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Evaluation of an automated blood culture system for the isolation of bacteria from equine synovial fluid

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ABSTRACT

The objective of this study was to evaluate an automated blood culture system for the isolation of microorganisms from infected equine synovial fluid (SF). Samples were collected from 220 severely inflamed synovial joints and classified as either presumably infected (group A: $n = 149$) or not infected (group B: $n = 71$), based on a combination of clinical history, clinical signs and cytological analysis of the SF. Samples were inoculated into blood culture bottles and after incubation were subcultured onto agar media to confirm the results and to facilitate full bacterial identification. Microorganisms were isolated from 107 group A samples (71.8%) and from three group B samples (4.2%). Overall, the detection system identified 117 bottles as positive and 103 as negative, including nine instrument-false-positives and two instrument-false-negatives. The median time-to-detection for Gram-positive bacteria, Gram-negative bacteria, and for fungi was 14.3 (interquartile range [I.R.] 10.0) h, 8.8 (I.R. 12.8) h, and 72.0 (range 60.8–74.8) h, respectively. It was concluded that culture of infected SF using the automated system combines the advantages of enrichment in specialised medium with the rapid detection of bacterial growth.

Introduction

Synovial infection is a serious and relatively common condition in horses that occurs when microorganisms colonise a synovial joint, tendon sheath or bursa following a penetrating wound, injection, surgery or haematogenous spread of bacteria (Schneider, 2006). Isolation of microorganisms from the synovial fluid (SF) is essential to confirm an aetiological cause and to allow antimicrobial susceptibility testing. Both detection of the bacterial cause and the results of antimicrobial susceptibility testing should be rapidly available to the clinician to facilitate decisions on the most appropriate treatment and antimicrobial agent. However, isolation of microorganisms from infected SF of horses is challenging as the concentration of viable microorganisms is often low and phagocytosed microorganisms cannot be recovered by routine culture (Hughes et al., 2001). Furthermore, successful culture may be affected by intrinsic inhibitors present in SF and by previous antibiotic administration (Hughes et al., 2001). As a consequence, negative culture results have been reported in up to 45% (Madison et al., 1991) and, more recently, in 72% (Payne et al., 2007) of horses suspected of having a synovial infection.

Previous research has illustrated the advantages of using blood over conventional culture methods to isolate microorganisms from SF in humans (von Essen, 1997; Yagupsky and Press, 1997), dogs (Montgomery et al., 1989) and horses (Pille et al., 2007). In the latter study, the use of a manual blood culture system (BD BBL Septi–Chek blood culture bottles, Becton Dickinson) had a high sensitivity (77.6%) for the detection of synovial bacteria relative to conventional culture on agar plates (37.8%) and infection was never detected by plate isolation without incubation in blood culture medium. Compared to plate culture, enrichment in liquid media offers the advantages of a larger volume of inoculum together with the dilution of growth inhibitors. Furthermore, commercial blood culture media contain resins and lytic agents to inactivate inhibitors and release phagocytosed organisms, respectively (Hughes et al., 2001).

In humans, automated blood culture systems have been used successfully in the isolation of bacteria from normally sterile body fluids, including SF using paediatric bottles (BACTEC Peds Plus/F bottle, Becton Dickinson) incubated in a BACTEC system (BACTEC 9000 series, Becton Dickinson) (Yagupsky et al., 1992, 2001; Fuller and Davis, 1997; Hughes et al., 2001; Akcam et al., 2006). The BACTEC system is an automated blood culture system, where the detection of bacterial growth is based on monitoring the carbon dioxide (CO_2) produced (Fig. 1). Any increase in CO_2 production creates changes in the output of a fluorochrome-sensor located

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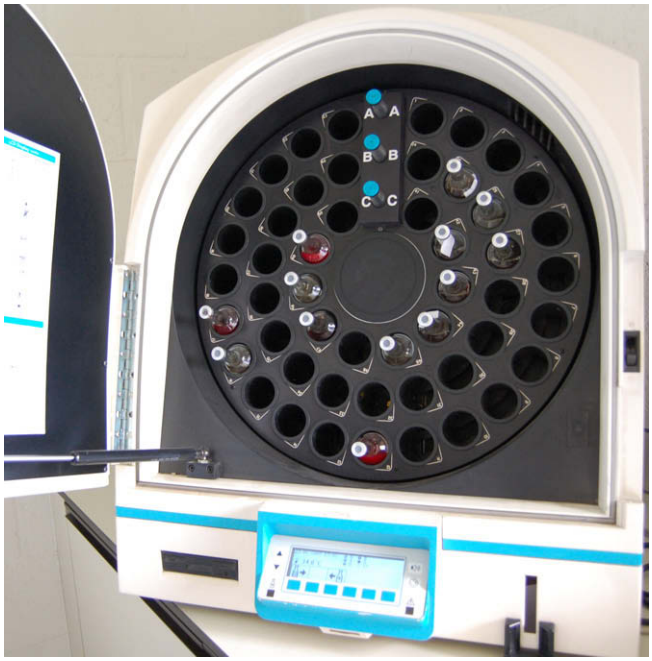

