

Bacterial culture of septic synovial structures of horses: Does a positive bacterial culture influence prognosis?

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Summary

Reasons for performing study: The influence of synovial fluid culture on short- and long-term prognosis of cases with septic synovitis requires study.

Hypotheses: Horses with a positive bacterial culture from septic synovial fluid are less likely to survive or return to successful athletic function than those with a negative bacterial culture from septic synovial fluid.

Methods: Records of mature horses presented to 2 equine referral hospitals for investigation of suspected septic synovitis were examined. Horses (n = 206) were included in the study if synovial fluid was submitted for full laboratory examination, including bacterial culture. A diagnosis of septic synovitis was based on a nucleated cell count >30 x 10⁹ cells/l or >90% neutrophils and other clinical, cytological and bacteriological parameters. Long-term follow-up was obtained by telephone questionnaire. Univariate analysis, using the Fisher Exact Test, was used for all outcomes.

Results: Fourteen (20.9%) of 67 horses with a positive bacterial culture from synovial fluid were subjected to euthanasia because of persistent synovial sepsis compared to 2 (1.44%) of 139 with negative bacterial cultures (P<0.001). Overall survival and successful long-term return to function in horses with a positive bacterial culture was 50% (24/48 horses) compared to 70.5% (74/105) in culture negative horses (P = 0.01). In horses that survived to be discharged, successful long-term return to function was not significantly different between culture positive and culture negative groups. Growth of *Staphylococcus aureus* from synovial fluid did not affect short-term survival to discharge from the hospital compared to other positive bacterial culture; however, successful long-term return to function was only 30.4% (4/13) in horses from which *S. aureus* was cultured compared to 73.9% (17/23) of horses in which other bacteria were cultured (P = 0.015).

Conclusions and potential clinical relevance: Horses with a positive bacterial culture from a septic synovitis have a poorer prognosis for survival to discharge from hospital and overall long-term return to function than horses that yielded no bacterial growth. When *S. aureus* was cultured, the long-term prognosis was poorer.

Introduction

Synovial sepsis, of a joint, tendon sheath or bursa, are common, potentially career-ending and life-threatening problems in horses (van Pelt 1974; Morris 1980; Lugo and Gaughan 2006) and is a direct effect of the sequestration of pathogenic bacteria or their toxins in a synovial structure (McIlwraith 1983). Three routes by which synovial structures can become septic are: haematogenous spread, occurring most frequently in foals and rarely in mature horses (Martens *et al.* 1986); wounds, particularly puncture wounds, into or adjacent to the structure (McIlwraith 1983; Wright *et al.* 2003); and iatrogenic inoculation of the synovium by intrasynovial injection or from surgical invasion (Lapointe *et al.* 1992; Bertone 1996). Wounds are the most common cause of synovial sepsis in mature horses with introduction of foreign material, along with microorganisms, exacerbating the infection (Honnas *et al.* 1991; Schneider *et al.* 1992; Wereszka *et al.* 2007). The clinical signs of septic synovitis include heat, effusion and rapidly developing lameness.

The most common microorganisms cultured in a septic synovial structure are aerobes or facultative anaerobes and the most commonly cultured genus of bacteria is *Enterobacteriaceae*. The second and third most commonly cultured genera of bacteria are *Streptococcus* and *Staphylococcus* (Moore *et al.* 1992). The most common infective organism resulting from synoviocentesis is *Staphylococcus aureus* (Lapointe *et al.* 1992). Diarthrodial joints, tendon sheaths and bursae are lined by a mesenchymal synovium that produces and maintains a selective physical, cellular and biochemical environment within the synovial structure (Dyce *et al.* 1996), and has mechanisms that prevent or control proliferation and colonisation of infective microorganisms (Bertone 1996). The factors that determine whether or not a synovial structure can withstand an inoculation of microorganisms include: the pathogenicity, virulence and numbers of organisms involved (Schneider *et al.* 1992; Bertone 1996); the presence of foreign material, other than microorganisms (Reginato *et al.* 1990); and the immunological status of the animal (Martens *et al.* 1986). The pathophysiology of septic synovitis has been well described and includes cartilage degradation and synovial ischaemia, fibrin deposition leading to pannus formation and adhesion formation (McIlwraith 1983; Bertone 1996; Frees *et al.* 2002; McIlwraith

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2002; Bertone 2003; Morton 2005). The objective of the present study was to investigate the influence of synovial fluid culture results on both the short- and long-term prognosis for horses treated for septic synovitis.

