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Bactericidal Efficacy of a Disinfectant Solution Composed to Povidine-iodine Against *Salmonella typhimurium* and *Brucella ovis*

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ABSTRACT - *Salmonella* spp. and *Brucella* spp. are associated with considerable diseases of both humans and animals. In addition, these microorganisms cause the economic loss in animal farming and food industry. In this study, the disinfection efficacy of a commercial disinfectant, composed to povidone-iodine was evaluated against *S. typhimurium* and *B. ovis*. A bactericidal efficacy test by broth dilution method was used to determine the lowest effective dilution of the disinfectant following exposure to test bacteria for 30 min at 4°C. The disinfectant and test bacteria were diluted with hard water (HW) or organic matter suspension (OM) according to treatment condition. On HW condition, the bactericidal activity of the disinfectant against *S. typhimurium* and *B. ovis* was 400 and 150 fold dilutions, respectively. On OM condition, the bactericidal activity of the disinfectant was 5 and 20 fold dilutions against *S. typhimurium* and *B. ovis*, respectively. As the disinfectant composed to povidine-iodine possesses bactericidal efficacy against animal pathogenic bacteria such as *S. typhimurium* and *B. ovis*, the disinfectant solution can be used to control the spread of bacterial diseases.

Key words : Povidine-iodine, *Salmonella typhimurium*, *Brucella ovis*, Disinfectant efficacy

Salmonella is a genus of gram-negative and facultatively anaerobic, rod-shaped bacteri[1)] which extensively causes self-limiting enteritis, fatal infection in animals, food-borne infection, and typhoid fever in humans[2-4)]. *Salmonella* infections of food animals play an important role in public health and particularly in food safety, as food products of animal origin are considered to be the major source of human *Salmonella* infections[5)]. *Salmonella typhimurium* (*S. typhimurium*) is one of the most frequently isolated serotypes from pig farms, slaughtered swine, and associated with human foodborne diseases[6,7)].

The Brucellae are non-motile gram-negative coccobacilli or short small rods 0.6-1.5 × 0.5-0.7 μm without bipolar staining, not encapsulated bacteria[8)]. Based on differences in pathogenicity and host preference, six species are recognized within the genus *Brucella*[9,10)]. *Brucella abortus*, *Brucella melitensis* and *Brucella suis* are responsible for bovine brucellosis, ovine and caprine brucellosis, and swine brucellosis, respectively. These three *Brucella* species may cause abortion in their hosts, which could result in huge economic losses. In addition, *Brucella ovis* (*B. ovis*) is responsible for lamb epididymitis[11)].

As antibiotic-resistant strains of *Salmonella* and *Brucella* are increasing due to abuse and overuse of antibiotics, the effective cleaning and disinfection regimes are essential for the prevention of infections and outbreaks[12,13)]. The cleaning and disinfectant regimes depend on the proper use of biocides, and there is the concern that the resulting increased use of biocides in farming, food production, and hospital settings, and the home could contribute to the selection of antibiotic-resistant strains as some mechanisms of biocide resistance also confer antibiotic resistance[14)]. Biocides are often composed of a mixture of ingredients that act upon a wide range of cellular mechanisms and targets, which makes it difficult for bacteria to become resistant to biocides[12)].

Salmonellosis and brucellosis in livestock animals and human cause enormous economic loss in the world[15,16)]. The use of disinfectant is very effective for successful control of diseases from bacteria, fungi and parasites in farm animals[17,18)]. Several disinfectants including chlorine dioxide, betaine hydrochloride and propylene glycol have been used for decontamination of farmed animal and food borne diseases[19-22)]. However, there is not the efficacy test for the disinfectant composed of povidone-iodine (PVI) against

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pathogenic bacteria. Therefore, this study was carried out to examine bactericidal efficacy of a disinfectant solution against *S. typhimurium* and *B. ovis*.

