Assessing techniques for disinfecting sites for inserting intravenous catheters into the jugular veins of horses

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The sites of insertion of catheters into the jugular veins of six horses were investigated to determine common isolates and to assess the effectiveness of two disinfection protocols with the hair coat left long, clipped or shaved. Skin commensals (Staphylococcus, Streptococcus and Micrococcus species) and environmental contaminants (Bacillus, Enterobacteriaceae, Aspergillus and Mucor species) were the microorganisms most frequently isolated. Chlorhexidine gluconate and povidone-iodine-based skin disinfection protocols resulted in significant reductions in the number of bacterial isolates from clipped sites. With chlorhexidine, there were no significant differences between the reductions observed at sites with the hair coat left long, clipped or shaved.

INTRA VENOUS catheters provide continuous, secure venous access for the administration of therapeutic agents to, and the collection of blood samples from, horses. However, they can be associated with undesirable complications such as thrombosis, septic thrombophlebitis and septicemia (Deem 1981, Bayly and Vale 1982). Microorganisms on the skin are sources of contamination of intravenous catheters in humans (Elliot and others 1997) and horses (Ettlinger and others 1992) and a correlation has been observed in people between the number of colony-forming units (cfu) of bacteria on swabs taken from skin over the sites of insertion of catheters and the bacterial colonisation of the catheters (Bjornson and others 1982). In people, bacteria from the skin and the catheter hub are the most common sources of colonisation of catheters (Lipton and others 1985) reported that 70 per cent of the organisms causing catheter-related septicemia originated at the catheter hub, and Elliot and others (1997) reported that all the organisms isolated from the tips of catheters within an hour of their insertion originated from the skin of the patient, despite the skin having been disinfected before the catheter was inserted. Inadequate disinfection of the skin before the insertion of an intravenous catheter has been shown to significantly increase the rates of catheter infection in cattle (Pusterla and Braun 1996), dogs (Burrows 1982) and human beings (Smallman and others 1980).

The colonisation of an intravenous catheter by bacteria can result in damage to endothelial cells and platelets, activation of clotting cascades and initiate thrombus formation (Sellon 2004). Bacteria may also migrate from the skin to colonise an existing thrombus or perivascular tissues, and initiate septic thrombophlebitis, septicemia or perivascular abscessation (Gardner and others 1991). These conditions require medical treatment with appropriate antimicrobial drugs and, occasionally, surgical intervention to improve drainage or replace occluded jugular veins (Stein and Pruitt 1970, Wiemer and others 2005), increasing the cost of hospitalisation.

Disinfection techniques at the sites of intravenous catheters have been studied in human beings and dogs, either by comparing a disinfected group with an untreated group (Smallman and others 1980, Coolman and others 1998), or by comparing the efficacy of different disinfectants (Maki and others 1991, Dorey-Phillips and Murison 2008). These studies have resulted in recommendations for the preparation of catheter sites. However, no such studies have been made in horses and there have been few studies of skin disinfection at other sites. Hague and others (1997) reported a significant reduction in numbers of bacterial cfu after the disinfection of clipped and unclipped skin over the intercarpal joint of horses with povidone-iodine and 70 per cent alcohol, and Zubrod and others (2004) found that four different disinfectant techniques based on povidone-iodine were equally effective at reducing cfu numbers from the skin overlying the distal interphalangeal joint. Galuppo and others (1999) observed that povidone-iodine based disinfectants significantly reduced bacterial numbers on the skin of the ventral abdomen of horses undergoing coeliotomy.

More information about the bacterial populations at the sites of insertion of venous catheters and the efficacy of disinfection techniques is required to reduce the likelihood of catheter-related infection in horses. The aims of this study were to investigate the microbial flora of the skin qualitatively and quantitatively, compare the effectiveness of commonly used disinfection techniques, and assess the effect on their effectiveness of clipping and shaving the hair coat before the sites were disinfected.

The studies were approved by the Animal Ethics and Welfare Committee of the Faculty of Veterinary Medicine, University of Glasgow.

Materials and methods
Five clinically normal female horses were used. They ranged in age from 14 to 29 years (mean 19 years). A visual inspection, palpation...
and ultrasonographic investigation of the external jugular veins and overlying skin of each horse revealed no abnormalities. They were housed in adjacent stalls in a single building and kept under identical conditions.

