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Bactericidal Efficacy of Vital-Oxide®, Disinfectant Solution Against *Salmonella Typhimurium* and *Brucella Ovis*

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ABSTRACT - *Salmonella* spp. and *Brucella* spp. have caused a considerable disease of farmed animals and economic loss in animal farming and food industry. In this study, the disinfection efficacy of Vital-Oxidel®, a commercial disinfectant, composed to chlorine dioxide, betaine hydrochloride, and propylene glycol was evaluated against *S. typhimurium* and *Brucella 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. Vital-Oixdel® and test bacteria were diluted with distilled water (DW), hard water (HW) or organic matter suspension (OM) according to treatment condition. On OM condition, the bactericidal activity of Vital-Oixdel® against S. typhimurium and Brucella ovis was lowered compared to that on HW condition. As Vital-Oxidel® possesses bactericidal efficacy against animal pathogenic bacteria such as *S. typhimurium* and *Brucella ovis*, this disinfectant solution can be used to control the spread of bacterial diseases.

Key words: Vital-Oxide®, *Salmonella typhimurium*, *Brucella ovis*, Disinfectant efficacy

Salmonella extensively causes self-limiting enteritis, fatal infection in animals, food-borne infection, and typhoid fever in humans[1-4]. *Salmonella* infections are zoonotic disease and can be transferred between humans and nonhuman animals. Many infections are due to ingestion of contaminated food.[4] The etiologic agents of salmonellosis are *Salmonella* spp. characterized by motile, Gram-negative, rod-shaped bacteria and facultative intracellular pathogens that can multiply within professional and nonprofessional phagocytes.[4] *Salmonella* can survive for weeks outside a living body and are not destroyed by freezing[5,6].

Salmonella typhimurium (*S. typhimurium*) is one of the most frequently isolated serotypes from pig farms, slaughtered swine and human foodborne illness[7,8]. Also, *S. typhimurium* can survive in different reservoirs and is easily transmitted through water and poultry to humans[9,10].

Brucellae are Gram-negative, facultative, and intracellular bacteria that can infect many species of animals and human. Based on differences in pathogenicity and host preference, six species are recognized within the genus *Brucella*.[11] *B. abortus*, *B. melitensis* and *B. 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, *B. ovis* is responsible for lamb epididymitis[12].

As *Salmonella* and *Brucella* infections are becoming harder to control because of resistance to commonly used antibiotics, the effective cleaning and disinfection regimes are essential for the prevention of infections and outbreaks[13,14]. 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[15]. Biocides are often composed of a mixture of ingredients that

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act upon a wide range of cellular mechanisms and targets, which makes it difficult for bacteria to become resistant to biocides[13].

Salmonellosis and Brucellosis in livestock animals and human may cause enormous economic loss[16,17]. The stress on livestock animals caused by intensive farming practices, and the development of antibiotic-resistant bacteria are among the major reasons for the increased frequency of bacterial disease outbreaks[18]. Highly hygienic measures including the use of disinfectant are very effective for successful control of diseases from bacteria, fungi and parasites in farmed animals[19,20]. Several disinfectants including chlorine dioxide, betaine hydrochloride and propylene glycol have been used for decontamination of farmed animal and food borne diseases[21-24]. However, there is not the efficacy test for the disinfectant composed of chlorine dioxide, betaine hydrochloride and propylene glycol against bacterial animal diseases. Therefore, this study was carried out to examine bactericidal efficacy of a disinfectant solution against *S. typhimurium* and *Brucella 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* cells were cultured in Luria-Bertani (LB) broth containing 1.5% agar. *Brucella ovis* were spread in Brucella broth containing 5% fetal bovine serum and incubated at 37°C under CO_2 condition.

Disinfectant

The active ingredients for Vital-Oxide®, the tested disinfectant solution, are chlorine dioxide (0.01% v/v), betaine hydrochloride (0.50% v/v) and propylene glycol (0.30% v/v). Vital-Oxide® was provided by Dae Han New Pharm Co. (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, Plant and Fisheries Quarantine and Inspection Agency Regulation No. 2008-14, Korea.