Materials and methods

Database

The medical records of all horses suspected of having synovial sepsis admitted to 2 equine referral hospitals (Royal Veterinary College Equine Referral Hospital: Hospital 1 and Bell Equine Veterinary Clinic: Hospital 2) over a 7 and 10 year period (1993–2006) were examined. Horses excluded from the study included foals aged <6 months and horses from which synovial fluid was not submitted for laboratory analysis, including bacterial culture.

Data extracted from the case records included: age, breed and sex; use at time of admission; suspected cause of infection; synovial structure infected; duration of clinical signs; synovial nucleated cell count (NCC) at the time of admission; results of bacterial culture; treatments performed; and whether or not the horse was discharged from the hospital. Synovial fluid was examined grossly for turbidity, viscosity and the presence of erythrocytes and submitted for NCC, cytological examination, determination of concentration of total protein and bacterial culture.

Diagnosis

Synovial sepsis was diagnosed if the horse had an NCC >30 x 10⁹ nucleated cells/l or >90% neutrophils, regardless of the total NCC, and 2 or more of the following: lameness in the affected limb, increased heat in the affected region and/or effusion of the affected synovial structure, bacteria seen during cytological examination of Gram-stained synovial fluid and concentration of total protein >30 g/l.

Sample collection and processing

Synovial fluid was collected under strict aseptic conditions at a site remote from the suspected synovial penetration. Synovial samples were collected into EDTA tubes for cytology, NCC and determination of concentration of total protein. Hospital 1 collected synovial fluid into sterile, plain tubes for aerobic and anaerobic bacterial culture at an on-site commercial bacteriology laboratory, using MacConkey, blood and chocolate agars as culture media. At Hospital 2, synovial fluid was placed in a sterile plain tube and, in addition, a sterile Amies bacteriology swab of the synovial fluid was taken. These samples were posted to an external commercial bacteriology laboratory for aerobic and anaerobic culture using the same methodology but after 24 h in a nutrient broth; therefore the second clinic had culture results at least 24 h later than the first clinic. If bacterial colonies were observed after 24 h of culture, testing for antimicrobial sensitivity was performed.

Treatment

The standard treatment protocol for each horse consisted of surgical lavage of the synovial structure with sterile isotonic solution with further treatment such as instillation of gentamicin (1000 mg), sodium benzyl penicillin (5 x 10⁶) or amikacin (250–500 mg) into

the synovial cavity just prior to closure at the surgeons discretion. Each horse was also treated empirically with systemic procaine penicillin (22,000 iu/kg bwt i.m. q. 12 h) or sodium benzyl penicillin (20,000 iu/kg bwt i.v. q. 8 h) and gentamicin 6.6 mg/kg bwt i.v. q. 24 h) for 2 to 9 days (median 7 days). Flunixin meglumine (1.1 mg/kg bwt i.v.), or phenylbutazone (2.2 mg/kg bwt) was given perioperatively to all horses for the first 24 h, with 50% of horses requiring repeated nonsteroidal anti-inflammatory (NSAID) treatment for a further 2–10 days (median 3 days) with the remaining 50% receiving no further NSAID.

Clinical progression for each horse was assessed on a daily basis and, if improving, no change to the standard treatment protocol was made. A change to oral potentiated sulphonamides (5 mg/kg bwt trimethoprim and 25 mg/kg bwt sulphadiazine *per os* q. 12 h) was based on clinical progression, surgeon preference and synoviocentesis culture results, prior to the horse being discharged from hospital. If clinical progression was not acceptable and a positive bacterial culture had been obtained, then culture and sensitivity results were considered when the alteration of antibiotic treatment was made. Further synoviocentesis and additional surgical lavage would be performed when appropriate.

