Domestic chickens (Gallus gallus domesticus) are used for egg and meat production, are commonly used as research models, and are becoming more widely kept as companion animals. Surgical procedures are routinely performed on chickens for research purposes or for the treatment of reproductive, musculoskeletal, or gastrointestinal disease, among others.

Saline, chlorhexidine, and povidone-iodine alone or in combination with iodine povacrylex are effective antiseptics in chickens (Gallus gallus domesticus)

Greta Doden, DVM1; Akhilesh Ramachandran, BVS, AH, PhD, DACVM2; Ian Kanda, RVT, VTS3; Nicola Di Girolamo, DMV, PhD, DECZM, DACZM4; Jessica Robertson, DVM, DACZM5; Danielle Dugat, DVM, MS, DACVS1; João Brandão, LMV, MS, DECZM, DACZM1*

1Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK
2Oklahoma Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK
3Pet Hospital of Peñasquitos, San Diego, CA
4Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY
5VCA West Los Angeles Animal Hospital, Los Angeles, CA
*Corresponding author: Dr. Brandão (jbrandao@okstate.edu)

OBJECTIVE
To evaluate the topical antiseptic activity of saline, chlorhexidine (CHX), and povidone-iodine (PI) scrubs on the skin of chickens with or without the addition of DuraPrep (DP).

ANIMALS
7 healthy adult Orpington hens (Gallus gallus domesticus).

METHODS
The right apterium corporale laterale was swabbed for standard aerobic bacterial culture and colony-forming unit (CFU) determination. The apterium was divided into 3 areas and treated with sterile saline, CHX, or PI. Samples were collected by swabbing each area before and after additional treatment with DP. CFU’s were counted after 48 hours of incubation. Statistical analysis was performed using a linear mixed model with a continuous outcome.

RESULTS
Compared to saline, CHX and PI treatment without DP decreased CFU count by 119 (95% CI, 55 to 183; P < .001) and 123 (95% CI, 58 to 187; P < .001), respectively. The application of DP after CHX and PI further decreased CFU counts by 6 (P = .01) and 9 (P = .01), respectively. DP after saline treatment decreased counts by 128 CFU (95% CI, 63 to 192; P < .001). No significant difference was detected between saline, PI, or CHX after DP application (~1.0 CFU; 95% CI, 63.4 to –65.4; P = .98 for both PI and CHX).

CLINICAL RELEVANCE
CHX or PI provided greater reductions in bacterial CFU than saline, and all combinations with DP provided similar results. No notable cutaneous reactions were detected at any point. This data suggests that a scrub protocol including CHX or PI with DP is acceptable in surgical site preparation of chickens.

Keywords: chicken, Gallus gallus domesticus, DuraPrep, chlorhexidine, povidone-iodine

Postoperative surgical site infections lead to increased health care costs due to additional treatment, drug administration, extended hospital stay, and patient morbidity and mortality. This creates emotional and financial stress for owners and affects the welfare of the animal. Most surgical site infections are caused by endogenous microorganisms of the host’s skin. Therefore, sterile preparation of the surgical field is crucial for the prevention of postoperative surgical site infections.

Historically, chlorhexidine (CHX) and/or povidone-iodine (PI) have been recommended as the...
antiseptics of choice for birds. However, this information is not scientifically validated and is adapted from mammalian medicine. Studies in various mammals, including horses, dogs, and rodents, have evaluated various preoperative surgical scrubs, concluding that CHX and PI are similarly effective in reducing bacterial contamination of the surgical site. Sites treated with sterile 0.9% NaCl also exhibit lower contamination than untreated sites but to a lesser extent than antiseptics. It may not be appropriate to extrapolate these results from mammalian to avian species due to significant differences in skin anatomy (e.g., presence of feathers instead of hair), endogenous cutaneous bacteria, and hygiene. The evaluation of preoperative surgical preparation is understudied in avian species.