Materials and methods

Bacteria and culture

The test bacteria, *S. typhimurium* (G-B-14-21-62) and *Brucella ovis* (ATCC 25840) were obtained from the Korean Veterinary Culture Collection (KVCC, Seoul, Korea). The strains were maintained as frozen glycerol stock. *S. typhimurium* were cultured in Luria-Bertani (LB) broth containing 1.5% agar. *B. ovis* were spread in Brucella broth containing 5% fetal bovine serum and incubated at 37°C under 5% CO_2 condition.

Disinfectant

The active ingredient for the tested disinfectant solution, Betadine® Solution-Concentrate, is PVI 10 g per 100 mL. Betadine® Solution-Concentrate was provided by Korea Parma Co., Ltd. (Seoul, Korea). The disinfectant solution was stored in the dark in room temperature and prepared for dilution on the day of evaluation. Determination of the antimicrobial efficacy of the disinfectant was based on Animal and Plant Quarantine Agency (Anyang, Korea), Regulation No. 2013-34.

Diluents and treatment condition

Testing was based on bactericidal effects of disinfectant diluents in two treatment conditions (standard hard water (HW) condition and organic matter (OM) condition) and pathogen control (disinfectant negative control) in Table 1. HW, an ingredient of HW treatment condition, was made by adding anhydrous $CaCl_2$ 0.305 g and $MgCl_2 \cdot 6H_2O$ 0.139 g into one liter distilled water. Organic suspension, an ingredient of OM treatment condition, is a solution of 5% (w/v) yeast extract in HW. The test organisms were prepared by titration of each cultural broth into at least 10^8 CFU/mL viable organisms with the same kind of diluents of treatment condition.

Table 1. Experimental design for the determination of the bactericidal efficacy of the disinfectant composed to PVI

Treatment condition*	Contents according to treatment condition**			
	HW	OM	Disinfectant	Bacteria
HW condition	+	-	+	+
OM condition	+	+	+	+
Bacteria control	+	-	-	+

*HW, standard hard water; OM, organic matter.
**+, presence; -, absence

Experimental procedures

For the efficacy test against *S. typhimurium*, the disinfectant was diluted 320, 360, 2.5, 400, 440, 480 and 520 times with HW, and diluted 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 times with OM, respectively. For the efficacy test against *B. ovis*, the disinfectant was also diluted 120, 135, 150, 165, 180 and 195 times with HW, and diluted 16, 18, 20, 22, 24 and 26 times with OM, respectively.

To verify the lowest effective dilution of the disinfectant, five serial dilutions of the disinfectant were prepared and placed at 4°C prior to test reaction. 2.5 ml of each disinfectant dilution was mixed with the same amount of test organism followed by contact time of 30 min at 4°C. During this period, the mixture was shaken at 10 min interval. At the end of 30 min contact period, one mL of the mixture was neutralized with 9 mL of Nutrient broth containing 5% inactivated horse serum (BD Korea Co., Ltd., Incheon, Korea) at 37°C. 0.1 mL of the neutralized reaction mixture was subcultured into 10 mL of recovery each cultural broth at 37°C for 48 h in incubator. For the test of each bacteria control, 2.5 ml of hard water was mixed with the same amount of each test organism followed by contact time of 30 min at 4°C, and then all procedure were undertaken in parallel for the disinfection test.

The valid dilution of the disinfectant was determined that the greatest dilution showing no growth in four or more in the five replicates was confirmed. The final dilution time was statistically determined by a median value among three valid dilution of the triplicate test, but each value of which should be within 20% experimental error. In each bacteria control, the number of bacterial growth in the five replicates was counted.