Groups

Horses were divided into 2 groups: those horses from which bacteria were cultured from the synovial fluid and those from which no bacterial growth was yielded. Short-term outcomes evaluated included: survival to discharge from hospital and influence of culture of *S. aureus* from synovial fluid on survival and discharge from hospital. A successful long-term outcome was defined as a horse that, in the owner or carer's opinion, was performing at or above the level of performance that it had achieved prior to sustaining sepsis of the synovial structure.

Statistics

Fisher Exact Tests were performed on discrete data to analyse the association between culture of bacteria from synovial fluid and both the short- and long-term outcomes for horses treated for synovial sepsis. The results were considered significant if the P value was <0.05; odds ratio (OR) and confidence interval (CI) were reported for each significant P value.

Results

Database

The criteria for inclusion in the study were met by 206 horses. Of these, 198 horses were presented with sepsis in a single synovial structure and 8 with suspected involvement of 2 separate synovial structures. Of these 214 septic synovial structures, 10 were bursae, 41 were tendon sheaths and 163 were joints. Sixty-nine samples, from 67 horses, yielded a positive bacterial culture; whereas 145 samples, from 139 horses, yielded no bacterial growth.

The predominant cause of synovial sepsis was a wound that penetrated the synovial structure, and of the 155 (75.2%) synovial structures that became septic due to a wound, 51 (32.9%) yielded a positive bacterial culture. The most common site affected was the tarsocrural joint (22.4%) followed by the metacarpo/metatarsophalangeal joint (20.1%) and the digital flexor tendon sheath (18.7%) (Table 1).

A synovial structure of 13 horses became septic due to iatrogenic causes, of which 9 (69.2%) were due to intrasynovial injection, the remainder occurring subsequent to surgery. Only one of these 9 horses (11.1%) that developed synovial sepsis subsequent to intrasynovial injection yielded a positive bacterial growth. Of the 4 horses that developed infection of a synovial structure subsequent to surgical intervention, 3 (75%) yielded positive bacterial culture.

Survival

Of the 67 horses that yielded a positive bacterial culture, 50 (74.6%) survived to be discharged from the hospital, 14 (20.9%) were subjected to euthanasia due to failure to resolve the sepsis, one horse (1.5%) was subjected to euthanasia due to financial reasons and 2 horses (3.0%) due to concomitant injuries. Of the 139 horses that yielded no bacterial growth, 135 (97.1%) survived to be discharged from the hospital, 2 (1.44%) were subjected to euthanasia, due to failure to resolve the synovial sepsis, and 2 (1.4%) were subjected to euthanasia due to concomitant injuries. Those horses that yielded a positive bacterial culture from their synovial fluid were significantly more likely to be subjected to euthanasia compared to those horses where no bacteria were cultured from the synovial fluid ($P < 0.001$; OR 18.9; CI 4.15–86.13). Furthermore, culture of *S. aureus* was 29.5 times more likely to result in euthanasia than negative culture ($P < 0.001$; CI 5.65–154.5) while culture of another bacterial genus was 13.9 times more likely to result in euthanasia compared to negative culture ($P < 0.001$; CI 2.76–69.93).

Therapy

Of the 206 horses in the study, 173 (84%) received antimicrobial therapy prior to synovial fluid being aspirated for bacterial culture. Fifty-two (77.6%) horses that yielded a positive bacterial culture, and 121 (87.1%) with a negative bacterial culture were administered systemic antimicrobial therapy prior to aspiration of synovial fluid. Administration of systemic antimicrobial drugs prior to synoviocentesis was not significantly different between

culture positive and culture negative groups and unlikely to have decreased the likelihood of positive culture ($P = 0.06$).

Time to first treatment was based on the history provided by the owner. For horses with a culture positive septic synovitis the median time to first treatment was 5 days, for horses discharged from hospital (range 0.2–56 days) and 4.5 days for those subjected to euthanasia due to sepsis (range 0.1–14 days) and was not known in 9 horses. Median time to first treatment for horses with a culture negative septic synovitis was one day for those discharged from hospital (range 0.1–42 days) and 4 days for those subjected to euthanasia due to sepsis (range 1–7 days) and was not known in 18 horses. Time to first treatment was not significantly different between culture positive and culture negative groups ($P = 0.29$).