Diluents and treatment condition

Testing was based on bactericidal effects of disinfectant diluents in three treatment conditions (distilled water (DW) condition, standard hard water (HW) condition, and organic matter (OM) condition), pathogen control (disinfectant negative control) and DW control (both disinfectant and pathogen 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 Vital-Oxide®

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

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

Experimental procedures

For the efficacy test against *S. typhimurium*, Vital-Oxide® was diluted 2.0, 2.25, 2.5, 2.75, and 3.0 times with DW and HW, and diluted 1.0, 1.1, 1.2, 1.3, and 1.4 times with OM, respectively. For the efficacy test against *Brucella ovis*, Vital-Oxide® was also diluted 2.4, 2.7, 3.0, 3.3, and 3.6 times with DW, and diluted 2.0, 2.25, 2.5, 2.75, and 3.0 times with HW, and diluted 1.0, 1.1, 1.2, 1.3, and 1.4 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 (Becton Dickinson & Co., MD, USA) 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. The valid dilution was determined that the greatest dilution showing no growth in two 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.

Results

Table 2 shows the final valid dilution of Vital-Oxide®

Table 2. Final valid dilution of Vital-Oxide® against *S. typhimurium* and *Brucella ovis*

Bacterial strains	Treatment condition*											
	DW				HW				OM			
	DT	1	2	3	DT	1	2	3	DT	1	2	3
S. typhimurium	3.0	○	○	○	3.0	○	○	○	1.4	○	○	○
	2.75	○	×	○	2.75	○	○	×	1.3	○	○	○
	2.5	×	×	×	2.5	×	×	×	1.2	○	○	○
	2.25	×	×	×	2.25	×	×	×	1.1	○	×	×
	2.0	×	×	×	2.0	×	×	×	1.0	×	×	×
	Valid		2.5		Valid		2.5		Valid		1.1	
Brucella ovis	3.6	○	○	○	3.0	○	○	○	1.4	○	○	○
	3.3	○	×	○	2.75	○	○	×	1.3	○	○	○
	3.0	×	×	×	2.5	×	×	×	1.2	○	○	○
	2.7	×	×	×	2.25	×	×	×	1.1	×	○	×
	2.4	×	×	×	2.0	×	×	×	1.0	×	×	×
	Valid		3.0		Valid		2.5		Valid		1.1	

*DW, distilled water; HW, standard hard water; OM, organic matter; DT, dilution time.
○ , growth; ×, growth inhibition

composed to chlorine dioxide, betaine hydrochloride and propylene glycol. On DW condition, *S. typhimurium* and *B. ovis* were completely inactivated with 2.5 and 3.0 fold dilutions of the disinfectant, respectively. When the bactericidal effect on HW condition was evaluated, the antibacterial activity of the disinfectant showed on 2.5 fold dilutions against both *S. typhimurium* and *B. ovis*. With the investigation of the bactericidal effect of the disinfectant on OM condition, both *S. typhimurium* and *B. ovis* were inactivated on 1.1 fold dilutions. Because 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 DM or HW conditions.

Comparing the results of the disinfectant against two pathogenic bacteria in the present study, the bactericidal effect of Vital-Oxide® against *B. ovis* was higher than that against *S. typhimurium* on the DM condition and was same on the HW and OM condition.

