet Sci* 2014;34:651-655
140. Hillström A, Tvedten H, Lilliehöök I: Evaluation of an in-clinic Serum Amyloid A (SAA) assay and assessment of the effects of storage on SAA samples. *Acta Vet Scand* 2010;52:8
141. Jacobsen S, Kjelgaard-Hansen M: Evaluation of a commercially available apparatus for measuring the acute phase protein serum amyloid A in horses. *Vet Rec* 2008;163:327-330
142. Canisso IF, Ball BA, Cray C, et al: Use of a Qualitative Horse-Side Test to Measure Serum Amyloid A in Mares With Experimentally Induced Ascending Placentitis. *J Equine Vet Sci* 2015;35:54-59

**Chapter 3: Serum and Synovial Fluid Serum Amyloid A Response in Equine**  
**Models of Synovitis and Septic Arthritis**

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Accepted for publication in *Veterinary Surgery* and included with kind permission from Wiley.

**ABSTRACT**

**Objective:** To investigate the serum and synovial fluid serum amyloid A (SAA) response in equine models of synovitis and septic arthritis and to compare handheld and validated immunoturbidometric assays for SAA quantification.

**Study Design:** Controlled, experimental study.

**Animals:** Healthy adult horses (n=9).

**Methods:** Synovitis (n=4) and septic arthritis (n=5) were induced using lipopolysaccharide and *S. aureus*, respectively, and serial serum and synovial fluid samples were collected. Serial synovial fluid cytologies were performed for both models

and synovial fluid from the septic arthritis model was submitted for bacterial culture. Serum and synovial fluid SAA were quantified by handheld test and immunoturbidometric assay. Cytologic and SAA data were compared within and between models (mixed model ANOVA) and SAA assays were compared using category-by-category analysis (weighted kappa coefficient).

**Results:** Synovial fluid total nucleated cell counts and total protein increased significantly following induction of both models. Serum and synovial fluid SAA remained normal in synovitis horses and increased significantly in septic arthritis horses. Serum SAA increased more rapidly than synovial fluid SAA. Agreement was 98% when SAA values were low (<50 µg/mL); however, the assays diverged when values were greater than ~100 µg/mL. Overall, however, there was good category-by-category agreement between SAA assays (weighted kappa = 0.824).

**Conclusion:** Because SAA values for the 2 assays diverged, a larger sample size is needed before direct numeric comparisons between the assays can be made. Serum and synovial fluid SAA may be useful adjuncts in diagnosing septic arthritis in horses.

Septic arthritis in adult horses occurs most commonly in association with traumatic wounds.<sup>1,2</sup> It can become a life-threatening problem due to difficulties clearing established infections and because of degenerative changes that frequently occur following ongoing inflammation.<sup>1,2</sup> Only 56-81% of horses return to their original function following treatment for septic arthritis.<sup>3,4</sup> Early diagnosis is critical to rapid resolution of infection and inflammation, avoiding cartilage degradation and osteoarthritis.

Synovial sepsis is diagnosed primarily based on clinical signs and synovial fluid analysis. In severe cases of septic arthritis, the diagnosis is straight forward and characterized by marked elevations of synovial fluid total protein (TP) and total nucleated cell count (TNCC). However, in a subset of insidious cases it can be challenging to distinguish between acute non-septic inflammation and infection based on clinical signs and synovial fluid cytology alone.<sup>1,5-9</sup> Horses with acute non-septic inflammation can show mild to moderate lameness and there can be significant overlap in TNCC and TP in some cases of synovitis and septic arthritis.<sup>5-9</sup>

A rapid assay to help confirm sepsis at the time of examination would be particularly valuable, especially for practitioners in the field without ready access to a laboratory.<sup>1,2</sup> Serum amyloid A (SAA) is a promising diagnostic tool recently described in horses for screening of systemic inflammation and infection and may be equally promising for screening synovial inflammation and infection.<sup>10-14</sup> Serum amyloid A, the major acute phase protein in horses, increases systemically in response to injury, infection, and inflammation.<sup>13,15,16</sup> It is found in very low or undetectable concentrations (0.5-20 µg/mL) in the serum of healthy horses,<sup>12,13,17,18</sup> and is produced primarily in the

liver.<sup>13,19,20</sup> Serum amyloid A quickly rises in response to infection and inflammation and is increasingly used as a diagnostic tool in the horse as a sensitive and reliable indicator of the presence of active inflammation.<sup>13,15</sup> The rapid peak at 36 to 48 hours and short half-life (24 hours) make SAA ideal for monitoring the progression of disease and response to treatment.<sup>13,15,19</sup> A variety of disease states are known to induce increased SAA concentrations in horses, including bacterial and viral infections, septic arthritis, surgery, gastrointestinal tract disease, reproductive disease, and local inflammation.<sup>13,14</sup> Available data suggest that SAA may be an order of magnitude higher with bacterial infection versus inflammation.<sup>13,16</sup> SAA is produced locally within synovial joints<sup>11,14,21</sup>; however, information on SAA in equine joints is limited.<sup>11,21</sup> SAA is present in low or undetectable levels in normal equine synovial fluid,<sup>10,12</sup> does not increase in synovial fluid following repeated arthrocentesis,<sup>10,12</sup> and does increase in synovial fluid in response to sepsis.<sup>12,14</sup>

Equine SAA levels are commonly determined by immunoturbidometric assay using an automated chemistry analyzer and monoclonal anti-human SAA antibodies.<sup>18</sup> Because development and validation of the assay may not be cost-effective for smaller laboratories, samples are frozen and shipped to a commercial laboratory, delaying receipt of results. Recently, a handheld lateral flow immunoassay (handheld test) has become commercially available to measure SAA in equine whole blood or serum. The assay can be performed immediately following sample collection and produces results in 10 minutes and the result can be read by visual categorical analysis or a portable colorimetric reader (StableLab, Epona Biotech Limited, Sligo, Ireland). Data are not available for use of this handheld test to measure SAA in equine synovial fluid. The

ability to determine serum and/or synovial fluid SAA using the handheld test could be used in conjunction with conventional data to facilitate diagnosis of septic arthritis.

The objectives of our study were to investigate the serum and synovial fluid SAA response in equine models of synovitis and septic arthritis and to compare SAA results of a new handheld test with those from a validated immunoturbidometric assay using the same equine models of acute synovitis and septic arthritis. We hypothesized that SAA in serum and synovial fluid from horses with septic arthritis would be significantly elevated compared to those with synovitis and there would be good agreement between the handheld test and the validated SAA immunoturbidometric assay in equine serum and synovial fluid.

## **MATERIALS AND METHODS**

### *Study Design*

Synovitis (n=4) or septic arthritis (n=5) were induced in 1 randomly assigned radiocarpal joint of adult horses free of orthopedic disease. The study was approved by the Institutional Animal Care and Use Committee (IACUC) and the Biosafety Committee. Blood and synovial fluid were collected before and following model induction for synovial cytology and SAA analysis by a handheld test and a previously validated immunoturbidometric assay and compared across time, between models, and between SAA assays.

