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Original article

An *in vitro* Investigation into the efficacies of Chlorhexidine Gluconate, Povidone-iodine and Green Tea (*Camellia sinensis*) to Prevent Surgical Site Infection in Animals

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1 **Original article**

2

3 **An *in vitro* Investigation into the efficacies of Chlorhexidine Gluconate, Povidone-iodine 4
and Green Tea (*Camellia sinensis*) to Prevent Surgical Site Infection in Animals**

5

6 **Abstract**

7 Surgical site infections are common in veterinary practice; their prevention is based on the 8
preoperative use of topical antimicrobials at the surgical site to reduce resident bacteria to sub9
pathogenic levels. Chlorhexidine gluconate (CHG) and Povidone-iodine (PI) are the most 10 popular
options for preoperative skin preparation in veterinary practice due to their broad11 spectrum
antibacterial properties. However increasing bacterial resistance to CHG and PI have
12 been reported, therefore investigation into alternative antimicrobials such as *Camellia sinensis*
13 (green tea: GT) is required. The Kirby-Bauer disk diffusion method was used to test the
14 antibacterial activity of four dilutions of CHG, PI and GT on the normal flora of animal skin,
15 represented by *S. aureus*, *S. intermedius*, *S. uberis* and *S. pyogenes*. Zones of inhibition (ZOI)
16 were measured to assess antimicrobial action. Kruskal-Wallis analyses with Mann-Whitney 17
post-hoc tests determined differences in efficacy between the dilutions of antimicrobials for 18 each
bacterium tested.

19 All antimicrobials inhibited bacterial growth, CHG was more efficacious than PI and GT
20 ($P < 0.0001$; mean CHG: $24.02\text{mm} \pm 2.05\text{mm}$; mean PI: $4.46\text{mm} \pm 1.35\text{mm}$; mean GT:
21 $2.90\text{mm} \pm 2.60\text{mm}$). Although GT produced smaller ZOIs than PI, no significant differences in 22
efficacy existed ($P > 0.05$). The results suggest that CHG is the best antimicrobial for
23 preoperative skin preparation. GT did produce an antibacterial effect on three of the four 24 bacteria,
although this was inferior to the existing veterinary products used. Therefore GT in
25 the formulation tested is not recommended for use as a veterinary antimicrobial, however,

25 further investigations into the potential of the active ingredients in different formulations and
26 concentrations are warranted.

27 *Key words: Antimicrobial resistance; Green tea; Surgical site infection; Veterinary nursing*

28 *Highlights:*

- 29 1. Chlorohexidine has a superior topical antimicrobial activity *in vitro* than povidone-iodine.
- 30 2. Green tea has limited antimicrobial activity but this is inferior to established veterinary
31 topical antimicrobials.
- 32 3. Chlorohexidine appears to be the most effective topical antimicrobial for surgical
33 preparation of the veterinary patient.

34

35

36 **Introduction**

37 Surgical site infections (SSIs) are a type of iatrogenic infection occurring in wounds
38 postoperatively which can be a potentially life-threatening surgical complication within human
39 and veterinary medicine (Eugster et al., 2004; Cho et al., 2008; Hemani and Lepor, 2009). SSIs
40 can result in pain, delayed healing and wound breakdown and, because preventable, could be
41 deemed ‘unavoidable suffering’ (Jennings and Berdory, 2010). Therefore effective SSI
42 prevention is essential to maintain the health and welfare of surgical patients.

43

44 It is widely believed that the patient’s skin is the main source of surgical wound contamination,
45 with *Staphylococci* and *Streptococci* bacterial species most frequently cultured from veterinary
46 SSIs (Darouiche et al., 2010; Hutchinson, 2012; Roberts, 2013). These species represent the

47 normal bacteria of the skin and are usually non-pathogenic, but can cause infection when they
48 enter a wound (Bowers, 2012; Roberts, 2013). SSIs account for 15% of human nosocomial
49 infections, prolong hospitalisation and increase morbidity and mortality, in turn increasing the
50 cost of surgery in humans (Durani and Leaper, 2008; Reichman and Greenberg, 2009).
51 Veterinary SSI incidence occurs at an estimated 3% in surgical patients (Frey et al., 2006;
52 Fitzpatrick and Solano, 2010; Turk et al., 2015) with livestock surgery that often occurs outside
53 the surgical environment *in situ* increasing SSI risk >30% (Fubini and Ducharme, 2004; Weaver
54 et al., 2005; Verwilghen and Singh, 2014). Investigations into the microbiology of SSIs have
55 demonstrated wide bacterial diversity (Owens and Stoessel, 2008; Wolcott et al., 2009; Turk et
56 al., 2015); causal bacteria implicated include *Staphylococci* species (74%), with *Staphylococcus*
57 *aureus* and coagulase-negative *Staphylococci* most commonly reported (Owens and Stoessel,
58 2008; Turk et al., 2015).

