GRANSKAD ARTIKEL

LITTERATURSTUDIE:

The clinical application of using serum amyloid A analysis of synovial fluid to diagnose synovial infection in horses

Denna artikel utgör det skriftliga arbetet av författarens specialistutbildning i hästens sjukdomar.

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Abstract
Several biomarkers in synovial fluid have been evaluated for their use in diagnosing synovial sepsis, of these serum amyloid A (SAA) is showing the most potential. Two studies showed synovial fluid SAA to be 80% and 75% sensitive and 73% and 92% specific respectively in diagnosing synovial sepsis. In experimentally induced synovial sepsis SAA values were shown to rise in all horses, but only between 16 and 24 hours after synovial sepsis was induced. This slow rise makes SAA measurements of synovia alone too inaccurate for clinical use in acute cases. Analysing SAA values in addition to total nucleated cell count (TNCC), total protein (TP) and neutrophil percentage in synovial fluid in cases with suspected bacterial contamination may be of value in diagnosing synovial sepsis and in monitoring disease progress and response to treatment. Further research is needed to establish cut-off values between septic and non-septic synovial pathology. Commercially available handheld devices for SAA analysis of blood samples were used successfully for analysis of SAA in synovia and may be of value in analysing synovial fluid in situations where other laboratory analyses are not available.

Introduction
Synovial sepsis is a common diagnosis in horses either due to a penetrating injury, hematogenous spread or as a complication of articular or intrathecal injections. A quick and accurate diagnosis is considered essential for a good prognostic outcome. The gold standard for diagnosis of synovial sepsis is a positive bacterial culture of synovial fluid or the presence of intracellular bacteria on a smear of synovial fluid (26). This is, however, not sensitive enough for diagnosis, because positive bacterial culture is found to be positive in only 55% or less of cases with septic arthritis (26,16). Traditionally synovial infections are diagnosed by measuring total nucleated cell counts (TNCC), percentage of neutrophils and total protein (TP) in synovial fluid, but cut-off values for what is considered normal and what is consistent with synovial sepsis vary. Values often used for diagnosis of synovial sepsis is TNCC over 20 x 109 cells/l, neutrophil percentages over 80% and a total protein count over 25 g/l. Normal synovial values are considered to be a TNCC of less than 1.0 x 109 cells/l, a total protein value of less than 20 g/l and a neutrophil percentage of less than 20% (26). Interpretation of values that are higher than normal values but not high enough to give a clear diagnosis of synovial sepsis can be difficult. To further complicate matters, sepsis following corticosteroid injections (29), sepsis following penetrating injuries causing draining wounds into synovial cavities and sepsis following infections of micro-organisms with low virulence can cause false negative results on total nucleated cell counts, neutrophil percentage and total protein in synovial fluid (16). For these reasons an adjunctive tool for diagnosing sepsis of synovial structures would be of value. Several biomarkers in synovial fluid have been evaluated for their use in diagnosing synovial sepsis in both experimental studies and in studies with clinical cases of synovial sepsis, including glucose, D-lactate and D-Dimer (1,13,19,20). Analysis of Serum amyloid A is so far showing the most potential in


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distinguishing between septic and non-septic synovial pathology and the analysis is considerably easier and faster than the other biomarkers studied (2,12,14,15,20,21,22,23,25). In this article results from studies measuring SAA in synovia were reviewed for the purpose of evaluating the diagnostic value of synovial fluid SAA measurements in diagnosing septic synovitis. Another question of clinical interest is if commercially available handheld devices for analysis of SAA of blood could be used for analysis of synovia in suspected cases of septic synovitis.

Serum amyloid A

Serum amyloid A (SAA) is an acute-phase protein synthesized primarily in the liver as a result of the acute phase response (APR), which is a nonspecific reaction to any type of tissue injury. In horses SAA is the major acute phase protein and is considered the most sensitive indicator of the APR (8,18,28,30). Inflammatory cytokines produced by monocytes and macrophages, such as interleukin-1β, interleukin-6 and tumour necrosis factor-α are responsible for the upregulation of SAA synthesis (9). SAA is also synthesized by extrahepatic sources including equine articular chondrocytes and fibroblast-like synoviocytes (5,10). Specific isoforms of SAA were detected in synovial fluid samples that were not detected in any blood samples, providing evidence that these isoforms were produced by the synovial membrane (10,11). In serum SAA rises rapidly in response to inflammation and peaks at 36-48 hours after injury (3,9). Because it also has a short half-life, it is a good tool for monitoring disease progress and response to treatment (3,8,9).