### *Experimental Horses*

Nine healthy adult horses (3 mares, 6 geldings) were determined to be free of musculoskeletal disorders related to the carpal joints based on physical and lameness examinations (mean  $\pm$  SD; 16.2  $\pm$  5.13 years; 479.7  $\pm$  38 kg). Breeds included Arabian or Arabian crosses (2), Paint (2), Tennessee Walking Horse (2), American Quarter Horse, American Saddlebred, and Thoroughbred (1 each). All horses had pain-free range of motion of the carpal joints, were evaluated at the walk and trot in a straight line and on circles, and carpal flexion tests were negative. Horses were housed at the Veterinary Teaching Hospital of the Virginia-Maryland College of Veterinary Medicine in 12' x 12' stalls for the duration of the study without forced exercise, fed free choice water and grass hay, and allowed a 24 hour acclimatization period prior to model induction.

On the day of model induction, a 14 gauge, 5.5 inch fluorinated ethylene propylene Teflon<sup>®</sup> IV catheter (Abbocath, Abbott Laboratories, Abbott Park, IL) was placed aseptically in one jugular vein with an extension set and 3-way stopcock attached. The catheter was sutured in place and maintained throughout the course of the study. Catheters were flushed with 10 mL heparinized saline (10 U/mL heparin in 250 mL 0.9% saline solution) every 6 hours and/or following administration of medications to maintain catheter patency. Physical examinations, including in-stall lameness evaluations, of each horse were performed every 6 hours. Lameness was evaluated subjectively in the stall as the horse was walked and turned. If horses became visibly lame in the stall, they received butorphanol (0.02 mg/kg IV and IM) every 4 hours until lameness was no longer visible. Originally, estimations of pain response based on the literature required that horses in the septic arthritis group receive phenylbutazone (2.2 mg/kg IV) at the time of model

induction and every 12 hours thereafter until euthanasia. However, based on our experience with the first horse in the septic arthritis group, the IACUC protocol was amended such that the remaining 4 horses in the septic arthritis group received phenylbutazone (2.2 mg/kg IV) if they became visibly lame in the stall and every 12 hours until the lameness was no longer visible or they were euthanized at the end of the study, whichever came first. Upon completion of final sample collection, each horse was humanely euthanized with pentobarbital sodium (86 mg/kg IV).

### *Synovitis and Septic Arthritis Models*

Synovitis was induced by lipopolysaccharide (LPS) injection of 1 randomly selected (RANDBETWEEN function; Microsoft Excel, Microsoft Corporation, Redmond, WA) radiocarpal joint using a low dose selected based on published studies.<sup>11,23-25</sup> Lyophilized LPS (*Escherichia coli* strain 055:B5, Sigma-Aldrich, Corp, St. Louis, MO) was diluted to 2.5 mg/mL in sterile saline, aliquoted, and stored at -20°C until use. Immediately prior to induction of synovitis (within 15 minutes), thawed aliquots of LPS were vortexed vigorously and serially diluted in sterile saline to 0.0125 ng/μL. The total dose of 0.5 ng LPS per radiocarpal joint was further diluted in 2 mL saline, vortexed, and drawn into a sterile syringe, sealed in a plastic bag, and stored on ice until injection. The low dose was selected based on the literature to induce changes in TP and TNCC similar to those anticipated for the septic arthritis model yet avoid systemic signs of endotoxemia.<sup>11,23-25</sup>

Septic arthritis was induced using a previously described bacterial model.<sup>6,26-28</sup> A strain of *Staphylococcus aureus* isolated from a case of naturally occurring bovine

mastitis and previously validated for use in a bovine model of mastitis was used to induce sepsis.<sup>29</sup> *S. aureus* inoculum preparation was conducted in Biosafety Level 2 approved facilities approved by the Institutional Biosafety Committee. The day prior to model induction, the frozen *S. aureus* culture was streaked on a bovine blood agar plate and incubated for 24 hours at 37°C. A single colony of bacteria was cultured in trypticase soy broth for 6 hours at 37°C with shaking, bacteria were pelleted by centrifugation at 2,500 x g for 10 minutes at 4°C (IEC CL31R, Thermo Fisher Scientific, Pittsburgh, PA), washed twice with phosphate buffered saline (PBS) and resuspended in PBS to achieve a concentration of approximately 1.5 x 10<sup>4</sup> colony forming units (CFU)/mL. Approximate concentration was based on a standard curve relating optical density (OD) to CFU for our bacterial isolate (Genesys 20 Spectrophotometer, Thermo Fisher Scientific, Pittsburgh, PA). Following preparation, the inoculation dose was brought up to a total injection dose of 2.5 mL in sterile PBS, transferred to a sterile syringe, sealed in doubled plastic bags, and stored on ice until injection. Intra-articular injection of the inoculation dose was performed within 30 minutes of dose preparation. To minimize variation from 1 bacterial inoculum preparation to another, horses were grouped such that 1-3 horses were injected on the same day from the same bacterial preparation (3 preparations total). CFU of the challenge inoculum were determined by delivering four 25 µL replicates of the final dilution to the surface of a trypticase soy agar plate, incubating for 18-24 hours at 37°C, manually counting the number of colonies. Inoculation doses showed minimal variation between the 3 preparations (median, 1.62 x 10<sup>4</sup> CFU/joint; range, 1.29-1.76 x 10<sup>4</sup>). The total dose of 1.5x10<sup>4</sup> CFU per joint was selected based on published literature in the

horse and on previous experience with this particular strain of bacteria in a bovine mastitis model.<sup>6,26-29</sup>

### *Model Induction and Sample Collection*

Prior to model induction, 6 mL blood was collected from the IV catheter for baseline serum levels of SAA. Horses were then sedated with detomidine (0.01mg/kg IV) and butorphanol (0.01 mg/kg IV) and the randomly selected radiocarpal joint clipped and prepared for arthrocentesis with povidone-iodine and alcohol using standard aseptic technique. With the carpus held in flexion by an assistant, a 20 gauge 1.5 inch hypodermic needle was placed in the dorsolateral synovial pouch and 4-6 mL synovial fluid collected and transferred to a blood tube containing EDTA to serve as the baseline control sample for each horse. Without removing the needle, the prepared dose of either LPS or *S. aureus* (depending on model) was injected using aseptic technique (and proper biosecurity precautions for *S. aureus*). Following injection, a light bandage consisting of a sterile non-stick pad, roll gauze, and elastic adhesive tape (Elastikon, Johnson & Johnson, New Brunswick, NJ) was applied over the arthrocentesis site to maintain cleanliness. A stack bandage of cotton sheets, gauze, and cohesive bandage was applied from the distal radius to the coronary band to control edema.