59 An estimated 40-60% of SSIs are avoidable (Uckay et al., 2010) and prevention of SSIs can be
60 relatively simple. Preoperative skin preparation¹ (PSP) with an appropriate antimicrobial is
61 commonly practiced by veterinary professionals to decrease resident bacteria to sub-pathogenic
62 levels, reducing SSI risk (Durani and Leaper, 2008; Roberts, 2013). Prophylactic antibiotics
63 were once recommended to prevent SSIs, however due to increasing bacterial antibiotic
64 resistance these are no longer advised (Knights et al, 2012; Turk, 2013). The most commonly
65 used antimicrobials in veterinary medicine are chlorhexidine gluconate (CHG) and
66 Povodineiodine (PI) (Hemani and Lepor, 2009; Bowlt and Gasson, 2013; Rutter et al., 2014).
67 Manufacturers recommend the use of undiluted product (BCM, 2013; Animalcare, 2014),
68 however in veterinary practice antimicrobials are commonly diluted in a solution with water

¹ Preoperative skin preparation: The reduction of resident and transient bacteria from the skin using swabs soaked with an antiseptic and applied to the skin in a methodological fashion, often followed by application of surgical spirit. It is impossible to make the skin sterile, so reduction of bacteria to sub-pathogenic levels is the aim (Bowers, 2012).

69 and soaked swabs used for application (McHugh et al, 2011; Aspinall, 2014). SSI risk increases
70 if the dilution is too weak, providing inadequate antibacterial action (Kampf and Kramer, 2004;
71 Evans et al., 2009). For example, it has been demonstrated that dilutions containing less than
72 3% CHG reduce but do not eliminate bacteria, and are ineffective against *Staphylococcus*
73 *aureus* (Evans et al., 2009; Montevecchi et al., 2013).

74

75 CHG is an aqueous antimicrobial, effective against Gram-positive and negative bacteria, yeasts
76 and some viruses (Hemani and Lepor, 2009; Reichman and Greenberg, 2009; Macias et al.,
77 2013). It has bactericidal action by disturbing bacterial cell membranes and increasing cell wall
78 permeability, facilitating bacteriolysis (Popovich et al., 2009; Karki and Cheng, 2012; Edmiston
79 et al., 2013). CHG binds to the surface of the skin to produce a residual effect, enabling lasting
80 antibacterial activity long after application (Anderson et al., 2010; Sogawa et al., 2010). It is
81 thought that CHG products are also able to delay the recolonization of resident bacteria (Rutter
82 et al., 2014). PI is another popular topical antimicrobial choice, as it is broad-spectrum and safe
83 for application to mucous membranes (Hemani and Lepor, 2009). It is a water-soluble complex
84 of iodine and a carrier, polyvinylpyrrolidone, which acts as a reservoir of the active ingredient
85 free iodine (Anderson et al., 2010). PI is an aqueous-based iodophor, whose antimicrobial
86 properties relate to its ability to destroy bacterial proteins and DNA (Hemani and Lepor, 2009).
87 PI is considered to have some level of persistence, although comparatively it is thought that
88 CHG has a longer residual effect (Art, 2005; Sogawa et al., 2010; Pelligand, 2012). Few studies
89 have compared the efficacy of PI with other antiseptics; however cultures from surgical sites
90 30 minutes post-application have suggested that CHG is more effective than PI (Culligan et al.,
91 2005). Other studies have also reported a superior effect of CHG when compared to PI, with
92 lowered colony counts at the surgical site after PSP (Art, 2007; Macias et al., 2013).
93 Interestingly, limited research has evaluated the synergy between antimicrobials; synergism

94 between CHG and PI has been reported but further research into the potential combination of
95 antimicrobials for SSI reduction in practice is needed (Anderson et al., 2010).