In humans and small animals, CHX and PI scrub may be followed by other disinfectants, such as DuraPrep (DP) (DuraPrep Surgical Solution; 3M). DP is a film-forming iodophor surgical solution that contains iodine povacrylex (0.7% available iodine) in isopropanol alcohol. It is commercially available as a sterile, single-use applicator with a sponge tip. DP can be used as a safe and effective one-step process but is commonly used in veterinary surgeries after an initial preparation with CHX or PI.

The purpose of this study was to compare cultured bacterial colony-forming unit (CFU) counts from the skin of chickens before and after the application of different surgical scrub protocols using saline, CHX, and PI, with or without the additional application of DP. The specific hypotheses were that CHX and PI would have lower CFU counts than saline and that the addition of DP would further reduce the CFU count.

Methods

Animals

This study was approved by the Oklahoma State University IACUC (VM-16-10). Seven adult client-owned Orpington hens were included in this study. Informed client consent was obtained prior to the study. All chickens originated from the same location. The chickens were housed in a barn with dirt substrate but had access to the outside. The chickens were kept at environmental temperature with fans during the summer and supplemental heat when temperatures reached below 40 °F. The chickens were fed a commercially available pelleted diet (Purina Layena layer feed; Purina Animal Nutrition LLC) with oyster shell and natural vegetation when outside. The chickens had free access to water. Each chicken was transported from its regular housing facility to the Oklahoma State University Veterinary Medical Hospital in pet carriers. On arrival, the chickens received a physical examination, with particular attention to the skin, to investigate the presence of dermatitis or other cutaneous abnormalities. At the end of the physical exam, blood was collected from the ulnar vein under physical restraint for hematologic and water was withheld in preparation for general anesthesia. The chickens used in this study fit the following inclusion criteria: normal physical examination results, lack of evidence of cutaneous disease, and normal hematology.

Procedures

Each chicken was manually restrained for anesthetic induction via face mask with 5% isoflurane in >95% oxygen at a rate of 2.0 L/min. Once anesthetic induction was achieved, the chickens were intubated with a 3.0- or 3.5-mm internal diameter uncuffed Murphy’s endotracheal tube. The size of the tube was selected based on visual assessment of the tracheal diameter. Anesthesia was maintained with isoflurane between 1.5% and 3% in oxygen at a flow rate of 2.0 L/min. Anesthetic monitoring included heart rate, electrocardiogram, oxygen saturation, end tidal CO2, and temperature via esophageal probe using a commercially available unit (VetGard; Vmed Technology).

Once they were anesthetized and all of the necessary monitoring equipment was in place, the chicken was placed in left lateral recumbency, the right leg was retracted caudally, and the right wing was retracted dorsally. The right apertium corporale laterale, which is located ventral to the pteryla spinales, cranial to the pteryla femoralis, and dorsal to the pteryla ventrales (pectoralis et abdominalis), was identified, and the surrounding feathers were secured with 1-inch medical tape (Transpore; 3M). The skin area was selected based on the natural lack of feathers in this area; therefore, plucking was not required. Markings were made with a pen on the surrounding tape to demarcate the 3 different treatment areas, approximately 15 cm2 each (Figure 1). Operators placing the tape used nonsterile gloves and avoided contact with the skin.