Timing of sample collection following model induction was customized for each model based on published information, real time evidence of changes in serum and synovial fluid SAA and synovial fluid TP and TNCC concentrations, and humane considerations related to animal welfare based on degree of lameness. Because the models do not progress at the same rates, the collection times for each model are

purposefully not identical so as to match sample collection with key changes in synovial fluid parameters. In addition to baseline samples, blood and synovial fluid were collected at 6, 12, 24, and 30 hours following LPS injection and at 12, 24, and 36 hours following *S. aureus* injection. Longer sampling periods were used for the first horse in each group until a known pattern of disease progression was established. Blood samples (6 mL) were collected from the IV catheter following removal of 10 mL discard sample to clear the catheter and extension set of stagnant blood diluted with heparinized saline. Serial radiocarpal synoviocentesis (4-6 mL) and bandaging were performed as described above. Bandages were maintained throughout the course of the study with replacement following each synoviocentesis.

### *Sample Processing*

Following collection, clotted blood samples were centrifuged at 2,000 x g for 10 minutes at 22°C and serum collected and divided into 2 aliquots: 1 for immediate SAA analysis using the handheld test and 1 stored in a polypropylene tube at -20°C for later SAA analysis using the equine immunoturbidometric assay and the colorimetric reader for the handheld test.<sup>15,18</sup> Following collection, synovial fluid was divided into aliquots. One was submitted for cytology without centrifugation (Virginia Tech Animal Laboratory Services, Virginia-Maryland College of Veterinary Medicine, Blacksburg, VA). Subjective assessment of synovial fluid color and viscosity were recorded and TP, TNCC, and white blood cell differential were quantified (ADVIA 2120 hematology analyzer, Siemens Healthcare Diagnostics, Inc., Tarrytown, NY). Total protein greater than 4.0 g/dL and TNCC greater than 30,000 cells/ $\mu$ L were used to confirm synovitis and

septic arthritis models.<sup>5</sup> A second aliquot of synovial fluid was centrifuged as above to pellet cells and debris and the supernatant removed and further aliquoted for immediate SAA analysis using the handheld test or frozen as described above for SAA analysis using the immunoturbidometric assay and the colorimetric reader for the handheld test. To confirm induction of sepsis, a 3rd synovial fluid sample (1 mL when TNCC and TP were highest) was transferred to a blood culture bottle (SIGNAL Blood Culture System, Oxoid Limited, Hampshire, UK) and submitted for bacterial culture and identification (Virginia Tech Animal Laboratory Services).

### *SAA Quantification*

SAA quantification of fresh, centrifuged serum or synovial fluid was performed using a handheld SAA test (StableLab, Epona Biotech Limited, Sligo, Ireland) according to manufacturer's instructions. Briefly, 5  $\mu$ L of serum or synovial fluid was measured using the supplied pipette, added to the supplied volume (~3.5 mL) of handheld test mix solution to dilute the sample, and gently inverted, as instructed. With the test cartridge placed on a flat surface, 4 drops of diluted sample were applied to the cartridge well of the test kit using the supplied dropper and the result read following the 10 minute wait period. Using the supplied StableLab reference card, SAA concentration was estimated based on color intensity of the test band and assigned to 1 of 4 categories (0-15, >15-50, >50-200, and >200-1,000  $\mu$ g/mL; Fig 1). All test cartridges were digitally photographed and the real time categorical SAA data used to monitor model progression along with synovial fluid cytology. Frozen serum and synovial fluid samples were batch shipped overnight on dry ice for SAA analysis via a previously validated equine

immunoturbidometric assay (Acute Phase Protein Laboratory, Miller School of Medicine, Miami, FL). Confirmation of SAA values using the handheld test was performed on frozen aliquots using a colorimetric reader (SAA quantification range 0-3,000  $\mu\text{g/mL}$ ; StableLab, Epona Biotech Limited) to generate continuous data for statistical comparisons.



**Figure 3.1** Reference card and representative cartridge for handheld SAA test run on serum from a septic arthritis horse 36 hours post inoculation showing comparison of band color intensity to the reference card for determination of an intermediate SAA concentration ( $>200$ - $1,000 \mu\text{g/mL}$  by handheld reference card;  $624 \mu\text{g/mL}$  by colorimetric handheld reader;  $357.7 \mu\text{g/mL}$  on immunoturbidometric assay). C=Positive control band; T=Sample test band.

### *Data Analysis*

Data were assessed for normality using normal probability plots. Data for weight, age, cytology and immunoturbidometric SAA were normally distributed. TNCC were log transformed (base e) to stabilize the statistical model. Data are presented as mean  $\pm$  SD and geometric mean [95% Confidence Interval] for normally distributed and TNCC data, respectively. Separately within synovitis and septic arthritis groups, the effect of time on cytology and immunoturbidometric SAA data were analyzed using mixed model ANOVA followed by Tukey's procedure for multiple comparisons for time points containing  $\geq 4$  data points. The linear model specified time as a fixed effect and horse identification as the random effect. The effect of time on continuous SAA data from the handheld test was assessed using Friedman chi-square test. Cytology and immunoturbidometric SAA data were also compared between synovitis and septic arthritis using mixed model ANOVA at 0, 12, and 24 hours when comparable times and  $\geq 4$  data points were available for both models. The linear model specified group (synovitis vs septic arthritis), time, and the interaction between group and time as fixed effects and horse identification nested within group as the random effect. To specifically compare groups at each time point, the slicediff option of proc glimmix was applied to the interaction between group and time. Only immunoturbidometric data were used to compare across time within models (synovitis and septic arthritis) and between models. Continuous data for the handheld test were skewed and are presented as median [range].

A preliminary assessment of agreement between paired continuous handheld and immunoturbidometric data was performed using a bias plot (i.e., values from the 2 assays for the same sample were plotted against each other). This plot was generated for only

samples from the septic arthritis horses, as all values for samples from synovitis horses were essentially 0 (<2.5 µg/mL). Because the scatter plot showed obvious divergence between assays after a concentration of ~100 µg/mL, formal Bland-Altman analysis was not performed. In addition, continuous data from both the handheld and immunoturbidometric assays were converted to categorical data using the following categories (µg/mL) derived from the handheld reference card and relating to clinical relevance: 0-15 (normal), >15-50 (mild elevation), >50-200 (moderate elevation), and >200 (severe elevation). Category-by-category analysis was then performed to assess assay agreement using a weighted kappa coefficient. All data analysis was performed using commercial software (SAS Version 9.4, SAS Institute Inc., Cary, NC). Real time categorical data generated from the handheld test were used only for assessment of model progression and were excluded from data analysis in favor of the continuous handheld data. Categorical data are, however, reported along with the continuous data from the same samples for transparency. Significance was set at  $P<.05$ .

## RESULTS

### *Model Induction*

As expected, variability existed between horses for both models of synovitis and septic arthritis; however, synovial fluid TP >4.0 g/dL and TNCC >30,000 cells/µL were achieved for all horses in both models (Tables 1 and 2).<sup>5</sup> All septic arthritis horses had positive synovial fluid cultures for *S. aureus*.