96

97 Resistance to both CHG and PI has been recorded, therefore it is becoming increasingly
98 important to establish novel, safe alternatives (Reichman and Greenberg, 2009; Anita et al.,
99 2015). There has been interest in the antimicrobial effects of tea for many years; recent *in vitro*
100 and *in vivo* studies into *Camellia sinensis* (green tea: GT) have provided a better understanding
101 of its antimicrobial properties (Kumar et al., 2012; Sharma et al., 2012). GT contains medically
102 important compounds including polyphenols (Taylor et al., 2005; Silva et al., 2013) and
103 flavonoids (Reygaert, 2014). Flavonoids, for example catechins, constitute approximately
104 30-40% of dry leaf weight and are thought to account for GT's antimicrobial properties (Taylor
105 et al., 2005; Almajano et al., 2008; Reygaert, 2014). Catechins have a direct antibacterial effect
106 by binding to the lipid bilayer of bacterial cell membranes, thus causing damage and preventing
107 bacteria forming biofilms (Reygaert, 2014). Fatty acids are a major component of the
108 phospholipid bilayer of the cell membrane and there is evidence that GT catechins, especially
109 epigallocatechin-3-gallate (ECGC), can also inhibit enzymes that are required for fatty acid
110 synthesis (Singh et al., 2011; Wang and Ma, 2013) and DNA replication, enabling them to
111 possess potent antibacterial activity (Grandišar et al., 2007).

112

113 Mechanisms of action differ between antimicrobials; CHG affects cell membranes, PI targets
114 intracellular molecules and GT uses a combination of both mechanisms (Li et al., 2006; Jones,
115 2007; Durani and Leaper, 2008; Reygaert, 2014). Research to directly compare the efficacies
116 of such antimicrobials has not yet been conducted, therefore this study aimed to provide a
117 framework for further research into the potential use of *Camellia sinensis* (GT) in veterinary

118 PSP, by comparing its efficacy at preventing bacterial growth of *Staphylococci* and *Streptococci*
119 species to that of CHG and PI *in vitro*.

120

121 **Materials and Methods**

122 The Kirby-Bauer disk diffusion method (disk diffusion) was used to determine the *in vitro*
123 susceptibility of *Staphylococcus aureus* (ATCC 25923), *Staphylococcus intermedius* (ATCC
124 29663), *Streptococcus uberis* (ATCC 9927) and *Streptococcus pyogenes* (ATCC 19615) to
125 dilutions of 4% CHG, 10% PI and GT (Block and Furman, 2002; Cappuccino and Sherman,
126 2008; Hudzicki, 2013; Vetbact, 2015).

127

128 Commercially available CHG (Hibiscrub™) and PI (Vetasept™) products contain 4%
129 chlorhexidine gluconate and 10% PI, the active ingredient respectively. Based on previous
130 research, 10%, 5%, 2.5% and 1.25% dilutions of CHG, PI and GT were prepared (Yassen et al.,
131 2011). A 0% dilution (distilled water) was used as a control. Fresh dilutions were prepared daily
132 to avoid degradation. The appropriate measure of undiluted CHG or PI was mixed with sterile
133 0.9% saline as a buffer to obtain 1ml of the required dilution; sufficient volume to impregnate
134 six filter disks per plate. For example, the 10% dilution was prepared using 0.9ml sterile saline
135 mixed with 0.1ml CHG in a sterile Eppendorf tube.

136

137 Dilutions of green tea were prepared using dried green tea leaves (Clipper®) and distilled water.
138 For the 10% dilution, 10g loose dried green tea leaves was added to 90ml water; this was
139 repeated for each dilution, for example 5g tea added to 95ml water for a 5% dilution. Following
140 the manufacturer's recommendations, distilled water was boiled, measured and left to cool for
141 one minute before pouring over the leaves. The solution was stirred once and left to infuse for

142 two minutes. Then 1.5ml of the liquid was decanted into a sterile Eppendorf tube using an
143 automatic pipette with a sterile tip, and allowed to cool before application to prevent heat
144 destroying the bacteria. The process was repeated for each dilution of tea.

145

146 *Inoculation and Application of Antimicrobials*

147 Sixteen plates were prepared for inoculation with each bacterium: *Staphylococcus aureus*,
148 *Staphylococcus intermedius*, *Streptococcus uberis* and *Streptococcus pyogenes*. The lawn
149 method of inoculation was used to inoculate Mueller-Hinton agar plates to promote an even
150 layer of bacterial growth, useful for susceptibility testing (Parija, 2009; Driscoll et al., 2012)
151 (Fig. 1).