Results

Table 2 shows the final valid dilution of the disinfectant composed to PVI. When the bactericidal effect on HW condition was evaluated, the antibacterial activity of the disinfectant showed on 400 and 150 fold dilutions against *S. typhimurium* and *B. ovis*, respectively. With the investigation of the bactericidal effect of the disinfectant on OM condition, *S. typhimurium* and *B. ovis* were inactivated on 5 and 20 fold dilutions, respectively. Because the organic material interferes with efficacy by either inactivating the disinfectant or blocking it from surface contact, the bactericidal activity of the disinfectant on the OM condition was lowered against animal pathogenic bacteria compared with HW conditions.

Comparing the results of the disinfectant against two pathogenic bacteria in the present study, the bactericidal effect of the disinfectant composed to PVI against *B. ovis*

Table 2. Final valid dilution of the disinfectant composed to PVI against *S. typhimurium* and *B. ovis*

Bacterial strains	Treatment condition*										
	HW				OM				BC		
	DT	1	2	3	DT	1	2	3	1	2	3
S. typhimurium	320	O	O	O	4.0	O	O	O	+	+	+
	360	O	O	O	4.5	O	O	O	+	+	+
	400	O	×	O	5.0	O	O	O	+	+	+
	440	×	×	×	5.5	×	×	×	+	+	+
	480	×	×	×	6.0	×	×	×	+	+	+
	520	×	×	×	6.5	×	×	×	+	+	+
	Valid		400		Valid		5.0			+	
B. ovis	120	O	O	O	16	O	O	O	+	+	+
	135	O	O	O	18	O	O	O	+	+	+
	150	×	O	O	20	O	×	O	+	+	+
	165	×	×	×	22	×	×	×	+	+	+
	180				24				+	+	+
	195	×	×	×	26	×	×	×	+	+	+
	Valid		150		Valid		20			+	

*HW, standard hard water; OM, organic matter; BC, bacterial control; DT, dilution time.
O, growth; ×, growth inhibition; +, all growth in each replicate.

was higher than that against *S. typhimurium* on the OM condition and was lower than that against *S. typhimurium* on the HW condition. In each bacterial control, the growth of *S. typhimurium* and *B. ovis* was verified in all replicates.

Discussion

The disinfectant composed to PVI is a potential antibacterial disinfectant. PVI is a stable chemical complex of polyvinylpyrrolidone (povidone, PVP) and elemental iodine[23]. In addition, PVI is a highly efficient broad-spectrum germicidal agent and effective against bacteria, viruses, fungi, and protozoa[24]. It is widely-used for topical cleansing and wound treatment. PVI releases free iodine, which has an important role in the bactericidal effect of PVI solution through the oxidizing effects of released iodine on proteins and fatty acids. Similarly, through the cytotoxic effects of free iodine, PVI is also an effective tumoricidal agent that may be used as an irrigation fluid to eradicate free cancer cells during head, neck, and colorectal cancer surgery[25-27]. In addition, in contrast to various antibiotic substances, which act on the cell walls, PVI not only destroys bacteria, but also effectively inhibits the release of pathogenic factors such as exotoxins, endotoxins and tissue-destroying enzymes[28].

Although the slow release of iodine from the PVI complex in solution minimizes iodine toxicity towards mammalian cells, the iodine is delivered to the bacterial cell surface where it penetrates the cell membrane and inactivates key cytosolic proteins, fatty acids, and nucleotides[29]. According to the MSDS of 10% PVI[30], PVI has been reported to be a mild skin and eye irritant in animals, and oral lethal dose 50 (LD_{50}) of PI in rat and mouse was over than 8.0 g/kg and 8.1 g/kg, respectively. In addition, Xia *et al*.[31] carried out the animal test to observe the toxicity and irritation to skin for PVI. In results, the oral toxicity LD_{50} of PVI for both rats and mice was 10 g/kg body weight, and PVI containing available iodine 6 g/L was non-irritating to rabbit skin and eye. Furthermore, Park *et al*.[32] investigated the toxicity of ten commercial disinfectants including PVI on chinook salmon embryo-214 cell line and flounder (*Paralichthys olivaceus*), black rockfish (*Sebastes pachycephalus*) and black sea bream (*Acanthopagrus schlegelii*). In results, the concentration of minimal toxicity for PVI and sodium hypochlorite dioxide was 50 and 12.5 mg/L, respectively, and the LC_{50} for PI and sodium hypochlorite in flounder, black rockfish and black sea bream was 51, 272, 187 mg/L and 30, 45, 48 mg/L, respectively. To determine a practical minimal disinfecting concentration for 10% PVI over different contact times and temperatures when added to water inoculated with *Escherichia coli* (*E. coli*), Heiner *et al*.[33] exposed *E. coli* to various dilutions of 10% PVI for 5, 15, and 30 min at 10, 20, and 30°C, neutralized with 0.5% sodium thiosulfate, and determined mean viable colony forming units (CFUs). In results, no CFUs were observed after exposure to the 1:100 dilutions and after 15 min of exposure to the 1:1,000 dilutions across experimental temperatures.

