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Evaluation of a routine antiseptic and two disinfectants for reducing bacterial population of cow hoof

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Abstract

A routine antiseptic and two disinfectant agents were used separately for reducing bacterial population of cow hoof: 1) 7.5% povidone–iodine scrub mixed with 10% povidone–iodine solution, 2) 10% copper sulfate, and 3) 8% formaldehyde. Swabbing for microbial colony counts were used to evaluate pre and post–scrub of hooves of eight cows. The results revealed no significant differences in reduction of bacterial colony count between post–scrubs of povidone–iodine and formaldehyde. Bacterial colony counts after the povidone–iodine scrub solution and formaldehyde scrub were significantly different from those obtained after the copper sulfate scrub. Significant reduction in number of microbial colony in post–scrub by povidone–iodine, formaldehyde, and copper sulfate were observed which were different from the control (warm tap water).

Keywords: Cow Hoof, Bacterial Population, Copper Sulfate, Formaldehyde, Povidone–Iodine

Introduction

Numerous disinfectants have been used to remove foreign and necrotic materials from surgical area. They reduce the number of surface microorganisms, so wound infection cannot develop (Hackett et al., 1983). Removal or killing the bacteria in the operative site leads to reduction in the postoperative infection rate (Ritter et al. 1980). Despite surgical preparation of skin, up to 20% of surface microorganisms are inaccessible to routine disinfection because of protection by hair follicle, crevices and lipids (Ritter et al., 1980). Hoof preparation of cattle is necessary for some digit surgeries, intra-articular injection and taking synovial fluid from interphalangeal joints. Although, hoof does not possess hair follicles, it does contain crevices and a lipid rich external layer, the priople. The outer wall and sole of hoof are usually in direct contact with manure, bedding materials or soil. So number and variety of resident and transient bacterial population of the hoof capsule may be higher than skin. Consequently, the likelihood of gross bacterial contamination and infection after surgery of hoof capsule may be increased (Hennig et al., 2001). Gram positive microorganisms are most commonly encountered on the skin (Osuna et al., 1990; Zamora, 1984). Probably the hoof environment might contribute to substantial population of gram negative bacteria of enteric and soil origin. Thus, antiseptics intended for the skin might not be as effective when applied for the hoof. Several preoperative agents and techniques have been identified in recent years. They are often based on laboratory studies.

Povidone–iodine has widespread usages as a surgical preparation agent. Its solution consists of iodine, which is the active antimicrobial element, bound to the organic carrier, polyvinyl pyrrolidone. Povidone–iodine interacts with cell walls of microorganism, causing pore formation or generating solid–liquid interfaces at the lipid membrane level that lead to loss of cytosol material, in addition to enzyme denaturation (Schreier, 1997). Povidone–iodine is effective against a broad spectrum of bacteria (Amber et al., 1983; Babb et al., 1991). It is relatively inexpensive, and it has no staining and odorless properties. It acts as a detergent to remove dirt and oils. Povidone–iodine is inactivated by blood and organic matter.

Formaldehyde is inexpensive and readily available solution with high consumption in dairy cow farms. Also, formaldehyde has been used frequently in combination with other components in dentistry (Yamano et al., 2002). Formaldehyde is a bactericidal solution that kills most bacteria, bacterial spore and viruses and is used as a reliable disinfectant and fumigant. It will also destroy superficial tissue and has been used in footbaths for treatment of foot rot infections of hoof corium and claws (Allen et al., 1993).

Various metals and metal salts are commonly employed to prevent microbial growth or kill microbes (Belcastro, 1994). One of themis copper sulfate ($CuSO_4$) has been used as a caustic dressing for debridement and coagulation in wounds, and in footbaths for the treatment of foot rot in cow. It is also used in feeds or salt mixtures to prevent copper deficiency in cow (Allen et al., 1993). Also, susceptibility of different bacterial species isolated from food animals to copper sulphate revealed potential and efficacy of copper sulphate antimicrobial activity (Aarestrup and Hasman, 2004).

However, there are several types of digit and claw surgery in cattle practice such as claw amputation, amputation of claw through the coronary band, surgical arthrodesis of the distal interphalangeal joint, declaw amputation and disarticulation at the distal interphalangeal joint (Acuña, 2008; Heppelmanna et al., 2009). Also, because of present of various types of available antiseptics and disinfectant agents and nature of structure of hoof, the present study was conducted to compare and evaluate the efficacy of a routine antiseptic and two disinfectant agents for reduction of bacterial load of cow hoof that are commonly available in dairies cow farms.