Serum amyloid A in synovial fluid of healthy horses

Serum amyloid A should not be detectable in synovial fluid of a healthy horse, since it is only produced in response to the acute phase response. In healthy horses SAA levels in synovial fluids were below or just above the detection limits of serum assays. Five studies reported SAA values in synovia below detection limit in all healthy horses studied (12,15,21,22,23). The detection limit for one of the studies was reported as 0,48 mg/l (12). Lindegaard et al. reported values of 0-0.8 µg/ml in 8 clinically healthy horses prior to experimentally induced synovitis (14). Robinson et al. reported values of 0-0.8 µg/ml in 8 healthy horses (20). All together 41 clinically healthy horses had SAA concentrations near or below detection rate (as 6 horses were used in all articles published by Sanchez-Teran these were counted as the same horses). No research has been done to see whether systemic inflammatory conditions with high serum concentrations of SAA could cause diffusion of SAA into otherwise normal synovial structures and subsequently increase SAA values in synovia.

The effects of various treatments of septic arthritis on SAA concentrations in synovial fluid

Synovial fluid SAA concentrations were not influenced by repeated arthrocentesis, intra-articular medication with amikacin, through-and-through joint lavage or arthroscopic lavage and debridement in healthy horses. In these studies, SAA values in synovial fluid did not increase significantly when total protein count and TNCC did (21,22,23). Antibiotic, anti-inflammatory or opioid medication may lower the SAA value in infected joints from initial high values (12,14). Low values of SAA were reported in two horses with septic arthritis where treatment had been started and TNCC and TP values had decreased from the initial values (12). Intra-articular medication with morphine decreased SAA peak values significantly compared to IV morphine administration in horses with experimentally induced synovitis (14). Medication with phenylbutazone caused a slower rise in SAA in synovial fluid in a horse with induced synovial sepsis (12).

Serum amyloid A in septic synovitis and in non-septic joint pathology

Synovial fluid SAA values were positively correlated with synovial fluid TNCC and serum SAA values in horses with naturally occurring synovial sepsis. Synovial fluid SAA values had moderate to high sensitivity of 80% and a specificity of 73% for the diagnosis of synovial contamination and sepsis, when a cut-off value of 1.14 µg/ml was used (20). Serum SAA values had a sensitivity of 82.4% and specificity of 88.9%, when a cut-off value of 60.7 µg/ml was used (20). Stack et al. reported a sensitivity of 75% and a specificity of 92%, when using a cut-off value of 132 µg/ml for SAA values in synovial fluid (25). Measuring both serum and synovial fluid SAA may improve sensitivity and specificity in diagnosing synovial contamination and sepsis (20). The cut-off values reported were markedly different from each other. The lower cut-off value used (1.14 µg/ml) overlapped values reported in synovial samples from joints with non-septic joint pathology (29.7 µg/ml) (20).

Reported values for SAA in naturally occurring septic synovitis cases ranged from 0-1400 mg/l in 7 horses and 0-368.9 µg/ml (median 39.2 µg/ml) in 38 horses (12,20). In two studies where septic synovitis was induced experimentally by lipopolysaccharide injections all horses had increased values of SAA in synovial fluid. Ludwig et al. reported SAA values in synovial fluid of 60-555 µg/ml (median 135 µg/ml) when measured by a handheld device and 144.3 ±114.5 µg/ml when measured by immunoturbidometric assay 36 hours after induced synovial sepsis in nine horses (15). Andreassen et al. reported peak values of SAA in synovial fluid of 50-900 mg/l (mean 220 mg/l) in 6 horses 48 hours after induced synovial sepsis (2). In the studies

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the SAA value in synovial fluid started rising at 16 hours with peak values at 48 hours (2) and 24 hours with peak values at 36 hours (15) after induction of synovial sepsis. In comparison peak values of TNCC in synovial fluid were reported at 8 hours post induction of synovial sepsis (2).