Sample collection
Two adjacent sample sites were selected over each of the left and right external jugular veins of each horse resulting in a total of 20 sites, chosen to represent typical sites of catheterisation. Lines were clipped into the hair coat dorsal to the jugular veins to demarcate the sample sites that were each 2.5 cm² in area.

All microbial samples were collected by T. E. G. while the horses were in a dry stable box that contained no bedding. Before the first sample was collected from each site, organic debris was removed with three strokes of a sterile currycomb. A sterile microbiological swab (Transwab; Medical Wire & Equipment) was then moistened in 1 ml of sterile distilled water in a sterile tube (Vacutainer; Becton Dickinson). Excess water was removed by rolling the swab on the inside of the tube. Wearing sterile gloves, the hair over the site was parted and the swab was directly applied to the skin surface; it was then rotated through 360° while maintaining good contact with the skin. The swab was returned to the tube containing sterile water, agitated in the water for 30 seconds, removed and replaced into transport medium (Transwab; Medical Wire & Equipment). Both the swab and the tube containing 1 ml of sample solution were transported to the laboratory for processing immediately after the samples had been taken.

Microbiological analysis
For the quantitative bacteriological investigation, each 1 ml sample was diluted serially by factors of 10 until a dilution of 1:10^{-3} was obtained. Ten µl of the undiluted sample and each dilution was transferred with a sterile pipette on to individual labelled 5 per cent sheep blood agar plate and incubated aerobically at 37°C. The plates were inspected after 24 hours and the numbers of cfu at each dilution were recorded. The numbers of cfu were also counted after the plates had been incubated for a further 72 hours at room temperature to allow slow-growing or temperature-inhibited organisms to grow. The total number of colonies was used to calculate a final cfu/ml for each sample. The minimum level of detection was 100 cfu/ml, equivalent to one cfu in 10 µl of the undiluted sample. If no growth was observed in any of the diluted samples or the undiluted sample, a nominal value of 50 cfu/ml was assigned to permit statistical analysis; this value allowed for the fact that small numbers of bacteria might not be detected by the culture technique and it was selected arbitrarily below the minimum level of detection, so that it would not influence the outcome of the statistical analyses.

For the qualitative bacterial investigation, the skin swabs were streaked on to sheep blood agar and MacConkey's agar plates and incubated as above. The organisms grown were identified to genus level by the morphological characteristics of the colonies, their Gram-stain reaction and microscopic morphology. When necessary, pure cultures were obtained by subculture and the organisms identified by biochemical testing (Automated Profile Index; bioMérieux). Staff of the Veterinary Diagnostics Services, Faculty of Veterinary Medicine, University of Glasgow, identified the organisms and were blinded to the origin of the samples. The microbial colonies growing on the plates used for the quantitative analysis were identified by the same methods.

Study design
Part 1 Skin swabs were taken from each of the four sites over the external jugular veins of each of the five horses to determine the commensal skin flora at the sites.

Part 2 The effectiveness of chlorhexidine gluconate for disinfecting shaved, clipped and unclipped hair coats and skin at the sites was assessed. Two groups (A and B), which contained one sample site from each vein, were selected randomly (each group contained 10 sites). The sites in group A were sampled on day 1 and day 28 and the sites in group B on day 14 of the study, giving 14 days between adjacent sites being used for a sample collection and 28 days between the same sites in group A being used for a second time.

The disinfection protocol was as follows: the site was scrubbed in small circles with five gauze swabs soaked in 2 per cent w/v chlorhexidine gluconate solution (Hibiscrub; Regent Medical). Each gauze swab was used for one minute so that the total scrub time was five minutes. Three gauze swabs soaked in surgical spirit (Dunlop's Veterinary Supplies) were used to remove excess lather from the site and the site was then allowed to dry completely by evaporation.

On day 1, each site was sampled before and after it was disinfected while the hair coat was left intact. On day 14, an initial sample was collected from each site with the hair coat intact. The hair over the site was then clipped with a sterile size 10 clipper blade and a second sample was collected. A third sample was collected after the clipped site had been disinfected. On day 28, an initial sample was collected with the hair coat intact. The hair was then shaved with a sterile razor blade and a second sample was collected. A third sample was collected after the site had been disinfected.