152 *Application of Filter Disks and Antimicrobials*

153 Following the Kirby-Bauer disk diffusion test protocol (Eucast, 2015), six filter disks were
154 applied to the surface of the inoculated agar of each plate and pressed down gently with forceps
155 to ensure complete contact with the agar. The disks were spaced evenly to allow visualisation
156 of clear zones of inhibition (ZOI). Each filter disk represented a repeat therefore there were six
157 repeats per antimicrobial dilution per bacterium (Parija, 2009). An automatic pipette was then
158 used to apply 10µl of antimicrobial to each filter disk, avoiding surface pooling which could
159 cause inaccurate results. Plates were then incubated at 37°C for 20 hours prior to collection of
160 results (Hudzicki, 2013).

161

162 *Data Collection*

163 The diameters of ZOIs (DZOIs) were measured in millimetres using Vernier callipers. In the
164 case that two ZOIs overlapped, the radius of the zone was measured and multiplied by two to
165 produce an estimate of the diameter. If there was no ZOI the bacterium was considered resistant,
166 with DZOIs recorded as 0mm (Hudzicki, 2013).

167

168 *Data Analysis*

169 The mean and standard deviation for DZOI for the six filter disks (n=92) on each plate (n=16)
170 for each bacterium were calculated using Microsoft Excel, version 2013. Data were analysed
171 using Statistical Package for the Social Sciences, version 20. A series of Kruskal-Wallis
172 analyses were undertaken to determine the presence of a difference between the DZOI, with
173 increased diameters representing enhanced efficacy, for each of the four bacterial species, for
174 dilutions within each antimicrobial, between the antibacterials and to compare synthetic versus
175 natural products (Field, 2009). Subsequent post-hoc testing was completed using MannWhitney
176 U tests with a bonferroni adjustment applied to the post-hoc alpha value due to the inclusion of
177 repeated groups, to prevent the occurrence of Type I errors (revised significance level: $P < 0.016$)
178 (Field, 2009).

179

180 **Results**

181 All dilutions of CHG were effective at reducing bacterial growth in all species, demonstrated
182 by clear zones of bacterial inhibition around the disks, and were larger than the other
183 antimicrobials; dilutions of PI and GT were less effective at bacterial reduction, demonstrating

184 largely similar ZOI (Fig. 2). Significant differences were found between mean DZOIs for the
185 three antimicrobials ($P=0.0001$). Subsequent post-hoc testing indicated CHG (mean ZOI:
186 $24.02\text{mm}\pm 2.05$) was more effective than both PI (mean ZOI: $4.46\pm 1.35\text{mm}$) and GT (mean
187 ZOI: $2.90\pm 2.60\text{mm}$). No significant difference in DZOI was found between PI and GT
188 ($P=0.022$).

189

190 *CHG versus PI*

191 CHG inhibited growth of all species of bacteria tested; in contrast, only 10% PI mimicked this
192 action. Mean DZOIs for CHG across all dilutions ($24.04\pm 2.05\text{mm}$) were larger than PI DZOIs
193 ($4.46\pm 1.35\text{mm}$), with the ZOI of 1.25% CHG 49% larger than 10% PI. Differences were
194 exposed between the mean DZOI for the different dilutions of CHG and PI for all bacteria tested
195 ($P<0.0001$) (Table 1). Enhanced efficacy was found for all dilutions of CHG compared to all
196 dilutions of PI for all bacteria ($P<0.001$) (Fig. 2). Further significant differences in antimicrobial
197 efficacy were exposed between the mean DZOIs of the variable dilutions of PI ($P<0.0001$);
198 DZOI diameter expanded with increased concentration
199 (10%: $10.32\pm 0.94\text{mm}$;

200 5%: $4.44\pm 3.65\text{mm}$; 2.5%: $1.37\pm 2.38\text{mm}$) (Table 1). In contrast, no significant difference in
201 CHG efficacy was found between all dilutions, across all bacteria ($P>0.05$).