Seimenis and Skyrianos[34] reported that dilutions of 10% PVI of up to 1:10,000 were contacted with suspensions of *Brucella melitensis* (*B. melitensis*) and *Brucella abortus* (*B. abortus*) and afterwards inoculated onto a suitable agar

medium after contact times ranging from 30 sec to 30 min, and then growth of *B. melitensis* and *B. abortus* was completely inhibited by contact with the 1:10,000 dilution for 30 sec and the 1:1,000 dilution for one min.

With the consideration of previous studies, the disinfectant composed to PVI is a more effective and safe disinfectant than sodium hypochlorite against pathogenic bacteria.

In the present study, the disinfectant efficacy of the disinfectant composed to PVI has limitation that the results are based on *in vitro* test. Organic material in suspension (OM condition) could not represent all possible parameters of *Salmonella* and *Brucella* contaminated farm and food-industry environment.

As the efficacy of the disinfectant composed to PVI against *S. typhimurium* and *B. ovis* was investigated *in vitro*, a controlled field trial is required to determine whether use of the disinfectant will be able to reduce new pathogenic bacteria infection in animal farm and food industry area.

Conclusions

In animal farm and food industry, salmonellosis and brucellosis were very important diseases because of high mortality for farmed animals, zoonoses and economic loss. In the study of the bactericide efficacy test of the disinfectant composed to PVI, the results suggest that the disinfectant has a safe and potential bactericidal activity against *S. typhimurium* and *B. ovis*. So, the disinfectant composed to PVI can be used to control the spread of zoonotic bacteria.

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요 약

살모넬라증과 부루셀라증은 가축에 심각한 피해를 유발하는 질병으로, 축산업과 식품산업에 많은 경제적 손실을 초래하고 있다. 본 연구에서는, 포비돈-아이오딘을 주성분으로 하는 소독제 베타딘® 농후액의 *Salmonella typhimurium*과 *Brucella ovis*에 대한 효력시험을 수행하였다.

배지희석법을 이용한 살균효력시험은 4ºC에서 30분 동안 시험 세균을 희석 소독제에 노출시켜 소독제의 가장 효과적인 낮은 희석배수를 결정하는 시험이다. 베타딘® 농후액과 시험 세균들을 처리조건에 따라 경수와 유기물로 희석하여 반응을 시켰다. 유기물 조건에서, *Salmonella typhimurium*과 *Brucella ovis*에 대한 베타딘® 농후액의 살균력은 경수조건에서의 살균력과 비교하여 낮게 나타났는데, 이는 유기물들에 의한 소독제의 살균 유효성분에 대한 저해작용에 따른 것으로 사료된다.

베타딘® 농후액은 *Salmonella typhimurium*과 *Brucella ovis*와 같은 가축병원성 질병들에 대해 살균효과를 가지므로, 살모넬라증과 브루셀라증과 같은 세균성 질병의 확산을 제어하는데 효과적으로 이용될 수 있을 것으로 사료된다.

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