Part 3 The efficacies of chlorhexidine gluconate and povidone-iodine for disinfecting the sites were compared with each other and with a saline control. The study was made three months after the conclusion of Part 2 and there had been considerable re-growth of the hair coat at all the sampling sites.

Chlorhexidine gluconate was used as in Part 2. The procedure with povidone-iodine was similar. Five gauze swabs soaked in 0.75 per cent povidone-iodine solution (Pevidine; Novartis Animal Health) were used for one minute each to scrub the site in small circles. Excess lather was then removed with three gauze swabs soaked in surgical spirit.

Similarly, for the saline control, five gauze swabs soaked in sterile saline were used to scrub the site for one minute each and excess saline was then removed with a sterile gauze swab.

Three sites were demarcated on each of the jugular grooves of each horse to produce 30 sites, each 2.5 cm² in area. Each treatment was applied to one site on each jugular vein so that each treatment was used 10 times, giving equal numbers of each treatment and avoiding any skewed distribution of the treatments between horses. Random allocation ensured that only one site from each vein would be in the same group; first, the sites were randomly allocated into three groups of 10 (A, B and C) that contained one site from each vein. Secondly, the 30 sites were randomly allocated into three groups of 10 to determine which treatment would be used on each site. Samples were collected from group A sites on day 1, from group B sites on day 14 and from group C sites on day 28.

Three samples were obtained from each site. The first sample was collected from the skin after the organic matter had been removed from the hair coat with a sterile currycomb. The hair over the site was then clipped with sterile size 10 clipper blades, and a second sample was collected. The site was then treated with its assigned disinfectant and a third sample was collected.

Statistical analysis
The statistical analyses were made by using Microsoft Excel 2000 and Minitab Release 14 Statistical Software (Minitab). The bacterial counts were log transformed to normalise the data. A probability of P less than 0.05 was considered significant for each analysis.

In Part 2, paired t tests were used to determine whether there were significant reductions in bacterial counts after the disinfection of sites with an intact hair coat. When the hair coat had been clipped or shaved before being disinfected, the reductions in log cfu/ml after hair coat preparation only, disinfection only and combined hair coat preparation and disinfection were assessed by using paired t tests.

In Part 3, a paired t test was used to determine whether there was a significant reduction in numbers of bacterial cfu after clipping alone, disinfection alone and combined clipping and disinfection for each group. The percentage reductions in cfu after clipping alone, disinfection alone, and clipping and disinfection combined were calculated for each of the three treatments. A one-way analysis of variance was
used to determine whether there were significant differences between the percentage reductions in cfu numbers between the three treatments.

Results

Part 1

Bacterial counts from the 20 skin sites ranged from 100 to 300,000 cfu/ml, with a mean (sd) of 46.5 x 10^3 (79.6 x 10^3). Sixty-seven per cent of the organisms identified were Staphylococcus species and 15 per cent were Streptococcus species (Fig 1); Bacillus, Enterobacteriaceae and Micrococcus species were less common.

Part 2

Table 1 shows the numbers of bacteria isolated from the skin swabs obtained from the sites before and after the hair coat had been clipped or shaved, and after the site had been disinfected. Table 2 shows the log-transformed data.

No organisms were grown from any of the samples taken after the skin had been disinfected, whether or not the hair coat had been clipped or shaved.

Clipping the hair coat resulted in a non-significant reduction in mean cfu/ml, but there was a significant reduction (P=0.03) after the hair had been clipped and the skin disinfected. There was also a significant reduction (P=0.01) after the site had been disinfected with the hair coat left intact.

No organisms were isolated from the skin after the sites had been shaved, either before or after they were disinfected.

Part 3

Table 3 shows the mean (sd) numbers of bacteria isolated from each group and Table 4 shows the log-transformed data.

At the sites disinfected with chlorhexidine there were significant reductions in bacterial numbers as a result of clipping alone (P=0.03), disinfection alone (P=0.02) and by their combined effects (P<0.01). At the sites disinfected with povidone-iodine there was no significant reduction after clipping alone but there was a significant reduction after they were disinfected (P=0.04). At the sites treated with sterile saline there were no significant reductions in bacterial numbers after the hair coat was clipped or after the skin was disinfected or after both treatments.