Follow-up

Long-term follow-up was obtained for 141 horses (68.4%). Of the horses that yielded a positive bacterial culture, 14 (28%) were lost to follow-up and 36 (72%) horses followed: of these 2 (5.6%) were subjected to euthanasia due to unrelated conditions, 2 (5.6%) were subjected to euthanasia due to conditions related to the septic synovial structure, 8 (22.2%) were considered to be reduced performers and 24 (66.7%) were considered to have returned to either their previous or a higher level of function. Of the horses with a negative bacterial culture, 30 (22.2%) were lost to follow-up, and long-term follow-up was available for 105 (77.8%) horses. Of these, 2 (1.9%) were subjected to euthanasia due to unrelated conditions, 3 (2.9%) were subjected to euthanasia due to conditions related to the septic synovial structure, 26 (24.7%) were considered to have poorer performance and 74 (70.5%) were considered to have returned to at least their previous level of function.

Discounting horses lost to follow-up and those subjected to euthanasia for unrelated conditions, the number of horses with an unfavourable outcome was 50% (24 of 48) for horses with a culture positive septic synovitis compared to 29.5% (31 of 105) for horses with a culture negative septic synovitis. The overall short and long-term survival and successful return to function was significantly poorer in horses with a culture positive septic

TABLE 1: Aetiology and synovial structure infected in 206 horses with a septic synovitis

	Wound		Injection		Surgery		Not known		Multiple		Totals	
	+C	NC	+C	NC	+C	NC	+C	NC	+C	NC	+C	NC
DIP	3	4	0	0	0	0	4	2	0	BCG	7	9
Navicular bursa	0	5	0	0	0	0	0	1	0	BC	0	8
PIP	5	5	0	0	0	0	0	3	0	0	5	8
MtC/MtT phalangeal joint	7	24	0	2	0	0	2	3	A	DDEH	10	33
Digital tendon sheath	6	22	0	2	0	0	3	2	FF	EGH	11	29
Carpus	1	11	0	2	1	1	1	2	0	0	3	16
Elbow	3	5	0	0	0	0	0	1	0	0	3	6
Shoulder	0	1	0	0	0	0	0	0	0	0	0	1
Tarsocrural joint	19	20	1	1	0	0	0	6	A	0	21	27
Tarsal sheath	0	0	0	0	1	0	0	0	0	0	1	0
Calcaneal bursa	2	0	0	0	0	0	0	0	0	0	2	0
Stifle	5	7	0	1	1	0	0	0	0	0	6	8
Coxofemoral	0	0	0	0	0	0	0	0	0	0	0	0
Totals	51	104	1	8	3	1	10	20	4	12	69	145

Number of synovial structures affected and cause +C = bacteria cultured, NC = bacteria not cultured. In multiple infections the letter indicates which synovial structures were affected in each case: e.g. Horse A had synovial sepsis in a metacarpo/metatarsophalangeal joint and a tarsocrural joint, both of which were culture positive with *Enterobacter*; Horse F had synovial sepsis in a fore and hind digital flexor tendon sheath, both of which cultured positive for *Bacillus*; all other horses did not yield a positive culture.

synovitis ($P = 0.01$; OR 0.42; CI 0.20–0.85). If a horse survived to be discharged from hospital, however, the long-term return to successful function was not significantly different between culture positive and culture negative groups ($P = 0.41$). Time of follow-up ranged from 6 months–3.5 years (median 18 months).

Culture

Staphylococcus aureus was cultured from the synovial fluid in 23 horses (34.3% of all positive cultures) and of these, 16 (69.6%) were discharged from the hospital, and 7 (30.4%) were subjected to euthanasia because of failure to resolve the synovial sepsis. Other bacterial genera were cultured from a septic synovial structure from 44 horses (Table 2). Of these, 34 (77.3%) were discharged from hospital, 7 (15.9%) were subjected to euthanasia because of synovial infection and 3 (6.8%) were subjected to euthanasia for previously described reasons. Culture of *S. aureus*, when compared to culture of other bacterial species, was not found to affect the likelihood of survival to discharge from hospital ($P = 0.18$).