Prior to the application of any treatment, the site was swabbed (BBL CultureSwab; Becton, Dickinson and Company) in a routine fashion for aerobic bacterial culture. For the determination of CFU, sterile cotton-tipped applicators were used. All procedures were performed using aseptic technique with sterile gloves, which were replaced between each swab and each application of treatment. Each of the 3 study sections received a different testing treatment. Treatment area and order were not randomized and were performed in the same order in all chickens. For each treatment, 3 sterile gauze squares (2 X 2 inches; Medline Industries Healthcare) soaked in saline or disinfectant were used to scrub the skin surface in a circular motion 10 times. Special attention to avoid overlap of the treatments was made, and all swabs were taken from the center of each treatment area. Area 1 was treated with CHX digluconate 2% (ChlorHex-Q scrub; VEDCO Inc), area 2 with sterile saline (0.9% sodium chloride irrigation, USP; Baxter Healthcare Corporation), and area 3 with PI 7.5% (Povidine; VetOne). After 2 minutes, the selected area was wiped with 1 gauze of sterile saline to remove excess treatment product. First treatment was allowed to dry for 2 minutes, and then a new swab for CFU calculation was collected.
Thereafter, a commercially available product containing iodine povidone (0.7% iodine) and isopropyl alcohol (74% w/w) (DuraPrep Surgical Solution; 3M) was applied, and 2 minutes were allowed for drying prior to collection of a new sample for CFU determination. A new DP applicator was used for each treatment area, and care was taken to ensure that DP solution had soaked the sponge prior to application. The first scrub, DP scrub, and swabs were performed consecutively for each area before moving on to the next treatment area (eg, area 2 was begun only after performing the second swab for area 1). All bacterial culture swabs were taken in duplicate, and swabs for CFU determination were placed in neutral-buffered sterile saline. When all procedures were complete, anesthesia was discontinued, all monitoring equipment was removed, and the chickens were allowed to recover. Chickens were returned to their owner. No further evaluations were performed by the investigators, but the owner, a registered veterinary technician specialist (anesthesia), was advised to report abnormal behavior, such as excessive preening or obvious irritation to the skin, similar to the recommendations for a surgical case.

Microbiological procedures

All microbiological procedures were performed by a single operator not blinded to the treatments. Swabs were kept in a refrigerator at 4 °C (39.2 °F) until processing, which occurred within 2 hours of collection. A routine bacterial culture was performed using blood agar plates incubated at 37 °C in a 5% CO₂ environment. Each swab for CFU determination was suspended in 1 mL of PBS and vortexed for 1 minute to suspend the collected bacteria. Three drops (25 μL each) of the suspension were then plated on blood agar and incubated at 37 °C (98.6 °F) in a 5% CO₂ environment. Three dilutions of this suspension (1:10, 1:100, and 1:1,000) were created and plated on blood agar in the same manner. Visible bacterial colonies were manually counted from the preparation with least crowding at 48 hours after incubation, then used to calculate CFU/mL. Three drops of PBS were also plated on blood agar as a negative control to evaluate for potential contamination.

Statistical analysis

Continuous variables were reported as means and ranges. A linear mixed model (LMM) with a continuous outcome (number of CFU) was developed to identify the effect of different treatments, accounting for individual chicken as a random effect and the CFU number at baseline as a covariate. Fixed effects included in the model were: baseline CFU, treatment type (saline/CHX/PI), application of DP (yes/no), and the interaction between treatment type and application of DP. Saline and no application of DP were the referent variables in this model. An additional LMM was built with identical random and fixed effects but with the groups that received DP as the referent variable. Results of the LMM were reported as estimated marginal means, 95% CIs, and P values. Data were analyzed with commercial software (SPSS Statistics, version 26.0; IBM Corp). Two-tailed values of P < .05 were considered statistically significant.

Sample size

The sample size was determined based on similar studies in other species. Although the number of animals in such studies was similar or slightly higher, each animal received 1 treatment. In contrast, each animal received multiple treatments in our study design. Therefore, a smaller sample size was utilized due to increased statistical power.

Table 1—Common bacterial species isolated from the skin (right apterium corporale laterale) of 7 client-owned Orpington hens (Gallus gallus domesticus) prior to application of surgical scrub.