**Table 3.1** Synovial fluid cytology results from horses with lipopolysaccharide-induced synovitis.

Post Model Induction (Hours)	N	Total Protein g/dL	Total Nucleated Cell Count† cells/ $\mu$ L x $10^3$	Neutrophil %	Lymphocyte %	Macrophage %
0	4	1.6 $\pm$ 0.5 <sup>a</sup>	0.1 [0-0.7] <sup>a</sup>	5.3 $\pm$ 9.8 <sup>a</sup>	41.2 $\pm$ 7.0 <sup>a</sup>	52.5 $\pm$ 11.4 <sup>a</sup>
6	4	3.6 $\pm$ 1.5 <sup>b</sup>	52.0 [1.2-2,303.6] <sup>a</sup>	82.5 $\pm$ 16.4 <sup>b</sup>	3.0 $\pm$ 2.8 <sup>b</sup>	14.2 $\pm$ 14.3 <sup>b</sup>
12	4	5.1 $\pm$ 0.7 <sup>b</sup>	76.0 [18.6-310.2] <sup>b</sup>	80.5 $\pm$ 14.8 <sup>b</sup>	3.8 $\pm$ 3.6 <sup>b</sup>	15.8 $\pm$ 11.9 <sup>b</sup>
24	4	4.7 $\pm$ 0.6 <sup>b</sup>	31.9 [13.4-76.0] <sup>b</sup>	59.6 $\pm$ 23.9 <sup>b</sup>	10.4 $\pm$ 10.2 <sup>b</sup>	29.8 $\pm$ 14.8 <sup>b</sup>
30	4	4.4 $\pm$ 0.5 <sup>b</sup>	19.7 [4.3-90.9] <sup>b</sup>	56.2 $\pm$ 27.4 <sup>b</sup>	10.8 $\pm$ 11.0 <sup>b</sup>	33.0 $\pm$ 17.4 <sup>b</sup>

N = number of horses. Mean  $\pm$  SD except †TNCC; Geometric Mean [95% CI]. Superscript letters indicate significant differences between values within the same column.

**Table 3.2** Serum and synovial fluid serum amyloid A (SAA) from horses with lipopolysaccharide-induced synovitis. SAA quantified using a commercial handheld test (Handheld; median [range]) and a validated immunoturbidometric assay (Immuno; mean  $\pm$  SD).

There were no significant differences in SAA values within either assay over time in either serum or synovial fluid.

Post Model Induction (Hours)	N	Serum SAA $\mu\text{g/mL}$		Synovial Fluid SAA $\mu\text{g/mL}$	
		Handheld	Immuno	Handheld*	Immuno
0	4	0 [0-0]	0.2 $\pm$ 0.2	0 [0-0]	0.4 $\pm$ 0.3
12	4	0 [0-0]	0.4 $\pm$ 0.4	0 [0-0]	0.3 $\pm$ 0.4
24	4	0 [0-0]	0.6 $\pm$ 0.7	0 [0-0]	0.6 $\pm$ 1.0
30	2	0 [0-0]	-	0 [0-0]	-
48	2	0 [0-0]	-	0 [0-0]	-

N = number of horses.

### *Synovitis*

Following induction of synovitis, the gross appearance of synovial fluid changed rapidly; becoming serosanguinous and lacking normal viscosity. Changes were moderate at 6 hours but became marked by 12 hours and remained markedly abnormal throughout the duration of the study. No horses exhibited signs of lameness within their stalls, all maintained normal physical examination parameters (temperature, pulse, and respiratory rate; TPR), and had no visible soft tissue swelling at the arthrocentesis sites. Synovial fluid TP increased by 6 hours, was highest at 12 hours, and remained elevated throughout the study (Table 1;  $P < .001$ ). Synovial fluid TNCC and percent neutrophils were highest 12 hours following LPS injection and gradually decreased, remaining elevated at the end of the study (Table 1;  $P < .001$  for both). Percent lymphocytes and macrophages decreased precipitously following induction of synovitis and remained decreased throughout the course of the study (Table 1;  $P < .001$  for both).

Serum and synovial fluid SAA was 0  $\mu\text{g/mL}$  at all sampling time points when measured by the handheld test using both the reference card and the colorimetric reader (Table 2;  $P = 1.00$  for both). When measured by immunoturbidometric assay, serum and synovial fluid SAA were measurable but very low at all time points and did not change over time (Table 2;  $P = .480$  and  $.532$ , respectively).

### *Septic Arthritis*

Grossly, synovial fluid changes following induction of septic arthritis were similar to those in the synovitis group, with the addition of fibrin present in the fluid of 2 horses at 24 and 36 hours. The first horse induced with septic arthritis received a

prophylactic dose (2.2 mg/kg IV) of phenylbutazone at the time of model induction in anticipation of the increased pain associated with the septic arthritis model compared to synovitis and in accordance with the original IACUC protocol. Serial sampling revealed a delayed onset of synovial fluid changes compared to what was expected based on the literature and the horse did not show signs of lameness in the stall. As a result, the 36 hour dose of phenylbutazone (2.2 mg/kg IV) was withheld until 66 hours post-induction when lameness became apparent at 66 hours. Analgesic administration and data collection for subsequent horses was adjusted accordingly and approved by the IACUC and expected synovial fluid changes were achieved. Horses with septic arthritis maintained normal physical examination parameters (TPR) and had no visible soft tissue swelling at the arthrocentesis sites. Two horses showed mild lameness in the stall 12 hours following model induction (occasional lame steps when turning), but remained weight bearing. The degree of lameness remained unchanged despite administration of phenylbutazone and butorphanol. Sampling end points were determined based on humane considerations.

Synovial fluid cytology values were not significantly different for synovitis compared to septic arthritis horses (Tables 1 and 3; TP  $P=$ .997; TNCC  $P=$ .150; % neutrophils  $P=$ .213; % lymphocytes  $P=$ .395; % macrophages  $P=$ .334). Septic arthritis synovial fluid TP increased by 12 hours and was highest between 36 and 48 hours (Table 3;  $P<$ .001). Synovial fluid TNCC and percent neutrophils were highest 24 hours following *S. aureus* injection and gradually decreased, remaining elevated at the end of the study (Table 3;  $P<$ .001 for both). Percent lymphocytes and macrophages decreased

following induction of septic arthritis and remained decreased throughout the course of the study (Table 3;  $P < .001$  and  $= .045$ , respectively).

Serum SAA following induction of septic arthritis began to increase at 24 hours and was highest at 36 hours (Tables 4 and 5). Synovial fluid SAA began to increase more slowly than in serum, but was still highest at 36 hours (Tables 4 and 5). Once SAA levels increased above normal, serum values were higher compared to synovial fluid. Immunoturbidometric SAA values increased significantly in both serum ( $P < .001$ ) and synovial fluid ( $P = .007$ ) over time (Table 4). Immunoturbidometric SAA values were significantly increased in the septic arthritis model compared to the synovitis model at 24 hours in serum (0 hours  $P = .882$ ; 12 hours  $P = .977$ ; 24 hours  $P = .005$ ) and synovial fluid (0 hours  $P = .874$ ; 12 hours  $P = .948$ ; 24 hours  $P = .038$ ).