Of the horses that yielded a positive bacterial culture, long-term follow-up data were available for 13 of 16 horses that had *S. aureus* sepsis and 23 of 34 horses that yielded other bacterial isolates. Of the 13 cases of *S. aureus* sepsis, only 4 (30.8%) returned to their former level of activity compared to 73.9% of those with non *S. aureus* sepsis. Culture of *S. aureus* from a synovial structure significantly increased the likelihood of a horse being unable to return to its previous level of performance ($P = 0.015$; OR 6.38; CI 1.42–28.6).

Hospital comparisons

Short-term survival was not significantly different between the hospitals with 16 of 124 horses (12.9%) being subjected to

TABLE 2: Bacteria cultured from the synovial fluid of 206 horses with septic synovitis

Bacteria cultured	Total	Hospital 1	Hospital 2	Percentage
<i>Acinetobacter</i>	2	1	1	2.9
<i>Actinobacillus</i>	3	0	3	4.5
<i>Bacillus</i>	3	1	2	4.5
Cocci (unidentified)	1	1	0	1.5
<i>Corynebacterium</i>	1	0	1	1.5
<i>Escherichia coli</i>	3	1	2	4.5
<i>Enterobacter</i>	2	0	2	2.9
<i>Enterococcus</i>	5	0	5	7.5
Mixed growth	7	6	1	10.5
<i>Proteus</i>	1	1	0	1.5
<i>Pseudomonas</i>	2	0	2	2.9
<i>Rhodococcus equi</i>	1	1	0	1.5
Rods (unidentified)	1	1	0	1.5
<i>Staphylococcus aureus</i>	23	3	20	34.3
<i>Staph</i> spp. (coag neg)	5	5	0	7.5
<i>Streptococcus</i>	7	0	7	10.5
Totals	67	21	46	67
% Cultured		31.3	68.7	100

Mixed growth culture results:

1. *Escherichia coli* and *Enterococcus faecalis*.
2. *Escherichia coli* and coagulase negative *Staphylococcus*.
3. *Enterococcus faecalis* and *Clostridium* species.
4. *Staphylococcus aureus* and *Streptococcus dysgalactiae equisimilis*.
5. *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis*.
6. *Staphylococcus aureus* and *Candida*.
7. Coagulase negative *Staphylococcus* (x 2).

euthanasia in Hospital 2 compared to 5 of 82 horses (6.1%) ($P = 0.086$). Long-term return to function was also not significantly different between hospitals. Where follow-up was available, 56 of 81 horses discharged from one Hospital 2 returned to at least the same level of function as before surgery compared to 42 of 60 horses at Hospital 1 ($P = 0.53$). The ability to culture bacteria was, however, significantly different between the 2 laboratories, with one returning a positive culture from 46 of 119 horses (38.7%) compared to 21 of 87 horses (24.1%) at ($P = 0.019$; OR 1.98; CI 1.07–3.66).

Discussion

Of the 206 horses in the study, 185 (89.8%) survived to be discharged from the hospital, comparable to previously reported case series of septic synovitis (Schneider *et al.* 1992; Wright *et al.* 2003). However, unlike the study by Schneider *et al.* (1992), where culture of bacteria from a septic synovial structure did not affect the likelihood of survival to discharge from the hospital, this study found that a positive bacterial culture significantly decreased the likelihood of survival. There are also differing reports in the human literature regarding prognosis in culture positive and culture negative children with septic synovitis. Chang *et al.* (2005) found that children with a culture positive synovial fluid finding had significantly increased morbidity compared to children with a culture negative synovial finding whereas Lyon and Evanich (1999) demonstrated a nonsignificant increase in morbidity in patients with a positive bacterial culture from a synovial structure compared to patients with a negative bacterial culture.

Of the horses discharged from the hospital, 69.5% returned to their previous level or better of function, a percentage that falls between those described by Schneider *et al.* 1992 (56.5%) and Wright *et al.* 2003 (81%). Of the horses with a positive synovial culture, 66.7% returned to function compared to 70.5% of horses where no growth was yielded from their synovial fluid ($P = 0.41$). If, however, the septic synovial structure yielded a positive bacterial culture of *S. aureus* these horses were significantly less likely to return to their previous level of performance than horses where other bacterial isolates were found, although culture of *S. aureus* did not alter the likelihood of short-term survival to discharge compared to culture of other microorganisms. A similar outcome has been found in the medical literature: <50% of human subjects recovered without serious articular cartilage damage after *S. aureus* infection whereas recovery rates after infection with non-*Staphylococcal* bacteria were 75% (Goldenberg and Reed 1985). Treatment of a pre-existing condition may affect long-term outcome in horses in which synovial sepsis is caused through synovial injection or surgery. *S. aureus* was the most common bacterial isolate cultured from septic synovial structures after joint injections or surgery in previous case series (McIlwraith 1983; Schneider *et al.* 1992). However, in the present study, only 2 of the 13 cases of iatrogenic sepsis yielded *S. aureus* with 9 yielding no culture. This study found no correlation between synovial injection or surgery and culture of *S. aureus* and return to function.