Materials and Methods

Eight healthy adult Holstein dairy cows with normal hooves were selected as followed. Gross debris of their feet was cleaned by a hoof pick, brush and running tap water. Based on physical examination, cows were determined to have normal healthy hooves. They were housed in a wood floored pen for 24 hours before trial. The cows were allowed free access to water and feed (hay and grain).

The hoof pick, brush, knife, and rasp used during the study were washed with ammonium chloride disinfectant (Hopkins & Williams Co., UK), rinsed with sterile water and air dried between each use. Before beginning of procedure, the cows were restrained in a hoof trimming chute and the hooves were rinsed 5 minutes with tap water, separately before swabbing and scrubbing. Their hoof wall (axial and abaxial surface of claw) and hoof sole was rolled on with a sterile swab moistened with citrate buffered solution to obtain a specimen for determination of a base line microbial count. After the specimen was obtained the hoof was dried with a sterile terry towel.

Rotating the solutions and control among the hooves of all the cows, a Latin square design was used, the cows as rows and the limbs as columns. The solutions include 1) 7.5% povidone–iodine scrub (7.5% povidone–iodine, surgical scrubs (Iran Nazhu Co., Tehran, Iran) and 10% povidone–iodine solution (10% povidone–iodine, Rasht, Iran), 2) 10% copper sulfate (Somshoo, Sahand Co., Tabriz, Iran), 3) and 8% formalin (Formalin, Merck Co., Germany), were used as scrub treatments and warm (38–40°C) tap water as control. For each scrub, 10 ml of warm tap water and 10 ml of scrub solution were prepared in a syringe and applied to 3×3 inch surgical sponge for scrubbing. Each region of hoof was scrubbed three times for 30 seconds with warm water rinses between each scrub. The progression of hoof scrubbing was as follows: dorsum, interdigital space and solar surface of hoof. A period of 5 minutes was allowed for air drying. Then post–scrub sterile moistened swab was obtained from the same area mentioned above. The swabs were immediately placed in citrate buffered solution tubes separately.

Serial ten–fold dilution, using phosphate–buffered saline solution (pH=7.4), were prepared, using the sample dissolved in the citrate buffer as the 10^{-0} dilution. The dilutions were carried out to 10^{-7}. One milliliter of each dilution was plated in duplicate on Mueller–Hinton agar plates (Merck Co., Germany), using the spread plate method. The plates were incubated for 48 hours at 37°C in 5% CO_2. Plates were examined for colony types and total colony count per specimen.

The total number of colony–forming units was determined from a countable plate, which was defined as having between 30 to 300 isolated colonies. Colonies representative of the sample population were sub cultured onto differential media. Identification was carried out by the following criteria: cell and colony morphologic features and conventional biochemical testing. Gram negative isolates were confirmed by use of API 20E identification strips (Analytab Products, Plain view, NY).

Statistical Analyses

The data were analyzed as a Latin square with repeated measures over time, using the square root transformation. Means and variances of the control group were determined separately and techniques of comparison with unequal variances were used to compare the control to scrub solutions.

Results and Discussion

The colony counts for each of pre-operative preparations on all eight cows are compared in Table 1. When the three scrub solutions (povidone–iodine, formaldehyde, and copper sulfate) were evaluated, a downward tendency was seen from pre–scrub to the post–scrub period. Solutions were compared by colony counts obtained from specimen collected as the pre–scrub stages showed no differences. After the scrubbing procedure, results associated to povidone–iodine scrub,

Table 1: Means ± SE of bacterial colony counts at different stages of preoperative preparation with a routine antiseptic and two disinfectant agents on hooves of cows (n=8).

Antiseptic or Disinfectant	Bacterial colony count	
	Pre–scrub	Post–scrub
Povidone–iodine	3348.5 ± 312.64 (57.3 ± 2.84)[a]	29.7 ± 5.13 (5.4 ± 0.45)[c]
Formaldehyde	4797 ± 457.08 (68.6 ± 3.57)[a]	36 ± 2.99 (6.0 ± 0.24)[c]
Copper sulfate	3679.5 ± 419.49 (60.0 ± 3.37)[a]	59.3 ± 5.35 (7.7 ± 0.34)[b]
Tap water (control)	3760.7 ± 243.73 (61.1 ± 1.98)[a]	1759.5 ± 223.4 (41.3 ± 2.7)[a]

[a-d] Mean values bearing different superscripts in a column differ significantly (P<0.05). Means ± SE in parenthesis are from transformation, SQRT (X+1), of the data

formaldehyde scrub, and copper sulfate scrub were significantly different from the control (P<0.05). There were no significant differences between povidone–iodine and formaldehyde after scrub period (P≥0.05). Results obtained after the povidone–iodine scrub solution and formaldehyde scrub were significantly different from those obtained after the copper sulfate scrub.