SAA values for paired samples measured by the immunoturbidometric assay and the handheld colorimetric reader were similar below 50  $\mu\text{g/mL}$  and diverged above  $\sim 100$   $\mu\text{g/mL}$  (Fig 2). When evaluated using category-by-category analysis there was good agreement between the two SAA assays (Fig 3; weighted kappa = .824).<sup>30</sup>

**Table 3.3** Synovial fluid cytology results from horses with *S. aureus*-induced septic arthritis.

Post Model Induction (Hours)	N	Total Protein g/dL	Total Nucleated Cell Count† cells/ $\mu$ L x 10 <sup>3</sup>	Neutrophil %	Lymphocyte %	Macrophage %
0	5	1.4 $\pm$ 0.5 <sup>a</sup>	0.2 <sup>a</sup> [0-0.3]	18.5 $\pm$ 24.5 <sup>a</sup>	37.0 $\pm$ 12.5 <sup>a</sup>	43.5 $\pm$ 27.4 <sup>a</sup>
12	5	4.1 $\pm$ 1.4 <sup>b</sup>	42.4 <sup>b</sup> [6.4-280.1]	74.0 $\pm$ 24.3 <sup>b</sup>	5.8 $\pm$ 5.9 <sup>b</sup>	20.4 $\pm$ 25.2 <sup>ab</sup>
24	5	5.8 $\pm$ 0.9 <sup>cd</sup>	151.0 <sup>b</sup> [100.9-226.1]	89.4 $\pm$ 5.2 <sup>b</sup>	4.4 $\pm$ 5.7 <sup>b</sup>	6.2 $\pm$ 4.0 <sup>b</sup>
36	4	6.0 $\pm$ 1.1 <sup>d</sup>	110.4 <sup>b</sup> [20.5-594.8]	84.2 $\pm$ 9.9 <sup>b</sup>	1.2 $\pm$ 2.5 <sup>b</sup>	14.5 $\pm$ 7.8 <sup>ab</sup>
48	2	6.6 $\pm$ 0.8	152.6 -	93.0 $\pm$ 2.8	1.5 $\pm$ 0.7	6.0 $\pm$ 1.4

N = number of horses. Mean  $\pm$  SD except †TNCC; Geometric Mean [95% CI]. Superscript letters indicate significant differences between values within the same column for times with sample sizes of  $\geq 4$ .

**Table 3.4** Serum and synovial fluid serum amyloid A (SAA) from horses with *S. aureus*-induced septic arthritis quantified using a commercial handheld test (Handheld; median [range]) and a validated immunoturbidometric assay (Immuno; mean  $\pm$  SD).

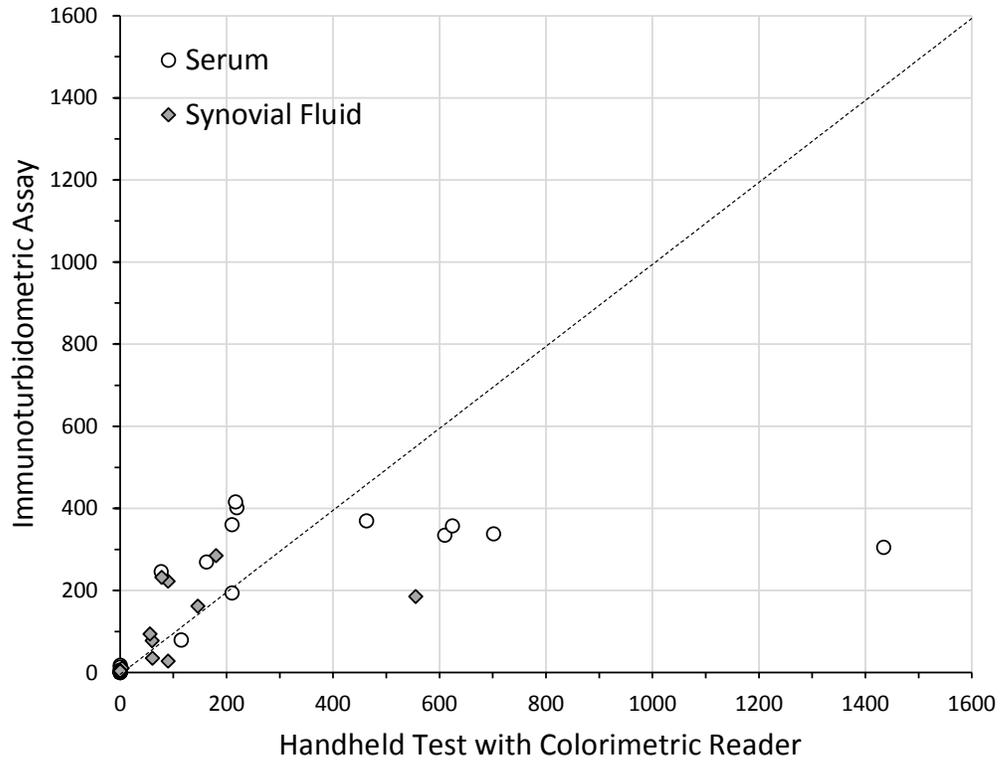
Post Model Induction (Hours)	N	Serum SAA $\mu\text{g/mL}$		Synovial Fluid SAA $\mu\text{g/mL}$	
		Handheld	Immuno	Handheld	Immuno
0	5	0 <sup>a</sup> [0-0]	5.4 $\pm$ 7.6 <sup>a</sup>	0 <sup>a</sup> [0-0]	2.3 $\pm$ 2.4 <sup>a</sup>
12	5	0 <sup>a</sup> [0-0]	1.4 $\pm$ 0.8 <sup>a</sup>	0 <sup>a</sup> [0-0]	1.1 $\pm$ 1.1 <sup>a</sup>
24	5	115 <sup>a</sup> [0-210.0]	111.9 $\pm$ 116.3 <sup>a</sup>	0 <sup>a</sup> [0-61]	27.3 $\pm$ 40.1 <sup>a</sup>
36	4	663 <sup>b</sup> [217-1434]	354.3 $\pm$ 46.4 <sup>b</sup>	135 <sup>b</sup> [60-555]	144.3 $\pm$ 114.5 <sup>b</sup>
48	2	410 [210-610]	347.7 $\pm$ 18.6	75 [4-146]	86.7 $\pm$ 106.3

N = number of horses. Superscript letters indicate significant differences between groups within the same column for times with sample sizes of  $\geq 4$ .