<table>
<thead>
<tr>
<th>Bacteria group</th>
<th>Bacteria species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus</em> spp</td>
<td>B cereus</td>
</tr>
<tr>
<td></td>
<td>B subtilis</td>
</tr>
<tr>
<td></td>
<td>B megaterium</td>
</tr>
<tr>
<td></td>
<td>B thuringiensis</td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp</td>
<td>S equorum</td>
</tr>
<tr>
<td></td>
<td>S xylosus</td>
</tr>
<tr>
<td></td>
<td>S capitis</td>
</tr>
<tr>
<td>Other bacteria</td>
<td>Acinetobacter lwoffii</td>
</tr>
<tr>
<td></td>
<td>Brevibacillus laterosporus</td>
</tr>
<tr>
<td></td>
<td>Cellulosimicrobium cellulans</td>
</tr>
</tbody>
</table>
Results

All 7 chickens met the inclusion criteria and were therefore used in this study. No morbidity or mortality were detected at any point, and no evidence of cutaneous irritation was noted during the study or reported by the owner after the study.

Twelve bacterial species were isolated by aerobic culture (Table 1). *Bacillus* sp (n = 10 species) and *Staphylococcus* sp (n = 6 species) were the most common.

Following treatment with saline only, 340.00, 286.70, 40.00, and 26.65 CFU and 2 negative cultures were obtained (Table 2). When treated with PI only, each swab cultured 20.00, 13.35, and 6.65 CFU (in 2 chickens), and 3 cultures were negative. When treated with CHX only, each swab grew 53.30, 13.35, and 6.65 CFU, and 4 cultures were negative. All treatment areas that received CHX and DP or PI and DP had no growth, except for 1 in each treatment group that had 6.65 CFU. All treatment areas that received saline and DP had no growth, except for 1 that had 13.35 CFU. The negative control with PBS on blood agar produced no CFU. A mean of 717.11 CFU (range, 380.00 to 1,846.65) were detected from skin swabs of the 7 chickens before treatment (Table 3).

Compared to saline, CHX and PI resulted in a decrease of 6 and 9 CFU, respectively (P = .01 for both). Application of DP after saline resulted in a decrease of 128 CFU (95% CI, 63 to 192; P < .001). The baseline estimate for the LMM was 0.01 (95% CI, –0.04 to 0.05). A similar LMM, which included presence of DP as the referent variable, showed no significant difference between saline, PI, or CHX after DP application (–1.0 CFU; 95% CI, 63.4 to –65.4; P = .98 for both PI and CHX).

Discussion

In this study, both PI and CHX significantly reduced bacterial CFU as compared to saline, as has been reported in many other species. Additionally, both double-scrub protocols with DP significantly reduced the bacterial contamination of the sites. The improved efficacy of double scrubbing has been demonstrated in humans, although DP tends to be evaluated as a one-step option. A 2020 study in laboratory mice reported comparable results between a standard application of PI with alcohol rinse and 3 commercially available products, including DP, but did not evaluate a double-scrub protocol. Multiple studies have shown similar efficacy of PI and CHX solutions; however, PI is often associated with a higher incidence of contact dermatitis and skin irritation. In contrast, surgical preparation with CHX led to more skin damage in African clawed frogs (*Xenopus laevis*), with histologic evidence of necrosis and higher incidence of clinical illness at the site as compared to PI. Although the

Table 2—Average colony-forming units (CFU) of duplicate swabs from the skin of 7 client-owned Orpington hens (*Gallus gallus domesticus*).

<table>
<thead>
<tr>
<th>Chicken</th>
<th>Baseline</th>
<th>Saline</th>
<th>Saline + DP</th>
<th>PI</th>
<th>PI + DP</th>
<th>CHX</th>
<th>CHX + DP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>660.00</td>
<td>340.00</td>
<td>13.35</td>
<td>6.65</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>600.00</td>
<td>286.70</td>
<td>0.00</td>
<td>20.00</td>
<td>0.00</td>
<td>53.30</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>386.70</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>4</td>
<td>1846.65</td>
<td>40.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>6.65</td>
<td>0.00</td>
</tr>
<tr>
<td>5</td>
<td>413.30</td>
<td>213.30</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>380.00</td>
<td>0.00</td>
<td>0.00</td>
<td>6.65</td>
<td>0.00</td>
<td>6.65</td>
<td>0.00</td>
</tr>
<tr>
<td>7</td>
<td>733.15</td>
<td>26.65</td>
<td>0.00</td>
<td>13.35</td>
<td>0.00</td>
<td>13.35</td>
<td>6.65</td>
</tr>
</tbody>
</table>

Baseline represents the number of CFU before any treatment, while the remaining numbers represent the number of CFU after application of treatment: saline only (0.9% sodium chloride irrigation), saline and DP (Saline + DP; 0.7% iodine povacrylex and 74% w/w isopropyl alcohol), PI (7.5%) only, PI and DP (PI + DP), CHX (2%) only, and CHX and DP (CHX + DP).