202

203 *Dilutions of Green Tea*

204 GT appeared effective against three of the four bacteria tested; 10%, 5% and 2.5% dilutions of
205 GT were effective against *S. pyogenes*, in contrast only 10% GT was effective against *S. aureus*
206 and *S. uberis*. *S. intermedius* was unaffected by the presence of GT, with no ZOI evident for

207 any dilution. The 10% dilution was most effective (mean DZOI: 7.17 ± 4.23 mm), whereas 2.5%
208 produced the smallest effect (mean DZOI: 2.08 ± 3.61 mm) and 1.25% GT was ineffective
209 against all bacteria (Table 2). No differences in efficacy ($P > 0.05$) were found between the
210 dilutions of GT when used on *S. intermedius* (Table 2). Efficacy did vary significantly ($P < 0.02$)
211 between dilutions against the remaining bacteria: the 10% dilution of GT exhibited an
212 antimicrobial action against *S. aureus* and *S. uberis*, however all dilutions of GT prohibited
213 growth of *S. pyogenes* (Table 3).

214

215 *Synthetic versus Natural Antimicrobials*

216 The synthetic antimicrobials produced a significantly higher mean DZOI ($P < 0.0001$) for all
217 bacteria species (mean DZOI: 14.24 ± 1.59 mm) when compared to GT (mean DZOI:
218 2.90 ± 2.60 mm) (Fig. 3). Interestingly, no significant difference ($P > 0.05$) was found between
219 mean DZOIs of PI and GT, with the exception of *S. intermedius* (where GT was ineffective at
220 all dilutions; $P < 0.001$). However CHG was significantly more effective than GT against all
221 bacteria tested ($P < 0.0001$).

222 **Discussion**

223 CHG proved the superior antimicrobial against the bacterial species tested in this study; CHG
224 has previously been reported to produce a superior effect in comparison to other antimicrobials
225 including PI, supporting the observed results (Darouiche et al., 2010; Jarral et al., 2011; Kunkle
226 et al., 2014). Manufacturers' guidelines indicate that Vetasept and Hibiscrub are recommended
227 for undiluted use during PSP (Animalcare, 2014, BCM, 2013), as there are no clear guidelines
228 and conflicting recommendations for PSP with CHG within the veterinary literature it could be
229 assumed that it could be used diluted or undiluted in veterinary practice (BCM, 2013; Aspinall,
230 2014). The antimicrobial action of the dilutions tested here suggest this assumption would not

231 enhance the risk of SSIs. In contrast, the application of diluted PI reduced its antimicrobial
232 action and could increase the risk of SSIs. In veterinary practice both PI and CHG are used
233 diluted, perhaps at insufficient concentrations, and applied using soaked swabs (McHugh et al.,
234 2012; Aspinall, 2014), often followed by application of iodophor alcohol (surgical spirit).
235 Alcohol has been shown to have a short acting broad spectrum antimicrobial activity which
236 complements the action of CHG (Hemani and Lepor, 2009). A dilution ratio of 50:50 CHG
237 solution: water followed by a final surgical spirit application is therefore recommended for use
238 for surgical site preparation in the veterinary patient (Roberts, 2013; Hemani and Lepor, 2009).
239 . However further investigations into antimicrobial dilutions used *in vivo* and incidence of SSIs
240 are warranted to determine the impact of common practices on SSI risk.

241

242 The comparison of relative dilutions of CHG and PI demonstrated that CHG produced a far
243 superior effect when used on the bacteria tested, indicating that CHG should be the preferred
244 choice for PSP in most surgeries. The superior antimicrobial action of CHG against the bacteria
245 tested in this study, may be related to CHG's 99% bacterial kill rate within 30 seconds of
246 application coupled with its residual activity; this may have initially eliminated the bacteria in
247 direct contact with the antimicrobial as it diffused through the agar, producing clear zones
248 around the disks as less bacteria were available to colonize (Orpet and Welsh, 2010; Macias et
249 al., 2013). Despite evidence of diffusion through the agar, PI and GT did not produce a
250 comparative residual effect; however it is possible some regrowth occurred during incubation.
251 It is possible that PI could produce a superior effect when used *in vivo*, therefore further research
252 to determine this is indicated (Osuna et al., 1992).