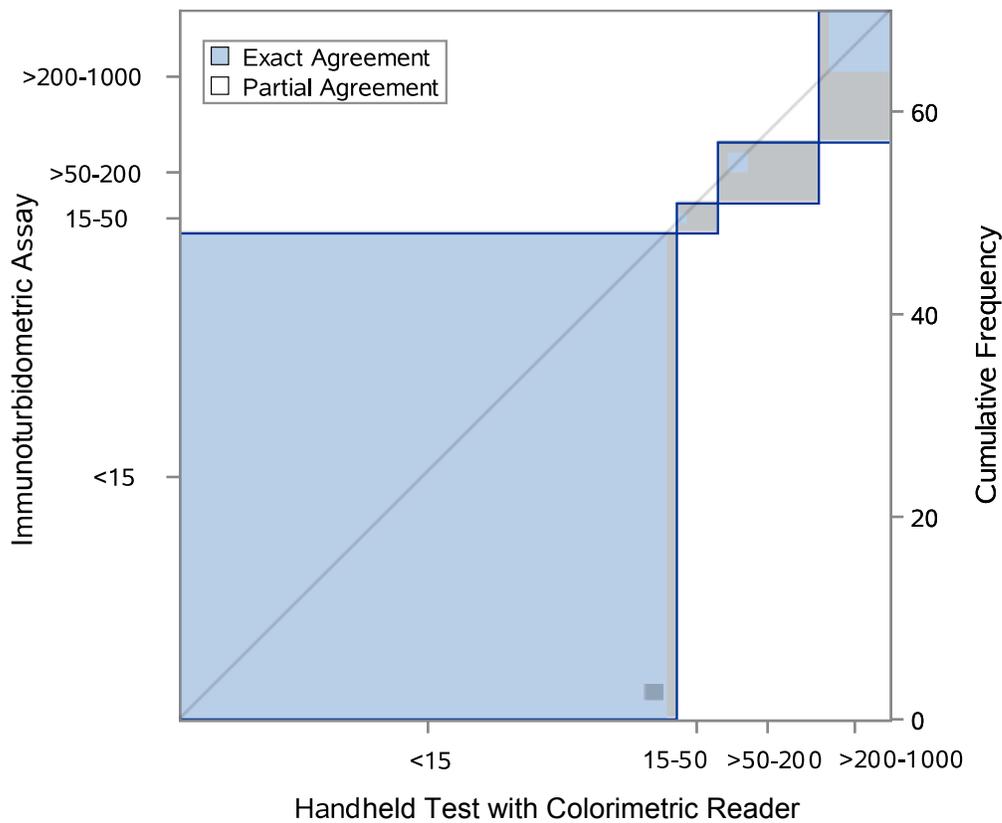
**Table 3.5** Numbers of samples by category for serum and synovial fluid serum amyloid A (SAA) from horses with *S. aureus*-induced septic arthritis quantified using the handheld reference card.

Post Model Induction (Hours)	N	Serum SAA µg/mL				Synovial Fluid SAA µg/mL			
		0-15	>15-50	>50-200	>200-1,000	0-15	>15-50	>50-200	>200-1,000
0	5	5	0	0	0	5	0	0	0
12	5	5	0	0	0	5	0	0	0
24	5	2	1	2	0	3	2	0	0
36	4	0	0	2	2	0	1	2	1
48	2	0	0	1	1	0	1	1	0

N = number of horses.



**Figure 3.2** Simple bias plot for SAA concentrations from paired serum and synovial fluid samples from 5 septic arthritis horses run using the handheld test with colorimetric reader and immunoturbidometric assay. The dotted line represents line of equality.



**Figure 3.3** Plot showing category-by-category agreement for SAA concentrations between the handheld test the immunoturbidometric assay for all 70 samples with pairwise data. The line represents perfect agreement. At low SAA concentrations ( $\leq 50$   $\mu\text{g/mL}$ ) there was 98% agreement (49/50) between the handheld test with colorimetric reader and the immunoturbidometric assay for SAA values. Below 50  $\mu\text{g/mL}$  agreement was 98% as shown by the large area of dark blue shading (exact agreement). At SAA concentrations  $>50$   $\mu\text{g/mL}$  40% of samples agreed exactly (8/20) and the remaining 60% showed partial agreement (12/20), clustering along the line of perfect agreement.

## DISCUSSION

Serum and synovial fluid SAA were successfully measured using both the handheld test and immunoturbidometric assay, remained within normal limits in samples collected from our model of synovitis, and were significantly increased in samples from our model of septic arthritis. Septic arthritis serum SAA began increasing earlier (24 vs. 36 hours) and was higher than synovial fluid SAA. Values obtained from the handheld test and the immunoturbidometric assay diverged above  $\sim 100 \mu\text{g/mL}$ ; however, overall there was good agreement between the 2 assays for SAA in serum and synovial fluid when agreement was assessed using category-by-category analysis. Our models of synovitis and septic arthritis both resulted in targeted synovial fluid TP and TNCC in all horses and had similar increases in TP and TNCC at the highest values.

Induction of synovitis using a range of dosages of intra-articular LPS has previously been reported.<sup>11,23,24,31</sup> The intra-articular dosage of 0.5 ng LPS per radiocarpal joint was selected for our study to induce cytology changes similar to acute synovitis<sup>5</sup> and those induced in the *S. aureus* model of septic arthritis. The low dose was selected as the lowest dose reported to achieve the desired effect and to avoid systemic signs of endotoxemia that might confound data interpretation. Use of higher LPS dosages of 1-3  $\mu\text{g}$  per joint resulted in increased serum and synovial fluid white blood cell counts and increased serum and synovial fluid SAA in a previous study.<sup>11</sup> However, the 4 horses receiving 1-3  $\mu\text{g}$  LPS developed fever, tachycardia, severe lameness, and swelling of the joints following intra-articular LPS injection, which was deemed undesirable for our study.<sup>11</sup> In contrast, our synovitis horses had no change in physical

examination parameters or lameness grades, nor did serum or synovial fluid SAA concentrations increase during synovitis, despite dramatic changes in synovial fluid cytology. Use of models for synovitis and septic arthritis does create some limitations; however, their use allowed us to design a controlled study with known start of synovitis and bacterial contamination, both of which were necessary to complete our stated objectives. Based on our data, clinical studies can be designed to evaluate the SAA response in naturally occurring cases of synovitis and septic arthritis.

*S. aureus*-induced septic arthritis has been previously reported in horses; however, to our knowledge this is the first study to use a bovine *S. aureus* isolate.<sup>6,26-28</sup> In addition to increased synovial fluid TP and TNCC, positive synovial fluid bacterial cultures for *S. aureus* were obtained for all septic arthritis horses in our study. This is the largest experimental study to date reporting the measurement of SAA in equine synovial fluid. Significant increases in serum and synovial fluid SAA were detected by both the handheld test and immunoturbidometric assay.

Serum SAA ranges from 0.5-20 µg/mL in normal horses<sup>12,13,22,32</sup> and increases rapidly in response to infection and inflammation.<sup>13,15,32</sup> Marked difference in serum SAA between infection and inflammation have been used to help distinguish between infectious and noninfectious disease.<sup>15,16,32,33</sup> Results of our study are consistent with these published reports and confirm normal SAA in serum and synovial fluid during acute synovitis and marked increases during septic arthritis in the models used. The relatively higher values in serum compared to synovial fluid are consistent with another recent report.<sup>14</sup> To our knowledge, this is the first in vivo septic arthritis study evaluating paired serum and synovial fluid SAA. We did not expect to see a temporal difference in

serum and synovial fluid SAA responses and the delayed SAA increase in synovial fluid may not be ideal for the timely diagnosis of septic arthritis. However, early increases in serum SAA along with clinical signs and synovial fluid changes consistent with septic arthritis may enable a prompt diagnosis of septic arthritis by simple blood collection and SAA quantification on the farm at the time of examination without the need for arthrocentesis, and facilitate timely referral. Synoviocentesis can be challenging in the field, whereas blood collection is commonplace. Used in conjunction with other diagnostics, elevated serum SAA may aid in the diagnosis of septic arthritis. Based on our results, we recommend measuring SAA in serum and synovial fluid in addition to synovial fluid TP, TNCC, cytology, and bacterial culture.