Non-septic joint pathology did increase synovial fluid SAA values in some horses. Robinson et al. reported a median value 0 µg/ml in 66 horses with non-septic joint pathology, however the range was 0-29.7 µg/ml. This group comprised of horses with osteoarthritis, osteochondrosis, intra-articular fracture and non-septic tenosynovitis. The number of horses that were above the cut-off value of 1.14 µg/ml used for septic synovitis in the same article was not recorded (20). Jacobsen et al. reported significantly higher SAA values in synovial fluid from horses with confirmed or suspected synovial sepsis compared to control horses and horses with non-septic joint pathology (only 4 of 13 horses with non-septic joint disease had values above the detection limit) (12).

Ludwig et al. also investigated serum SAA values in horses with lipopolysaccharide induced synovitis and did not find any rise in serum SAA (15), but when non-infectious arthritis was induced by intra-articular injection of amphotericin B in 24 horses SAA measured in serum did increase (7). In naturally occurring septic synovitis blood SAA values were higher than in control horses (20).

Synovial fluid serum amyloid A analysis using a hand-held point-of-care assay

Handheld devices for instant analysis of serum amyloid A are commercially available. The point-of-care test by Stablelab® is a proprietary, lateral-flow, membrane-based immunoassay. Semi-quantative and quantative measurements can be obtained from serum, heparinised blood, EDTA-anticoagulated whole blood and fresh nonanticoagulated whole blood, though serum is preferable. The working range for the device is 0-3000 µg/ml and the device provides acceptable accuracy and precision in equine serum/plasma up to 1000 µg/ml. Semiquantitative results are obtained by using a visual colour indicator compared with a reference card. Quantitative results are obtained in µg/ml by using a handheld reader. Results are obtained within 10 minutes (24).

Ludwig et al. successfully used the point-of-care handheld test to measure synovial fluid in healthy horses and horses with induced septic arthritis. A volume of 5 µl of synovial fluid was measured using the pipette provided with the device and mixed with the supplied test mix solution. Four drops of the diluted sample were dropped in the well of the test kit device and the result was read after 10 minutes. The test result was compared to a reference card and results obtained as between 0-15 µg/ml, 15-50 µg/ml, 50-200 µg/ml or 200-1000 µg/ml. After septic arthritis was induced, values of SAA in synovial fluid started rising above 15 µg/ml after 24-36 hours. There was good overall agreement between the handheld assay kit and the immunoturbidometric assay used more commonly for analysis of SAA in synovial fluid. In the same study serum SAA concentrations increased faster than synovial fluid SAA concentrations after induced septic synovitis. (15) Stack et al. compared results between another handheld device for SAA analysis (the EquiChekTM Analyser) and enzyme-linked immunosorbent assay (ELISA) test of SAA in inflamed septic and inflamed non-septic joints. Samples from 72 synovial structures of 62 horses were tested, 48 cases of inflamed non-septic joints and 24 cases of inflamed septic joints. This handheld device provided semi-quantitative results, with mild, moderate and marked inflammation as test results. The cut-off value considered positive for sepsis was moderate for the handheld device and 132 µg/ml for the ELISA test. Both tests showed the same sensitivity (75%) and specificity (92%) in differentiating septic from non-septic joints and excellent correlation was observed between tests. When results from structures sampled within 6 hours of onset of clinical signs

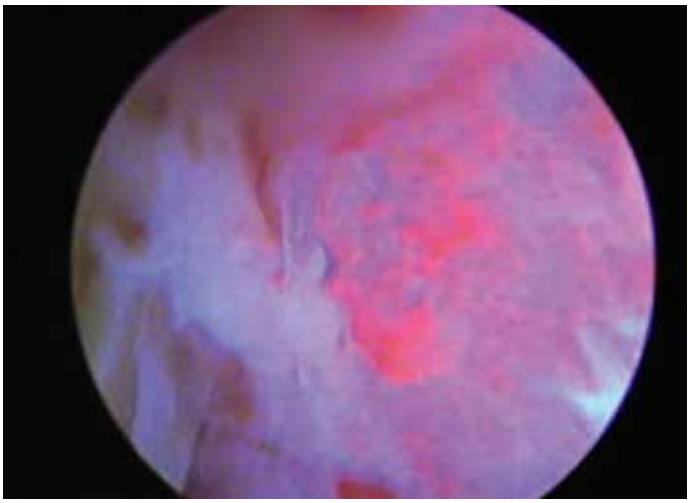
were excluded, sensitivity (84%) improved (25).