The most frequently isolated pre–scrub and post–scrub of microbial population was *Staphylococcus* spp., *Micrococcus* spp., and *Bacillus* spp. Although, fungi were isolated sporadically, they were not statistically analyzed. The most frequently isolated pre–scrub gram positive bacteria were *Staphylococcus epidermis*. Gram negative bacteria most frequently isolated before scrub were *Escherichia coli*, *Klebsiella* spp., and *Actinobacter* spp. It was noted that with all three scrub solutions, there was a reduction in the number of genera isolated from hooves after scrubbing for preparation procedure.

Complete sterilization of the surgical area is not possible. But, all three preoperative scrubbing protocols were effective in decreasing the microbial population of the hoof. Surgery and trimming is usually preceded by aseptic preparation of the hoof surface, which, due to its close association and contact with the ground and contaminated bedding, is commonly assumed to host a large population of bacteria, particularly those that are of enteric origin or are soil inhabitants.

Despite of some conflicting data that have demonstrated no significant difference in the bacterial counts immediately after performing a surgical preparation of canine paws with povidone–iodine (Swaim et al., 1991), our study revealed bacterial reduction following use of this solution.

The results of this study indicated that the activity of formaldehyde is comparable with povidone–iodine. In some situations such as presence of blood, organic material and serous exudates that limit activity of povidone–iodine, formaldehyde can be used with caution in the surgical site (Mertz et al., 1984). Results obtained after the povidone–iodine scrub solution and formaldehyde scrub were significantly different from those obtained after the copper sulfate scrub. Some studies revealed that copper sulfate products have antimicrobial effects (Faundez and Figueroa, 2001; Aarestrup and Hasman, 2004; Gant et al., 2007). Free iodine, the active antibacterial element, is released in the greatest amounts when povidone–iodine solutions are diluted (Swaim and Lee, 1987). Another study has reported that 10% povidone–iodine solution is a too effective antiseptics agent (Ghogawala and Furtado, 1990).

Formaldehyde as a lipid–dispersal agent is able to remove bacteria of periople. The vapors of formaldehyde are very irritating and it is classified as a potential carcinogen (IARC, 1995). Care must be taken to protect both staff and patients from the fumes when mixing and using formaldehyde solution, and do not dilute with chlorinated water as a dangerous gas can be produced. Staff should wear gloves to avoid skin contact (Gilman et al., 1990), protect eyes from splashes, limit exposure time and use these solutions only in a well ventilated area. Symptoms of toxic and side effects of formaldehyde in human are published frequently and attention to them is necessary (Adams et al., 1987; Alexandersson and Hedenstierna, 1988).

It is obvious that the presence of white line and other hoof cracks and defects can harbor and protect bacteria from removal. It was determined that not only scrub solutions were effective in reducing the bacteria, but also the mechanical action of scrubbing and rinsing the hooves were effective in reducing the microbial population as evidenced by the lowering of the colony counts of the hooves prepared with only tap water.

In this study, most bacteria isolated from samples after treatment were *Bacillus* spp, *Staphylococcus* spp, *Micrococcus* spp (results not shown). These finding can be explained by the nature of these organism. *Bacillus* spp. are able to produce endospores and possess an inherent ability to tolerate antiseptics and other more cleansing agents. A variety of *Staphylococcus* spp. and some *Micrococcus* spp. are considered as normal flora to cow skin and hoof and may be present in sites where antiseptics or the mechanical action of scrubbing is not effective (Krogh and Kristensen, 1976; Cox et al., 1988).

Cost of formaldehyde and copper sulfate is less than povidone–iodine. An effective preoperative

disinfection protocol should decrease the use of antibiotics, and the compounds used in this study for aseptic preoperative preparation of hoof reduced the number of microbial population. Therefore it is possible and economical to use formaldehyde and copper sulfate for reduction of bacterial population of hoof if povidone–iodine is not available in a dairy farm.

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