Discussion

Vital-Oxide® is a potential antibacterial disinfectant which was composed of chlorine dioxide, betaine hydrochloride and propylene glycol. Chlorine dioxide has been used for food sanitization as an alternative of chlorine-based sanitizer. Aqueous chlorine dioxide has much higher oxidation capacity than chlorine and doses not generate undesirable chemicals like trihalomethanes[25]. The main action of chlorine dioxide consists in the oxidation of cellular constituents. Chlorine dioxide has a direct action on cell membranes, either altering (at high concentrations) or disrupting their permeability (at low concentrations) and then penetrating into the cell and disrupting the protein synthesis[26]. At the various concentration of chlorine dioxide, the method of rapid bacterial kill appears to be the softening and destroying of the cell wall or viral envelope. However, human cells do not have cell walls and are apparently unaffected. Human skin and bodies are likely protected from the general oxidative effects of chlorine dioxide by the many reducing agents in human cells and blood[27]. Filby *et al*.[28] previously reported that in fish exposed to the chlorine dioxide-treated wastewater effluents, there was no induction of plasma vitellogenin or reduction in the weight of the fatpad, a secondary sex character in males. Daniel *et al*.[29] exposed groups of 10 male and 10 female Sprague-Dawley rats to chlorine dioxide in drinking water for 90 days at concentrations of 0, 25, 50, 100, or 200 mg/l. No exposure-related deaths and consistent alterations in hematologic parameters were reported. But, exposure to over than 50 mg/l resulted in significant reductions in terminal body weights and water consumption. In the short-term toxicological studies of chlorine dioxide[30,31], a group of 10 healthy male adults drank 1,000 ml of a 0 or 24 mg/l chlorine dioxide solution. Neither study found any physiologically relevant alterations in general health, vital signs, serum clinical chemistry and hematologic parameters. Chang and Schneider[24] reported that the treatment for 15 sec with chlorine dioxide-based disinfectant at the dose of 5 μg/ml reached a 3.0 logCFU/ml reduction on the surface of tomatoes contaminated with *Salmonella*. Trinetta *et al*.[32] investigated on the effectiveness of chlorine dioxide, ozone gas and e-beam irradiation treatments for inactivation of pathogens inoculated onto tomato, cantaloupe and lettuce seeds. The result suggested that chlorine dioxide, ozone gas and e-beam irradiation treatments

were 5.3, 4.4 and 4.0 log CFU/g reduction against *Salmonella* on contaminated tomato seeds, respectively. And Shams *et al*.[21] carried out the disinfectant efficacy test for the chlorine dioxide solution against *Brucella* species. At the concentration of 0.25 mg/l chlorine dioxide in portable water, *Brucella* species were inactivated by at least 3.0 logCFU/ml within 10 min.

With the consideration of previous studies, Vital-Oxide® is a more effective and safe disinfectant than chlorine and other treatments like ozone and beam irradiation against pathogenic bacteria.

In this study, disinfectant efficacy of Vital-Oxide® 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 environments.

As the efficacy of Vital-Oxide® against *S. typhimurium* and *Brucella ovis* was investigated *in vitro*, a controlled field trial is required to determine whether use of Vital-Oxide® 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 Vital-Oxide®, the results suggest that Vital-Oxide® has potential bactericidal activity against *S. typhimurium* and *Brucella ovis*. So, Vital-Oxide® composed to chlorine dioxide, betaine hydrochloride and propylene glycol can be used to control the spread of animal bacterial diseases like zoonoses.

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요 약

살모넬라와 부루셀라는 가축에 심각한 질병을 유발하며, 축산업과 식품산업에 많은 경제적 손실을 초래하고 있다. 본 연구에서는, 이산화염소와 베타인 염산염을 주성분으로 한 소독제 바이탈옥사이드의 *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. 바이탈옥사이드와 시험 세균들을 처리조건에 따라 증류수, 경수, 그리고 유기물 등으로 희석하여 반응을 시켰다. 유기물 조건에서, *Salmonella typhimurium*과 *Brucella ovis*에 대한 바이탈옥사이드의 살균력은 경수조건에서의 살균력과 비교하여 낮게 나타났는데, 이는 유기물들에 의한 소독제의 살균 유효성분에 대한 저해작용에 따른 것으로 생각된다.

바이탈옥사이드는 *Salmonella typhimurium*과 *Brucella ovis*와 같은 가축병원성 질병들에 대해 살균효과를 갖고 있기 때문에, 이 소독제는 세균성 질병의 확산을 제어하는데 이용될 수 있을 것으로 사료된다.