CHX = Chlorhexidine. DP = DuraPrep. PI = Povidone-iodine.

Table 3—Descriptive statistics and percent decrease of colony-forming units (CFU) from the skin of 7 client-owned Orpington hens (*Gallus gallus domesticus*).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean (CFU)</th>
<th>Minimum (CFU)</th>
<th>Maximum (CFU)</th>
<th>% Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>717.11</td>
<td>380.00</td>
<td>1,846.65</td>
<td>N/A</td>
</tr>
<tr>
<td>Saline</td>
<td>129.52</td>
<td>0.00</td>
<td>340.00</td>
<td>81.59–100.00</td>
</tr>
<tr>
<td>Saline + DP</td>
<td>1.91</td>
<td>0.00</td>
<td>13.35</td>
<td>99.28–100.00</td>
</tr>
<tr>
<td>PI</td>
<td>6.66</td>
<td>0.00</td>
<td>20.00</td>
<td>98.92–100.00</td>
</tr>
<tr>
<td>PI + DP</td>
<td>0.95</td>
<td>0.00</td>
<td>6.65</td>
<td>99.64–100.00</td>
</tr>
<tr>
<td>CHX</td>
<td>10.47</td>
<td>0.00</td>
<td>53.30</td>
<td>97.11–100.00</td>
</tr>
<tr>
<td>CHX + DP</td>
<td>0.95</td>
<td>0.00</td>
<td>6.65</td>
<td>99.64–100.00</td>
</tr>
</tbody>
</table>

Baseline represents the number of CFU before any treatment while the remaining numbers represent the number of CFU after application of treatment: saline only (0.9% sodium chloride irrigation), saline and DP (Saline + DP; 0.7% iodine povacrylex and 74% w/w isopropyl alcohol), PI (7.5%) only, PI and DP (PI + DP), CHX (2%) only, and CHX and DP (CHX + DP).

CHX = Chlorhexidine. DP = DuraPrep. N/A = Not applicable. PI = Povidone-iodine.

Unauthenticated | Downloaded 06/30/24 06:18 PM UTC
researchers did not assess the chickens after the sample collection period, the owner, an experienced registered veterinary technician, monitored for clinical signs and reported none. While PI and CHX were more effective, scrubbing with saline also significantly decreased bacterial load compared to pretreatment. This may indicate that mechanical motion of moist scrubbing during surgical preparation has a substantial effect on the reduction of bacterial load. One study comparing mechanical versus nonmechanical preparation with CHX in horses found no significant difference between treatments, but the reported CFU count was slightly lower for mechanical preparation than nonmechanical. This may be important in specific cases in which disinfectants need to be avoided, such as in cases of allergic reactions, mucous membrane preparation, or amphibian skin, among others. This also may introduce a confounding factor in the assessment of DP in this study. There was no baseline for the DP with disinfectant treatment, such as a second application of disinfectant or dry scrub with disinfectant, so it is possible the CFU counts may have been affected by the additional mechanical action during DP application.

Isopropyl alcohol is sometimes used in conjunction with CHX or PI rather than saline. However, alcohol was not used in this study due to the potential for hypothermia in avian species. Alcohol-based preoperative solutions have been shown to contribute to hypothermia in laboratory mice. Although not statistically analyzed, there was no obvious change in the temperature associated with the application of the treatments. We elected not to pursue this assessment due to the lack of adequate control groups (ie, a group of chickens undergoing anesthesia without application of treatments) and because the area of treatment application was small, which would be unlikely to impact body temperature in the authors’ opinion. Future studies investigating the impact of surgical scrub protocols on body temperature would be beneficial, especially in small-sized species.