Discussion

The diagnosis of synovial sepsis is sometimes challenging with the diagnostic tools currently in use. The gold standard for diagnosis, a positive bacterial culture, is not sensitive enough nor fast enough to use in clinical settings. TNCC and TP as well as neutrophil percentage are used commonly for synovial fluid analysis. These results have to be interpreted according to when the injury happened, whether the synovial structure is recently punctured or medicated and whether injury has caused draining of synovial fluid from the structure (26). For veterinarians working in the field the diagnostic options are even more limited. The simplest way to confirm joint involvement by distending the joint with saline and checking for drainage from the wound is only diagnostic if the penetration tract is still open at the time of assessment. Total protein of synovial fluid can easily be measured by a handheld refractometer directly from synovia in a field situation. The total protein value has the limitations discussed above and seems to be less accurate than a TNCC (26,28). Measuring TNCC of synovial fluid is not practical in these situations, as it requires at least a smaller lab setting with a microscope. When synovial samples are submitted for analysis, results must be interpreted in light of storage time and temperature having an effect on TNCC and neutrophil percentage in synovial fluid (6). On-site diagnostic tools, such as handheld devices measuring SAA in synovial fluid, could help in reducing time to analysis and facilitate field diagnosis. Two commercially available hand-held devices validated for blood/serum SAA measurements were used successfully to measure synovial fluid SAA in two studies and showed excellent correlation with ELISA and immunoturbidometric assays (15, 25). Testing synovial fluid SAA was done in the same way blood samples were tested, only exchanging the blood sample for synovia (15,25). However, the studies in which septic synovitis was induced experimentally showed a slow response in synovial SAA concentrations, with values only rising between 16 and 24 hours after lipopolysaccharide injection (15, 2). Stack et al. showed improved sensitivity and specificity of synovial fluid SAA values when samples taken within 6 hours of the onset of clinical signs were excluded (25). In conclusion handheld devices can be used successfully to measure SAA in synovial fluid, but samples taken early after the injury has occurred or early after onset of clinical signs may be falsely negative.

Even with laboratory analysis available diagnosis of synovial sepsis may be difficult. Recent arthrocentesis and certain medications can cause values comparable to septic synovitis in absence of bacterial contamination (17, 23, 27). Recent injuries and puncturing wounds, where synovia is draining from the structure, can show falsely low values even in presence of bacterial contamination (16). Inflammatory and infectious conditions may sometimes be difficult to distinguish between, when measuring TNCC, TP and neutrophil percentage.

All the studies showed SAA to be a good marker of an inflammatory response in synovia (2,12,15,20,25) and also showed potential for differentiating between septic and non-septic joint pathology (20). In experimentally induced septic synovitis all horses showed an increase of SAA in the synovial fluid (2,15). In



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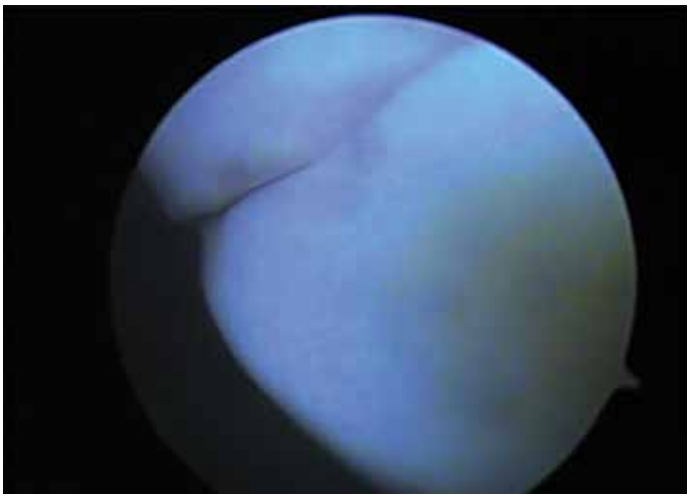