A lack of positive culture does not mean the synovial structure is not infected (Morris 1980; Orsini 1984) and this has also been described in human studies of septic synovitis where clinical signs and cytology, other than bacteriology, are insignificantly different between culture positive and culture negative patients. The reported success rate for a positive culture from synovial fluid is

highly variable (Lavoie *et al.* 1991; Madison *et al.* 1991; Honnas *et al.* 1992; Schneider *et al.* 1992) and it is recognised that culture of synovial fluid is difficult because of a number of potential factors, such as the sequestration of the bacterium into the synovial membrane (McIlwraith 1983) and the bactericidal qualities of the synovial fluid (DeGara 1943; Gruber *et al.* 2008).

In the present study, successful bacterial cultures were achieved in 32.5% of cases, which is within the range of culture success rates previously reported where 22–74.1% of synovial samples submitted yielded a positive bacterial culture (Lavoie *et al.* 1991; Madison *et al.* 1991; Honnas *et al.* 1992; Schneider *et al.* 1992). In a study of human septic arthritis, Weston *et al.* (1999) achieved a culture success rate of 67% using standard culture and 73% if blood culture bottles were used. However, Lyon and Evanich (1999) reported only a 34% culture success rate in children using a combination of standard plates, enrichment broth, blood culture bottles and blood culture. A study in greyhounds with known synovial sepsis showed that bacterial culture from synovial fluid, synovial membrane or blood culture medium that had been incubated for less than one hour, was significantly less reliable when compared to synovial fluid samples incubated for 24 h in blood culture medium (Montgomery *et al.* 1989). In the present study, a higher culture success rate was obtained by one hospital that used an enrichment broth prior to culture, despite having to post samples away; there was, however, no effect on overall outcome by the delay in bacterial culture. A higher bacterial culture success rate may have been achievable if an enrichment broth had been used by both hospitals prior to microbial culture or if blood culture bottles had been used for synovial sample collection.

Administration of an antimicrobial drug before aspiration of synovial fluid has been postulated to decrease the likelihood of obtaining a positive bacterial culture (Ince *et al.* 2004) and this could be inferred as contributing to the relatively low culture success rate obtained in the present study. However, administration of one or more antimicrobial drugs to horses prior to aspiration of synovial fluid did not affect the ability to culture bacteria from the sample. Ghanem *et al.* (2007) also found that preoperative antibiotic therapy did not interfere with isolation of bacteria from synovial samples in human patients.

Some studies have found that duration of clinical signs prior to treatment was associated with a poorer prognosis (Baxter 1987; Gibson *et al.* 1989) and in some human studies this is the most important prognostic indicator (Bennett and Namnyak 1992). However, in the present study, duration of clinical signs prior to treatment was not a significant factor in either survival to discharge from hospital or in long-term return to function, a perhaps surprising finding as longer-term synovial sepsis is likely to result in greater intrasynovial damage. This lack of association between duration of clinical signs prior to treatment and outcome has also been described by Frees *et al.* (2002) and Wright *et al.* (2003) and is therefore less important perhaps in overall prognosis than a confirmed bacterial presence within the synovial structure, in particular *S. aureus*.

The present study found that a positive bacterial culture had a negative effect on short-term survival to discharge from hospital, overall long-term prognosis and, where *S. aureus* was cultured, long-term return to function. However, resolution of synovial sepsis is a multifactorial issue with conflicting data regarding many of the factors involved and, therefore, the use of positive bacterial culture as a major prognostic indicator in synovial sepsis is not warranted without further study.

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