References

1. Cleaveland, S., Laurenson, M.K. and Taylor. L.H.: Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *B. Biol. Sci*. **356**, 991-999 (2001).
2. Kim, G.S., Kim, D.H., Lim, J.J., Lee, J.J., Han, D.Y., Lee, W.M., Jung, W.C., Min, W.G., Won, C.G., Rhee, M.H., Lee, H.J. and Kim, S.: Biological and antibacterial activities of the natural herb *Houttuynia cordata* water extract against the intracellular bacterial pathogen *Salmonella* within the raw 64.7 macrophage. *Biol. Pharm. Bull*. **31**, 2012-2017 (2009).
3. Kim, D.H., Lim, J.J., Lee, J.J., Jung, W.C., Shin, H.J., Lee, H.J., Kim, G.S. and Kim, S.: Antibacterial and therapeutic effects of *Houttuynia cordata* ethanol extract for murine salmonellosis. *Kor. J. Environ. Agricul.* **27**, 156-162 (2008).
4. Valle, E. and Guiney, D.G.: Characterization of salmonella-induced cell death in human macrophage-like THP-1 cells. *Infect. Immun*. **73**, 2835-2840 (2005).
5. Sorrells, K.M., Speck, M.L. and Warren, J.A.: Pathogenicity of *Salmonella gallinarum* after metabolic injury by freezing. *Appl. Environ. Microbiol*. **19**, 39-43 (1970).
6. Beuchat, L.R. and Heaton, E.K.: Salmonella survival on pecans as influenced by processing and storage conditions. *Appl. Environ. Micobiol*. **29**, 795-801 (1975).
7. Katsuda, K., Kohmoto, M., Kawashima, K. and Tsunemitsu, H.: Frequency of enteropathogen detechtion in sucking and weaned pigs with diarrhea in Japan. *J. Vet. Diagn. Invest*. **18**, 350-354 (2006).
8. Korsak, N., Jacob, B., Groven, B., Etienne, G., China, B., Ghafir, Y. and Daube, G.: Salmonella contamination of pigs and pork in an integrated pig production system. *J. Food Prot*. **66**, 1126-1133 (2003).
9. Garland, J.B., Frye, J.G., Gray, J.T., Berrang, M.E., Harrison, M.A., Cray, P.J.F.: Transmission of *Salmonella enterica* serovar Typhimurium in poultry with and without antimicrobial selective pressure. *J. Appl. Microbiol*. **101**, 1301-1308 (2006).
10. Sharan, R., Chhibber, S. and Reed, R.H.: A murine model to study the antibacterial effect of copper on infectivity of *Salmonella Enterica* Serovar Typhimurium. *Int. J. Environ. Res. Public Health*, **8**, 21-36 (2011).
11. Moreno, E., Cloechkaert, A. and Morivon, I.: *Brucella* evolution and taxonomy. *Vet. Microbiol*. **90**, 209-227 (2002).
12. Cloeckaert, A., Grayon, M., Grepinet, O. and Bounedine, K.S.: Classification of *Brucella* strains isolated from marine mam-