The analysis of variance showed there were no significant differences between the percentage reductions in mean log cfu/ml between the sites disinfected with chlorhexidine or povidone-iodine for the effects of clipping, disinfection, or both combined.

The organisms isolated in Parts 2 and 3 were predominantly Staphylococcus and Streptococcus species. Smaller numbers of Bacillus, Enterobacteriaceae, Macor and Aspergillus species were also isolated.

Discussion

The results provide quantitative and qualitative bacterial information for sites where catheters are inserted into the jugular veins of horses. In Part 1, the mean (sd) cfu/ml of samples taken from the skin over these sites was 46.5 x 10^3 (79.6 x 10^3). Hague and others (1997) isolated 2.18 x 10^3 (3.08 x 10^3) cfu/ml from skin over the midcarpal joint of horses and 20.7 x 10^3 (32.7 x 10^3) cfu/ml from skin over the distal interphalangeal joint. In both the present study and that of Hague and others (1997), the standard deviation was markedly greater than the mean value, indicating a very large range between individuals. This trend continued through Parts 2 and 3 in which the mean values for cfu/ml were 52.5 x 10^3 (18.2 x 10^3) and 2.74 x 10^3 (4.56 x 10^3), respectively. The wide ranges were probably due partly to differences between individual animals and partly to the poor sensitivity of the skin swab technique for the collection of bacteria. Skin swabbing samples only the transient bacteria and not the resident bacteria on adnexal structures that are estimated to constitute at least 20 per cent of the total bacterial population of the skin (Selwyn and Ellis 1972).

The sensitivity could have been improved by culturing skin biopsies or by using contact agar plates pressed on to the hair coat or surface of the skin (Selwyn and Ellis 1972). Skin biopsies can affect a horse’s welfare adversely. Contact plates have been used successfully in anaesthetised small animals (Lambrechts and others 2004), but it would probably be more difficult to maintain adequate contact between a plate and the jugular groove of a horse, and therefore more difficult to collect a representative sample of bacteria.

The qualitative examinations revealed predominantly commensal bacteria (Staphylococcus, Streptococcus and Micrococcus species) and less commonly environmental contaminants (Bacillus, Enterobacteriaceae, Aspergillus and Macor species). The species were similar to those reported by Hague and others (1997) and Zubrod and others (2004) who collected bacteria from the distal limbs. The results suggest that the necks of horses are frequently contaminated with environmental bacteria. Commensal and environmental species are common contaminants of intravenous catheters in horses (Ettlinger and others 1992) and have been associated with catheter-associated thrombophlebitis (Gardner and others 1991, Ettlinger and others 1992).

In Part 2, the disinfection of intact hair coat and clipped hair resulted in significant reductions in bacterial numbers. In contrast, there was no significant reduction after the disinfection of the shaved skin. The

| TABLE 1: Mean (sd) numbers of bacteria (cfu/ml) from skin swabs taken over the external jugular veins of five horses before and after the hair coat had been clipped or shaved and after the site had been disinfected |
|----------------------------------|----------------|----------------|
| State of hair coat               | Before clipping or shaving | After clipping or shaving | After disinfection |
| Normal                           | 12,055 (31,043) | NA | *50 (0) |
| Clipped                          | 2545 (4113) | 705 (906) | *50 (0) |
| Shaved                           | 1155 (3122) | *50 ±(0) | *50 (0) |
| * Below the level of detection   | NA Not applicable |

| TABLE 2: Log-transformed numbers of bacteria (cfu/ml) from skin swab samples taken over the external jugular vein of five horses before and after the hair coat had been clipped or shaved and after the site had been disinfected |
|----------------------------------|----------------|----------------|
| State of hair coat               | Before clipping or shaving | After clipping or shaving | After disinfection |
| Normal                           | 3.17 | NA | 1.70 |
| Clipped                          | 2.54* | 2.47* | 1.70* |
| Shaved                           | 2.15 | 1.70 | 1.70 |
| * Values within a row with different superscripts are significantly different |
| NA Not applicable |
process of shaving the skin in itself resulted in a marked reduction in bacterial numbers, which was probably caused by the physical removal of the transient bacterial population with the outermost cells of the epidermis. The small numbers of bacteria obtained before disinfection probably contributed to the lack of a significant reduction after disinfection. The small sample size and low power of the analysis may also have contributed to the lack of a significant reduction. No bacteria were isolated from the swabs taken after the sites were disinfected and it is reasonable to conclude that chlorhexidine gluconate was effective regardless of whether the hair coat was present, or had been clipped or shaved.