Surgical site infections can lead to significant morbidity and mortality and are not uncommon in veterinary medicine, with reports as high as 25.9% in dogs and cats, depending on the procedure. In birds, no studies assessing the incidence of postoperative surgical site infections have been published; however, these appear to be rare. It is unclear why this appears to be the case. One could hypothesize that the feathered coat provides a protective layer to the surgical site or that the use of antiseptic solutions for surgical preparation may be more effective in birds. These hypotheses have not yet been investigated and may prove to be promising areas of future research.

The addition of DP to a surgical preparation protocol incurs increased cost and waste, given that DP is a single-use product. Future studies may assess the benefits of DP for surgical preparation in comparison to CHX or PI in the context of financial and ecological impact.

This study has several limitations. The treatments were not randomized for each patient, although they were performed in the same manner in every chicken. Additionally, the close proximity of the 3 treatment sites within a single apterium may have affected the results. There was the potential for crossover between sites, although this was minimized by swabbing the center of each treatment site. A single apterium, a tract of skin naturally lacking feathers, was used, which limited the available testing surface. However, by doing such an approach, the potential impact of feather plucking was removed, and manipulation of the area prior to testing was minimized (ie, manual extraction of the feathers). The use of several apteria would provide a larger area; however, it would also increase the variability of the samples as different areas may have different bacterial loads. Nevertheless, the use of cutaneous areas with plucked feathers may mimic more clinical scenarios since many surgical approaches are performed in feathered areas, including the coelom and limbs. In veterinary medicine, shaving or plucking is almost always necessary for appropriate surgical site visualization. In mice, the use of a depilatory agent versus electric clippers with subsequent PI preparation showed a comparable reduction in bacteria and surgical site infections. Further research is necessary to evaluate different preoperative preparation agents after feather plucking. Future studies may also compare additional antimicrobial agents and protocols as many different types are used in clinical practice. Another limitation is the small sample size, which is a frequent problem in veterinary and zoological companion animal research. However, the results reached statistical significance despite the small sample size. This study also utilized aerobic bacterial culture, which may not detect some bacterial species, including anaerobes. Future studies may consider the use of molecular analysis or other culture techniques to maximize the detection of pathogens. A nonblinded operator performed all CFU counts, although since this is a quantitative measure, any resulting bias is likely minimal. Lastly, different methods for counting bacterial CFU have been described, including manual counting protocols similar to this study as well as Replicating Organism Detection and Counting plates.

As hypothesized, DP decreased CFU further in areas first prepared with CHX and PI, but simply preparing the site with saline before applying DP yielded the greatest decrease. Unfortunately, we could not statistically compare these results because the study was not designed for such comparisons.

In conclusion, this data suggests that a scrub protocol including DP with saline, CHX, or PI are all acceptable in the preparation of a sterile surgical field in chickens. Additional studies in other species are needed to determine if this applies to other species. Future studies using clinical cases undergoing surgical procedures and assessing the incidence of postoperative infections and/or occurrence of adverse reactions to treatments are warranted.

Unauthenticated | Downloaded 06/30/24 06:18 PM UTC
Acknowledgments

The authors wish to thank Dianne Hudson and the Oklahoma Animal Diagnostic Disease Laboratory staff for their assistance in this study.

Disclosures

3M provided supplies for this study. However, the study design, including antiseptic types, was determined prior to contacting the company.

Dr. Brandão is a member of the AJVR Scientific Review Board, but was not involved in the editorial evaluation of or decision to accept this article for publication.

No AI-assisted technologies were used in the generation of this manuscript.

Funding

Financial support was provided by the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Oklahoma State University. The authors would like to thank Wade Kyle and 3M for providing supplies for this study.

References


AJVR