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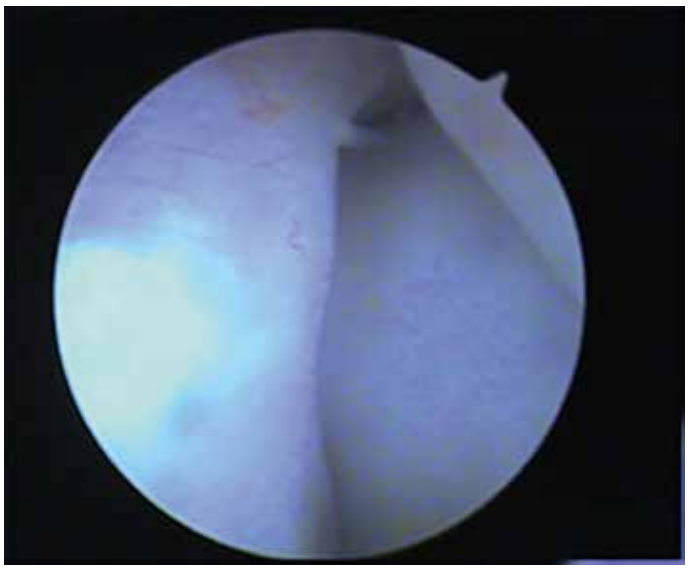

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studies where naturally occurring septic synovitis was studied, results of low SAA of synovial fluid in the presence of bacterial contamination were reported (12,20). This is most likely due to the reported slow rise in SAA values of synovial fluid after bacterial contamination. The slower rise of SAA in synovial fluid makes it less accurate than TNCC of synovial fluid for diagnosis of synovial sepsis in the acute stages of the disease.

Measuring SAA values of synovial fluid in addition to TNCC, total protein and neutrophil percentage may facilitate distinguishing septic from inflammatory conditions in cases where TNCC, TP and neutrophil counts are negative or only mildly increased. Jacobsen et al. reported two cases where horses with penetrating injuries into joints had normal values of TNCC, TP and neutrophil percentage in the synovial fluid, but an increase in SAA values. These horses then had a decrease in SAA values in response to treatment with joint lavage and systemic antimicrobials (12). Due to the low number of horses, further studies are needed to see whether SAA values in synovial fluid could be of value in cases where TNCC, TP and neutrophil counts are not diagnostic. This include diagnosing septic conditions in cases where penetrating injuries cause draining of synovial fluid from the synovial structure and when the structure has been recently medicated.

Different intra-articular medications may have effects on values measured in synovia. Some medications may cause a rise of TNCC, TP and neutrophil percentage in absence of bacterial contamination. Other medications may cause falsely low values of TNCC, TP and neutrophil percentage in a septic synovial structure. Jacobsen et al hypothesized that corticosteroid injections could cause falsely low SAA values in the same way that it can cause falsely low TNCC values in synovial fluid as one of the studied horses with septic arthritis showed a low synovial fluid SAA value 12 days after a corticosteroid injection (12). Other medications such as intra-articular amikacin, can cause a falsely high value of TNCC in synovial fluid, making it hard to distinguish septic conditions from merely inflammatory responses (17,23,27). In the study by Sanchez-Teran et al. the SAA value in synovial fluid of healthy horses was not affected by intra-articular medication with amikacin (23). Studies evaluating SAA response in synovial fluid following intra-articular medication with other commonly used substances would be of interest. Further research is needed to see whether SAA analysis of synovial fluid might aid in differentiating reactive inflammatory responses from septic conditions.

In a clinical setting measuring SAA in synovial fluid would have additional value in monitoring disease progress and response to treatment. TNCC, TP values can increase in response to common treatments such as arthrocentesis, intra-articular medication with amikacin, through-and-through lavage and arthroscopic joint lavage in healthy horses (23,22,21). These values might be elevated even though synovial sepsis is responding to treatment and using synovial TNCC, neutrophil percentage and TP for monitoring response to treatment especially after arthroscopic lavage is of limited value (4). SAA in healthy joints do not seem to increase in response to common treatments and it shows some evidence of decreasing from initial high values in response to treatment of septic synovitis. Therefore using synovial SAA analysis might be more valuable in evaluating response to treatment than traditional analysis of TNCC, TP and neutrophil percentage of synovial fluid only (21,22,23).