Fig. 1. The BACTEC 9050 automatic blood culture system accommodates up to 50 blood culture bottles and serves as an incubator, agitator and detection system.

at the bottom of each bottle (Fig. 2). Using computer algorithms, cultures are recognised by the system as positive based on an increasing rate of change as well as on a sustained increase in CO_2 production. Advantages of automated systems over manual systems, include full automation once the bottles are loaded, considerable labour savings and, most importantly, a shortened time-to-detection (TTD) of organism growth. However, in a small percentage of cases, subculturing the bottles reveals no growth despite a positive signal (instrument-false-positives), or growth may be present on subculture while the system gives no signal (instrument-false-negatives) (Nolte et al., 1993).

Fig. 2. Examples of BACTEC Peds Plus/F bottles. The resin granules can be observed in the medium as well as the fluorochrome-sensor at the bottom of the bottle.

The objective of the present study was to evaluate the BACTEC 9050 automated blood culture system for the isolation of microorganisms from presumably infected equine SF samples and to determine the reliability and speed of detection of this methodology.

Materials and methods

Between March 2005 and April 2007, SF specimens were collected from all horses admitted to the Ghent University teaching hospital with severe synovial inflammation. Only samples obtained on initial animal admission were included in the study and no follow-up samples were taken. A synovial infection was presumed if more than three of the following criteria were met: lameness; joint distension; a SF white cell count (WCC) >30 $\times$ 10^9 cells/L; a differential WCC with >80% neutrophils; a SF total protein concentration >40 g/L; and predisposing circumstances such as septicaemia (in foals), recent surgery, recent injection of the synovial cavity, or penetrating synovial injuries (Pille et al., 2007). Synovial cavities with draining, penetrating wounds existing for >7 days but with insufficient amounts of contained SF available for cytology and determination of total protein concentrations were also considered presumably infected (Pille et al., 2007).

SF was obtained by routine synoviocentesis and aspiration using aseptic techniques. Samples of 1–2 mL of fluid were inoculated into BACTEC Peds Plus/F blood culture bottles which were immediately placed in the BACTEC 9050 blood culture system. All bottles were incubated until microbial growth was detected by the automated system or otherwise for maximum of 14 days. The TTD for instrument-positive bottles was automatically recorded by the system.

All instrument-positive and negative bottles were subcultured onto agar media after bacterial growth was detected by the system or after 14 days as outlined above. The media used were Columbia agar with 5% defibrinated sheep blood (Oxoid) and Columbia agar with 5% defibrinated sheep blood supplemented with colistin and nalidixic acid (CNA agar, Oxoid). Agar plates were incubated at 37 °C in 5% CO_2. Microorganisms were identified to the species level according to standard bacteriological or mycological methods, including evaluation by Gram-stain, colonial morphology and biochemical characteristics (Quinn et al., 1999). If these methods were insufficient, identification by tDNA intergenic spacer PCR was applied (De Baere, 2004).

The influence of antibiotic treatment of animals within 24 h prior to sampling on culture positivity rate and on the TTD was assessed using the Pearson chi-square and non-parametric Mann–Whitney rank sum tests respectively. Data were collated and prepared for statistical analysis using spreadsheet software (Microsoft Office Excel 2003) and statistical analysis was performed using SPSS 15.0 with statistical significance set at $P < 0.05$. Time-to-detection data are presented as median values and interquartile ranges.

Results

During the period of study, a total of 220 SF samples were obtained from 189 horses. Based on the defined criteria, infection was presumed in 149 samples (group A) and was considered unlikely in the remaining 71 samples (group B). Of the 149 group A samples, 67 were obtained from horses treated with antibiotics within 24 h prior to sampling. The group A SF WCC averaged 75.3 $\times$ 10^9 cells/L (SD 68.6 $\times$ 10^9) with 80.6% (SD 12.8) neutrophils and 46.7 g/L (SD 12.3) total protein compared to group B samples which had a mean WCC of 5.8 $\times$ 10^9 cells/L (SD 8.0 $\times$ 10^9) with 39.9% (SD 32.6) neutrophils and 23.7 g/L (SD 8.4) total protein.

Microorganisms were isolated from 107/149 (71.8%) group A samples and from 3/71 (4.2%) group B samples making a total of 110 positive and 110 negative samples. The three bacteria cultured from the group B samples were as follows: 1 *Acinetobacter* spp., 1 *Aureobacterium* spp. and 1 (not fully identified) Gram-positive organism. The total number and number per group of the different microorganisms are summarised in Table 1. Overall, 25 different bacterial species, two *Candida albicans* isolates and one *Fusarium* species were identified. Twelve bacterial isolates could not be fully identified – 11 in group A and 1 in group B. Growth from 20 samples was considered polymicrobial and in 18 of these isolation and speciation of the different microorganisms was possible. For horses with presumed infected joints that had been treated with antibiotics within 24 h prior to sampling, 76.1% of samples were culture positive, compared to the 68.3% culture positive samples from