mals by infrequent restriction site-PCR and development of specific PCR identification tests. *Microb. Infect*. **5**, 593-602 (2003).
13. Whitehead, R.N., Overton, T.W., Kemp, C.L. and Webber, M.A.: Exposure of *Salmonella enteric* Serovar Typhimurium to high level biocide challenge can select multidrug resistant mutants in a single step. *PLoS ONE,* **6**, e22833 (2011).
14. Turkmani, A., Psaroulaki, A., Christidou, A., Samoilis, G., Mourad, T.A., Tabaa, D. and Tselentis, Y.: Uptake of ciprofloxacin and ofloxacin by 2 *Brucella* strains and their fluoroquinolone-resitant variants under different conditions. An *in vitro* study. *Dign. Microbiol. Infect. Dis*. **59**, 447-451 (2007).
15. Russell, A.D.: Biocide use and antibiotic resistance: the relevance of laboratory findings to clinical and environmental situations. *Lancet Infect. Dis*. **3**, 794-803 (2003).
16. Giammanco, G., Pignato, S. and Giammanco, G.M.: Recent trends in salmonellosis epidemiology. *J. Prev. Med. Hyg*. **40**, 19-24 (1999).
17. Munozdel, R.M., Montano, M.F., Renteria, T.B., Sanchez, E., Moreno, J.F., Perez, A. and Saucedo, S.: Assessment of the economic impact of a brucellosis control program in a dairy herd using the partial budget method. *J. Anim. Vet. Adv.* **6**, 146-151 (2007).
18. Mennerat, A., Nilsen, F., Ebert, D. and Skorping, A.: Intensive farming: Evolutionary implications for parasites and pathogens. *Evol. Biol*. **37**, 59-67 (2010).
19. Kahrs, R.F.: General disinfection guidelines. *Rev. Sci. Tech*. **14**, 105-163 (1995).
20. Ahmad, K.: Control of animal diseases caused by bacteria: Principles and approaches. *Pakistan Vet. J*. **25**, 200-202 (2005).
21. Shams, A.M., O'Connellm H,, Arduino, M.J. and Rose, L.J.: Chlorine dioxide inactivation of bacterial threat agents. *Lett. Appl. Microbiol*. **53**, 225-230 (2011).
22. Lindstedt, M., Allenmark, S., Thompson, R.A. and Edebo, L.: Antimicrobial activity of betaine esters, quaternary ammoniumamphipheles which spontaneously hydrolyze into nontoxic components. *Antimicrob. Agents Chemother*. **34**, 1949-1954 (1990).
23. Gotvajn, A.Ž. and Zagorc-Končan, J.: Laboratory simulation of biodegradation of chemicals in surface waters: closed bottle and respirometric test. *Chemosphere*, **38**, 1339-1346 (1999).
24. Chang, A.S. and Schneider, K.R.: Evaluation of overhead spray-applied sanitizers for the reduction of *Salmonella* on tomato surfaces. *J. Food Sci*. **71**, M45-69 (2012).
25. Kim, Y., Kim, M. and Son, K.B.: Combined treatment of fumaric acid with aqueous chlorine dioxide or UV-C irradiation to inactivate *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *Listeria monocytogenes* inoculated on alfalfa and clover sprouts. *Food Sci. Technol*. **42**, 1654-1658 (2009).
26. Opinion of the Scientific Panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the Commission: Treatment of poultry carcasses with chlorine dioxide, acidified sodium chlorite, trisodium phosphate and peroxyacids. *EFSA J*. **297**, 1-27 (2005).
27. Patrick, G.L.: An introduction to medicinal chemistry. I*n* An antibacterial agents, Oxford University Press, Oxford, pp. 157-159 (1995).
28. Filby, A., Shears, J., Drage, B., Churchley, J. and Tyler, C.: Effects of advanced treatments of wastewater effluents on estrogenic and reproductive health impacts in fish. *Environ. Sci. Technol*. **44**, 4348-4354 (2010).
29. Daniel, F.B., Condie, L.W., Robinson, M., Judy, A., York, R.G., Olson, G.R. and Wang, S.R.: Comparative subchronic toxicity studies of three disinfectants. *J. Am. Water Works Assoc*. **82**, 61-69 (1990).
30. Lubbers, J.R., Chauhan, S. and Bianchine, J.R.: Controlled clinical evaluations of chlorine dioxide, chlorite and chlorate in man. Fundam. *Appl. Toxicol*. **1**, 334-338, 1981.
31. Lubbers, J.R., Chauhan, S. and Bianchine, J.R.: Controlled clinical evaluations of chlorine dioxide, chlorite and chlorate in man. *Environ. Health Perspect*. **46**, 57-62, (1982).
32. Trinetta, V., Vaidya, N., Linton, R. and Morgan, M.: A comparative study on the effectiveness of chlorine dioxide gas, ozone gas and e-beam irradiation treatments for inactivation of pathogens inoculated onto tomato, cantaloupe and lettuce seeds. *Int. J. Food Microbiol*. **146**, 203-206 (2011).