Summary

Synovial sepsis is commonly diagnosed by measuring total nucleated cell count (TNCC), total protein (TP) and neutrophil percentage of synovial fluid. Interpretation of these values may sometimes be difficult as they may be affected by common treatments, such as medications and lavage of synovial structures. Analysis of serum amyloid A (SAA), an acute phase protein, has been evaluated for use in diagnosing synovial sepsis in several studies. The purpose of this paper is to review whether there is enough information to warrant using SAA as an additional diagnostic tool when synovial fluid is analysed and if it has the potential to be developed into a tool for veterinarians in the field to determine joint involvement in penetrating injuries. Analysing SAA in synovial fluid in two studies showed a sensitivity of 80% and 75% and a specificity of 73% and 92% respectively in diagnosing synovial sepsis. However, the SAA values in synovial fluid only started rising between 16 and 24 hours after induced synovial sepsis. The slow rise of SAA in synovia after injury makes SAA analysis of synovial fluid alone too inaccurate for clinical use in acute cases of penetrating injuries. In addition to measuring TNCC, TP and neutrophil percentage in synovial fluid, SAA could be beneficial in diagnosis and monitoring of treatment response in synovial sepsis, as it was not affected by common treatments such as through-and-through lavage, joint lavage and repeated arthrocentesis and intra-articular medications with amikacin. In healthy horses, SAA values in synovial fluid were below detection limits, but further research is needed to establish cut-off values between septic and non-septic conditions of synovial structures. Commercially available handheld devices for SAA analysis of blood samples were used successfully for analysis of SAA in synovial fluid and may have a value in analysing synovial fluid in field situations.

Sammanfattning

Infektion i synoviala strukturer, det vill säga leder, senskidor och bursor, är en vanlig komplikation vid sårskador och intrasynoviala behandlingar på häst och kan vara livshotande. Snabb och tillförlitlig diagnostik av synovial infektion kan vara avgörande för korrekt behandling och för en god prognos.

För att fastställa en diagnos av infektion i en synovial struktur analyseras mängden leukocyter (TNCC), totalprotein (TP) och procentuella mängden neutrofiler i synovia. Bedömning av resultaten försvåras av att dessa värden kan påverkas av tidsintervallet mellan provtagning och skadetillfället eller om leden blivit injicerad, medicinerad eller spolad före provtagning. Även i fall där sårskador orsakat dränage av den synoviala strukturen kan leukocytmängd, totalprotein och neutrofilprocent vara missvisande. Analys av akutfasproteinet serum amyloid A (SAA) i synoviala prover har använts som inflammationsmarkör i ett flertal studier och visar potential för användning som komplement i diagnostiken av synovial infektion. Syftet med den här litteraturstudien är att sammanställa resultaten från dessa studier och utifrån detta avgöra om analys av SAA i synoviaprover kan förenkla diagnostiken i misstänkta kliniska fall av synovial infektion. Enkel och snabb diagnostik av SAA i ledvätskeprover skulle kunna förenkla beslut

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om sårskador behöver remitteras för behandling. Analys av SAA i synovia hade en sensitivitet på 80% och 75% och en specificitet på 73% och 92% som diagnostiskt test i kliniska fall av synovial infektion. I fall där synovial infektion inducerades experimentellt och SAA värdet i synovia följdes över en tidsperiod, ökade SAA värdet mellan 16 och 24 timmar efter infektionens induktion. Två olika kommersiellt tillgängliga snabbanalyser (Stablelab och EquiCheck Analyser) för SAA analys av serum användes med framgång för analys av synovia i två studier. Då SAA stiger långsamt i synovia är det som enda analysmetod för otillförlitligt i akuta sårskador och infektioner. Som komplettering till analys av leukocytmängd, totalprotein och procentuell neutrofil mängd kan SAA vara av nytta för diagnos och uppföljning av behandlingsresultat, då det inte påverkades av ledspolning, artroskopiskt ingrepp eller upprepade behandlingar med amikacin. Hos friska hästar låg SAA värdet under gränsen för detektion, men mer forskning krävs för att kunna särskilja mellan septisk och ickeseptisk synovial patologi. •

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