The first septic arthritis horse received a prophylactic dose of phenylbutazone at the time of model induction, in expectation of pain and lameness. This horse had an unexpected delay in the serum and synovial fluid SAA response; however, by 72 hours SAA did reach values similar to those observed at 36 hours in other septic arthritis horses. While it is possible that this first septic arthritis horse was simply slow to respond to *S. aureus* inoculation, it raises the possibility that anti-inflammatory medications may dampen or delay the SAA response.

The acute phase response occurs following injury or infection and results in activation of inflammatory mediators and cytokines<sup>13,19,32,36</sup> that induce synthesis of acute phase proteins, including the rapid production of SAA.<sup>13,19,32</sup> Non-steroidal anti-inflammatory drugs inhibit the cyclooxygenase (COX) pathway and therefore the synthesis of prostanoids and thromboxanes.<sup>37-40</sup> Phenylbutazone resulted in decreased synovial fluid white blood cell counts following induction of equine synovitis with intra-

articular LPS compared to horses with induced synovitis not receiving phenylbutazone.<sup>41</sup> The effects of selective and non-selective NSAIDs on the human acute phase response have been reported.<sup>42,43</sup> Patients undergoing thoracotomy and treated with flurbiprofen, a non-selective NSAID, had lower levels of several acute phase reactants compared to patients who did not receive flurbiprofen, leading the authors to suggest that anti-inflammatory medications may contribute to attenuation of the postoperative inflammatory response.<sup>42</sup> In people treated with COX-2 selective NSAIDs for Rheumatoid Arthritis, C-reactive protein, an acute phase protein, was not decreased; however, in the few treated with a nonselective COX inhibitor, C-reactive protein did decrease, leading the authors to speculate that there may be a relationship between the effects of COX-2 selective and nonselective inhibitors.<sup>43</sup>

The handheld SAA assay provided a convenient and valuable means of measuring SAA rapidly. Based on the results of our study using models of synovitis and septic arthritis and in conjunction with clinical signs and synovial fluid analysis, an elevation in serum or synovial fluid SAA above normal values may be a useful indicator of synovial sepsis. However, a larger sample of clinical cases would be helpful to establish more distinct cut-off ranges using clinical cases. Serum SAA may serve as an earlier marker for sepsis than synovial fluid SAA based on our findings. Early differentiation between aseptic synovitis and septic arthritis has the potential to result in more rapid therapy and an improved prognosis for soundness and survival. Serum and/or synovial fluid SAA is a promising adjunct to existing laboratory analyses in the diagnosis of septic arthritis and warrants further investigation in clinical cases of suspected synovial sepsis.

## REFERENCES

1. Morton AJ: Diagnosis and treatment of septic arthritis. *Vet Clin North Am Equine Pract* 2005;21:627-649
2. Lugo J, Gaughan EM: Septic arthritis, tenosynovitis, and infections of hoof structures. *Vet Clin North Am Equine Pract* 2006;22:363-388
3. Schneider R, Bramlage L, Moore R, et al: A retrospective study of 192 horses affected with septic arthritis/tenosynovitis. *Equine Vet J* 1992;24:436-442
4. Wright I, Smith M, Humphrey D, et al: Endoscopic surgery in the treatment of contaminated and infected synovial cavities. *Equine Vet J* 2003;35:613-619
5. Steel CM: Equine synovial fluid analysis. *Vet Clin North Am Equine Pract* 2008;24:437-454
6. Tulamo R, Bramlage L, Gabel A: Sequential clinical and synovial fluid changes associated with acute infectious arthritis in the horse. *Equine Vet J* 1989;21:325-331
7. LaPointe JM, Laverty S, Lavoie JP: Septic arthritis in 15 Standardbred racehorses after intra-articular injection. *Equine Vet J* 1992;24:430-434
8. Bertone AL: Update on infectious arthritis in horses. *Equine Vet Educ* 199;11:143-152
9. McIlwraith CW, Billingham RC, Frisbie DD: Current and future diagnostic means to better characterize osteoarthritis in the horse-Routine synovial fluid analysis and synovial fluid and serum markers. *Proc Am Assoc Equine Pract* 2001;47:171-179
10. Sanchez Teran A, Rubio-Martinez L, Villarino N, et al: Effects of repeated intra-articular administration of amikacin on serum amyloid A, total protein and nucleated cell count in synovial fluid from healthy horses. *Equine Vet J* 2012;44:12-16

11. Jacobsen S, Niewold TA, Halling-Thomsen M, et al: Serum amyloid A isoforms in serum and synovial fluid in horses with lipopolysaccharide-induced arthritis. *Vet Immunol Immunopathol* 2006;110:325-330
12. Jacobsen S, Thomsen MH, Nanni S: Concentrations of serum amyloid A in serum and synovial fluid from healthy horses and horses with joint disease. *Am J Vet Res* 2006;67:1738-1742
13. Jacobsen S, Andersen P: The acute phase protein serum amyloid A (SAA) as a marker of inflammation in horses. *Equine Vet Educ* 2007;19:38-46
14. Robinson CS, Singer ER, Piviani M, et al: Can serum amyloid A, D-Lactate or L-Lactate aid diagnosis of synovial sepsis in horses? *Vet Surg* 2015;44:E64
15. Belgrave RL, Dickey MM, Arheart KL, et al: Assessment of serum amyloid A testing of horses and its clinical application in a specialized equine practice. *J Am Vet Med Assoc* 2013;243:113-119
16. Pepys M, Baltz ML, Tennent GA, et al: Serum amyloid A protein (SAA) in horses: objective measurement of the acute phase response. *Equine Vet J* 1989;21:106-109
17. Hulten C, Tulamo R-M, Suominen M, et al: A non-competitive chemiluminescence enzyme immunoassay for the equine acute phase protein serum amyloid A (SAA)—a clinically useful inflammatory marker in the horse. *Vet Immunol Immunopathol* 1999;68:267-281
18. Jacobsen S, Kjelgaard-Hansen M, Hagbard Petersen H, et al: Evaluation of a commercially available human serum amyloid A (SAA) turbidometric immunoassay for determination of equine SAA concentrations. *Vet J* 2006;172:315-319