253

254 It is commonly perceived that PI is effective at reducing resident skin bacteria so is often used
255 in veterinary practice, whereas the results obtained here suggest poor efficacy (Bowers, 2012).
256 These results may indicate an over-reliance on PI for SSI reduction in the veterinary industry
257 (Hedalgo and Dominguez, 2001; Hemani and Lepor, 2009; Bowlt and Gasson, 2013). This is
258 particularly true for large animal surgery where PI is often used, especially during field
259 procedures, despite having been demonstrated to be less effective than CHG in these species
260 (Desrochers et al., 1996, Wilson et al., 2011). The results suggest that CHG would be
261 recommended as the superior produce for PSP, however as its use is contraindicated in certain
262 surgeries such as ophthalmic, aural or neurosurgery, PI still plays an important role in SSI
263 prevention in some cases (Denton, 2001; Roberts, 2013). These results could assist veterinary
264 professionals make an informed decision on the best antimicrobial product for use during
265 general PSP.

266

267 pH can influence the antimicrobial activity of the products tested; PI is active at pH 3 to 5.5
268 whereas Mueller-Hinton agar is pH 7.4 ± 0.2 , which could account for the reduced performance
269 observed (Atlas and Snyder, 2006; Animalcare, 2014). It has been found that normal animal
270 skin is approximately pH 6; although more acidic than Mueller-Hinton, this is still outside the
271 active pH range of PI, which could adversely impact its efficacy *in vivo* (Meyer and Neurand,
272 1991). No recommendations for pH are available for CHG. Contact times are another important
273 factor in the determination of the efficacy of antimicrobials, with increasing time related to
274 superior efficacy (Koburger et al., 2009). However Evans et al. (2009) found that the majority
275 of veterinary nurses were unaware of contact times used during PSP, suggesting that educating
276 veterinary professionals in the appropriate use of antimicrobials may be an equally important
277 factor in future SSI reduction.

278

279 *Efficacy of Green Tea*

280 Demand for natural products is growing in many industries, with particular interest in natural
281 pharmaceuticals and an emphasis on antimicrobials due to increasing bacterial resistance
282 (Jacob, 2014; Sharif et al., 2014; Ling et al., 2015). However, GT was not found to have
283 comparative antimicrobial properties to CHG and PI. Ten percent GT was the most effective
284 dilution tested (mean DZOI 7.16 ± 4.23 mm) and 2.5% the least effective (mean: 2.08 ± 3.61 mm).
285 Growth within the GT ZOI was evident, suggesting some concentrations were not sufficient to
286 clear all bacteria. It is probable that the higher the concentration of GT, the more numerous the
287 water-soluble catechins present. As these possess antibacterial properties, a direct correlation
288 would be expected between increasing concentrations of GT and antibacterial activity (Cushnie
289 and Lamb, 2005). A manufactured antimicrobial based on GT may be more appropriate for PSP
290 than the infused preparation used here, to ensure adequate concentrations of catechins are
291 present. Synergistic effects have also been observed when GT and antimicrobials are used
292 concurrently. Therefore the addition of GT catechins to skin preparation products such as CHG
293 and PI could enhance their antibacterial activity (Tiwari et al., 2005; Archana and Abraham,
294 2011; Reygaert, 2014). Further research investigating GT used solely or in combination with
295 other antimicrobials for SSI prevention is therefore needed.

296

297 A sample of bacteria were selected here to represent normal which a diverse range of bacterial
298 species (Grice and Segre, 2011). Therefore to fully determine the efficacy of an antimicrobial
299 for skin preparation it should be tested on all bacteria that might be present *in vivo*. Future
300 research could culture swabs taken directly from animal skin to test the efficacies of
301 antimicrobials. It is also possible that *in vitro* results may not directly correlate with *in vivo*

302 efficacy (Hardy Diagnostics, 2015), consequently the conclusions drawn from this study should
303 be interpreted with caution.

304

305 **Conclusions**

306 The results of this study advocate the use of CHG as the most effective topical antimicrobial for
307 surgical preparation of the veterinary patient. Whilst GT was shown to exhibit some
308 antibacterial action against common skin bacteria, this was inferior to veterinary antimicrobials
309 currently used in PSP. Therefore GT in the formulation tested is not recommended for use as a
310 veterinary antimicrobial.

311

312 **Conflict of interest statement**

313 None of the authors has any financial or personal relationships that could inappropriately
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318

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528 *aureus*. *Antimicrobial Agents and Chemotherapy*, **45**(6): 1737-1742.