In Parts 2 and 3, there was a reduction in the bacterial count after the hair coat had been clipped. In contrast, Hague and others (1997) observed an increase in the numbers of bacteria retrieved from the skin surface after the hair on a distal limb had been clipped; however, it was not stated whether the clipper blades were sterile. Differences in the lengths of the hair coat, clipping technique, time between clipping and sampling, and swab technique may account for the difference between the studies. In this study, it was possible to make good contact between the swab and the skin even when the hair coat was long. Removing the hair coat may remove some of the environmental organisms present at the sample site.

Both the disinfectant treatments resulted in a significant reduction in cfu/ml and there were no significant differences between them, regardless of the preparation of the hair coat. The treatment with saline also reduced the bacterial count but not significantly, probably as a result of scrubbing the area and removing bacteria physically. The results show that the two disinfectants would be similarly effective in reducing the numbers of bacteria on the skin before inserting an intravenous catheter, but it was not possible to determine whether the treatments would have prevented catheter-related infections owing to the non-invasive nature of the study. In human beings, the risk of catheter-related infections was lower after the disinfection of catheter sites with chlorhexidine than with povidone-iodine (Maki and others 1991), possibly owing to its wider range of antimicrobial activity, longer duration of action and less inhibition by organic material (Freeman 2006).

The results of the study show that disinfecting the skin over the external jugular veins of horses with chlorhexidine was equally effective when the hair was left long, clipped or shaved, and that chlorhexidine and povidone-iodine were equally effective when used on skin after the hair had been clipped. The organisms isolated from the skin surface were those commonly associated with the colonisation of intravenous catheters.

However, clipping or shaving the hair also reduced the numbers of bacteria, and would make it easier to see the veins within the jugular groove, particularly in breeds with a long-hair coat and during the winter months when the coat is long, so reducing the risk of damaging the wall of the vessel as the catheter is being introduced. Furthermore, foreign material is more likely to be introduced with the catheter if the hair is left long.

Clipping or shaving the hair over the veins before the site is disinfected with either chlorhexidine or povidone-iodine is therefore recommended as an effective technique for reducing the numbers of bacteria on the skin before the catheter is introduced. However, the skin can be disinfected effectively with either agent without clipping or shaving the hair, when rapid intravenous catheterisation is necessary as a clinical priority.

TABLE 3: Mean (sd) numbers of bacteria (cfu/ml) from skin swabs taken over the external jugular veins of five horses before and after the hair coat had been clipped, and after the site had been disinfected with chlorhexidine gluconate, povidone-iodine or sterile saline

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Before clipping</th>
<th>After clipping</th>
<th>After disinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine gluconate</td>
<td>3680 (4068)</td>
<td>400 (630)</td>
<td>55 (16)</td>
</tr>
<tr>
<td>Povidone-iodine</td>
<td>2825 (6124)</td>
<td>500 (623)</td>
<td>250 (615)</td>
</tr>
<tr>
<td>Saline</td>
<td>1725 (3276)</td>
<td>135 (142)</td>
<td>1050 (3145)</td>
</tr>
</tbody>
</table>

TABLE 4: Log transformed numbers of bacteria (cfu/ml) from skin swab samples taken over the external jugular veins of five horses before and after the hair coat had been clipped, and after the site had been disinfected with chlorhexidine gluconate, povidone-iodine or sterile saline

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Before clipping</th>
<th>After clipping</th>
<th>After disinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine gluconate</td>
<td>3.01*</td>
<td>2.25*</td>
<td>1.72*</td>
</tr>
<tr>
<td>Povidone-iodine</td>
<td>2.57*</td>
<td>2.34*</td>
<td>1.89*</td>
</tr>
<tr>
<td>Saline</td>
<td>2.54*</td>
<td>1.98*</td>
<td>1.96*</td>
</tr>
</tbody>
</table>

* Values within a row with different superscripts are significantly different (P=0.05)

Acknowledgements

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