19. Uhlar CM, Whitehead AS: Serum amyloid A, the major vertebrate acute-phase reactant. *Eur J Biochem* 1999;265:501-523
20. McDonald TL, Larson MA, Mack DR, et al: Elevated extrahepatic expression and secretion of mammary-associated serum amyloid A 3 (M-SAA3) into colostrum. *Vet Immunol Immunopathol* 2001;83:203-211
21. Berg LC, Thomsen PD, Andersen PH, et al: Serum amyloid A is expressed in histologically normal tissues from horses and cattle. *Vet Immunol Immunopathol* 2011;144:155-159
22. Nunokawa Y, Fujinaga T, Taira T, et al: Evaluation of serum amyloid A protein as an acute-phase reactive protein in horses. *J Vet Med Sci* 1993;55:1011-1016
23. Palmer JL, Bertone AL: Experimentally-induced synovitis as a model for acute synovitis in the horse. *Equine Vet J* 1994;26:492-495
24. Palmer JL, Bertone AL, Malemud CJ, et al: Biochemical and biomechanical alterations in equine articular cartilage following an experimentally-induced synovitis. *Osteoarthritis Cartilage* 1996;4:127-137
25. Williams LB, Koenig JB, Black, B, et al: Equine allogeneic umbilical cord blood derived mesenchymal stromal cells reduce synovial fluid nucleated cell count and induce mild self-limiting inflammation when evaluated in an LPS induced synovitis model. *Equine Vet J* 2015 doi: 10.1111/evj.12477. Epub ahead of print
26. Brusie R, Sullins K, II NW, et al: Evaluation of sodium hyaluronate therapy in induced septic arthritis in the horse. *Equine Vet J* 1992;24:18-23

27. Whitehair KJ, Bowersock TL, Blevins WE, et al: Regional Limb Perfusion for Antibiotic Treatment of Experimentally Induced Septic Arthritis. *Vet Surg* 1992;21:367-373
28. Tulamo R, Bramlage L, Gabel A: The influence of corticosteroids on sequential clinical and synovial fluid parameters in joints with acute infectious arthritis in the horse. *Equine Vet J* 1989;21:332-337
29. Petersson-Wolfe C, Wolf S, Hogan J: Experimental challenge of bovine mammary glands with *Enterococcus faecium* during early and late lactation. *J Dairy Sci* 2009;92:3158-3164
30. Petrie A, Watson P: Additional techniques, in Petrie A, Watson P (eds): *Statistics for Veterinary and Animal Science* (ed 3). Ames, IA, Wiley-Blackwell, 2013, pp 200-227
31. Hawkins D, MacKay R, Gum G, et al: Effects of intra-articularly administered endotoxin on clinical signs of disease and synovial fluid tumor necrosis factor, interleukin 6, and prostaglandin E2 values in horses. *Am J Vet Res* 1993;54:379-386
32. Crisman MV, Scarratt WK, Zimmerman KL: Blood proteins and inflammation in the horse. *Vet Clin North Am Equine Pract* 2008;24:285-297
33. Hulten C, Sandgren B, Skiöldebrand E, et al: The acute phase protein serum amyloid A (SAA) as an inflammatory marker in equine influenza virus infection. *Acta Vet Scand* 1998;40:323-333
34. O'Hara R, Murphy EP, Whitehead AS, et al: Acute-phase serum amyloid A production by rheumatoid arthritis synovial tissue. *Arthritis Res* 2000;2:142-144

35. de Seny D, Cobraiville G, Charlier E, et al: Acute-phase serum amyloid a in osteoarthritis: regulatory mechanism and proinflammatory properties. *PLoS One* 2013;8:e66769
36. Cray C, Zaias J, Altman NH: Acute phase response in animals: a review. *Comp Med* 2009;59:517-526
37. Higgins A, Lees P: The acute inflammatory process, arachidonic acid metabolism and the mode of action of anti-inflammatory drugs. *Equine Vet J* 1984;16:163-175
38. Lees P, Higgins A: Clinical pharmacology and therapeutic uses of non-steroidal anti-inflammatory drugs in the horse. *Equine Vet J* 1985;17:83-96
39. Lees P, Landoni M, Giraudel J, et al: Pharmacodynamics and pharmacokinetics of nonsteroidal anti-inflammatory drugs in species of veterinary interest. *J Vet Pharmacol Ther* 2004;27:479-490
40. Tobin T, Chay S, Kamerling S, et al: Phenylbutazone in the horse: a review. *J Vet Pharmacol Ther* 1986;9:1-25
41. Morton AJ, Campbell NB, Redding WR, et al: Preferential and non-selective cyclooxygenase inhibitors reduce inflammation during lipopolysaccharide-induced synovitis. *Res Vet Sci* 2005;78:189-192
42. Esme H, Kesli R, Apiliogullari B, et al: Effects of flurbiprofen on CRP, TNF-alpha, IL-6, and postoperative pain of thoracotomy. *Int J Med Sci* 2011;8:216-221
43. Tarp S, Bartels EM, Bliddal H, et al: Effect of nonsteroidal antiinflammatory drugs on the C-reactive protein level in rheumatoid arthritis: a meta-analysis of randomized controlled trials. *Arthritis Rheum* 2012;64:3511-3521

## **Chapter 4: Final Comments**

This thesis investigated the serum and synovial fluid SAA response in equine models of synovitis and septic arthritis and compared the SAA results of a handheld SAA test with those from a validated SAA immunoturbidometric assay. The intent was to better understand the SAA response in controlled models of joint infection and joint inflammation, to determine if the handheld test and the immunoturbidometric assay would comparably measure SAA in serum and synovial fluid, and overall to assess whether SAA could be a useful aid in diagnosing septic arthritis in horses.

Our goal was to create a model of joint inflammation that was similar to clinical cases of synovitis, therefore a low dose of LPS was injected intra-articularly. This low dose of LPS was successful in producing synovial fluid TP and TNCC changes in our desired ranges without producing systemic signs of endotoxemia, which has been reported in other equine synovitis experiments that used high doses of LPS. Serum and synovial fluid SAA concentrations did not increase from baseline values in the synovitis horses. Our model of equine septic arthritis was induced with intra-articular *S. aureus*, which resulted in increased synovial fluid TP and TNCC, positive synovial fluid bacterial cultures, and significant increases in serum and synovial fluid SAA concentrations. Unexpectedly, the serum SAA concentrations in the septic arthritis horses began to increase earlier than synovial fluid SAA. The lag in synovial fluid SAA response may be due to diffusion of peripheral SAA proteins through the synovial membrane. While the delayed SAA response in synovial fluid may not be ideal for the timely diagnosis of septic arthritis, the early increases in serum SAA, in conjunction with clinical signs and synovial fluid cytology changes, may be supportive of a diagnosis of septic arthritis.

The handheld test seems to be an acceptable alternative to the immunoturbidometric assay for measurement of SAA in equine serum and synovial fluid and it has the advantage of being performed patient-side, delivering results in less than 10 minutes. As synoviocentesis for the collection of synovial fluid can be challenging to perform or should not be attempted due to wounds or trauma to a joint increasing the risks of iatrogenic intra-articular bacterial infection, the simple collection of blood allows for the quantification of serum SAA. Based on our experimental findings, serum SAA may serve as an earlier indication of joint infection than synovial fluid SAA, and early differentiation between aseptic synovitis and septic arthritis may result in more rapid therapy and an improved prognosis for soundness. The results of this study show that serum and synovial fluid SAA quantifications are promising additions to the diagnostic tools available for the identification of septic arthritis in horses and warrant further research in clinical cases of suspected septic arthritis.