529

530

531 **Tables**

532 Table 1: Significant differences observed between dilutions of Povidone-iodine.

533 *Zones of inhibition for each dilution of Povidone-iodine were tested to compare efficacy of the different dilutions*
534 *across all bacterial species evaluated: Staphylococcus aureus, Staphylococcus intermedius, Streptococcus uberis*
535 *and Streptococcus pyogenes; '>' indicates increased efficacy i.e. reduced diameters for zones of inhibition.*

536

| Dilution of PI | Result |
|----------------|----------|
| 10% > 5% | P=0.0001 |
| 10% > 2.5% | P=0.0001 |
| 10% > 1.25% | P=0.0001 |
| 5% > 2.5% | P=0.0001 |
| 5% > 1.25% | P=0.0001 |
| 2.5% and 1.25% | P=0.039 |

537

539 Table 2: Mean diameters of zones of inhibition (mm) for each dilution of Green tea for each
 540 bacterium; ZOI: zone of inhibition.

541 *Mean zones of inhibition were measured for each bacteria species tested and an average overall zone of inhibition*
 542 *was calculated.*

| Dilution rate | <i>Staphylococcus aureus</i> Mean ZOI (mm) | <i>Staphylococcus intermedius</i> Mean ZOI (mm) | <i>Streptococcus uberis</i> Mean ZOI (mm) | <i>Streptococcus pyogenes</i> Mean ZOI (mm) | Mean ZOI across all bacterial species ±standard deviation |
|----------------------|--|---|---|---|--|
| 10% | 9.67 | 0 | 8.25 | 10.75 | 7.16±4.23 |
| 5% | 0 | 0 | 0 | 9.33 | 2.33±4.04 |
| 2.5% | 0 | 0 | 0 | 8.33 | 2.08±3.61 |
| 1.25% | 0 | 0 | 0 | 0 | 0±0 |

543

544

545 Table 3: Differences observed between dilutions of Green tea; bold values represent
 546 significant results (Bonferroni adjusted P value<0.02; bold values indicate a significant
 547 difference exists).

548 *Zones of inhibition for each dilution of green tea were tested to compare efficacy of the different dilutions across*
 549 *the three bacterial species where growth was inhibited: Staphylococcus aureus, Streptococcus uberis and*
 550 *Streptococcus pyogenes; '>' indicates increased efficacy i.e. reduced diameters for zones of inhibition.*

| Dilution rate | <i>Staphylococcus aureus</i> | <i>Streptococcus uberis</i> | <i>Streptococcus pyogenes</i> |
|----------------------|-------------------------------------|------------------------------------|--------------------------------------|
| 10% > 5% | P=0.002 | P=0.015 | P=0.009 |
| 10% > 2.5% | P=0.002 | P=0.015 | P=0.002 |
| 10% > 1.25% | P=0.002 | P=0.015 | P=0.002 |
| 5% > 2.5% | P=1.000 | P=1.000 | P=0.015 |
| 5% > 1.25% | P=1.000 | P=1.000 | P=0.002 |
| 2.5% > 1.25% | P=1.000 | P=1.000 | P=0.002 |

551

552 **Figure legends**

553

554 Fig. 1: Method of Inoculation.

555 *As homogenous plating is essential for reliable results, a sterile inoculating loop was used to streak the agar*
556 *with the corresponding bacterium (Hendrikson, 2002). The surface of the agar was streaked from the top to the*
557 *centre, turned 90° and repeated until the plate had been turned a full 360° (Cappuccino and Sherman, 2008).*

558 Fig. 2: Mean zones of inhibition for each dilution of antimicrobial for each bacterium

559 *The diameters of the zones of inhibition (ZOI) for each dilution of the three antimicrobials: chlorohexidone (CHG),*
560 *povodiine-iodine (PI) and green tea (GT) were measured and a mean overall ZOI calculated for each dilution rate*
561 *to enable comparison of their efficacy across all bacterial species evaluated: Staphylococcus aureus,*
562 *Staphylococcus intermedius, Streptococcus uberis and Streptococcus pyogenes; '>' indicates increased efficacy*
563 *i.e. reduced diameters for zones of inhibition.*

564

565 Fig. 3: Mean diameter of zones of inhibition for natural and synthetic antimicrobials

566 *The diameters of the zones of inhibition (ZOI) surrounding each impregnated filter disk were measured and an*
567 *average total ZOI calculated for synthetic antimicrobials: chlorohexidine and povodine-iodine, and green tea to*
568 *enable a comparison of their efficacy to be undertaken.*







