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Antimicrobial efficacies of alkaline disinfectant solution and commercial disinfectants against *Brucella ovis*

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Abstract

Bruella spp. involves a considerable danger of public health and farm animal industry. In this study, we assessed the disinfection efficacy of alkaline disinfectant solution and three commercial farm disinfectants (quaternary ammonium compound, sodium dichloroisocyanurate, potassium monopersulphate/sodium dichloroisocyanurate) against *Brucella ovis*. A bactericidal efficacy test by broth dilution method was used to determine the lowest effective dilution of selected disinfectants following exposure to test bacteria for 30 minutes at 4℃. Disinfectants and test bacteria are diluted with distilled water (DW), hard water (HW) or organic matter suspension (OM) according to treatment condition. Three commercial disinfectant showed excellent antimicrobial activity (up to dilution of ×200 in OM treatment). Alkaline disinfectant solution demonstrated favorable bactericidal efficacy against *B. abortus* (at dilution of ×20 in OM treatment). Three commercial farm disinfectants possess excellent efficacy against *B. ovis*. Alkaline disinfectant solution has lower potency than commercial farm disinfectant but could help to limit the spread of brucellosis.

Key words : Alkaline disinfectant solution, *Brucella* spp., Disinfectant efficacy, Farm disinfectants

INTRODUCTION

Brucellosis is an important bacterial zoonosis in worldwide and it remains a cause of great economic loss in the animal farm industry by reproductive failure including late stage abortion and infertility (Glynn and Lynn, 2008). The causative agent, *Brucella* spp. is divided to eight species according to their host preference; cows (*B. abortus*), goats and sheep (*B. melitensis*), rams (*B. ovis*) pigs (*B. suis*), dogs (*B. canis*), wood rats (*B. neotomae*), marine mammals (*B. ceti, B. pinnipedialis*) (Foster et al, 2007; Glynn and Lynn, 2008). These small Gram-negative rods are intracellular organism and can invade phagocytic and non-phagocytic cells, by which they can avoid being detected and eliminated by the host immune system (Fugier et al, 2007). It leads to characteristic persistent infection shedding in reproductive and mammary secretion and major therapeutic limitation and difficulty in control and eradication of brucellosis in animal industry (Radostitis et al, 1994). In the Republic of Korea, brucellosis is endemic problem in dairy and beef cattle and dogs although the active control program has been executed since 1960s (Kang et al, 2009; Wee et al, 2008). Recently, as bovine brucellosis spread progressively, sporadic outbreak of human brucellosis was has been reported in live stock worker and veterinarians (Kim et al, 2006).

Because brucellosis is breeding-related problem and milk, contaminated tissues or fluid associated with birth or abortion achieves high concentration of *Brucella* and can be main risk factor of consistent brucellosis in animal husbandry (Shareef, 2006; Wanke, 2004). Highly hygienic measure including the use of disinfectant is extremely effective measure for successful brucellosis

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control especially in endemic area (Al-Majali et al, 2009; Al-Majali et al, 2008; Al-Talafhah et al, 2003; Radostitis et al, 1994). Several disinfectants including quaternary ammonia, iodides, and chlorhexidine are known to effective to brucellosis (Hall, 1979; Wanke, 2004). But, efficacy of alkaline disinfectant solution against *Brucella* spp. has not been reported.

Therefore, this study was carried out to examine bactericidal efficacy of alkaline disinfectant solution against *Brucella* spp. and compare its efficacy with those of commercial farm disinfectants.

MATERIALS AND METHODS

Bacteria and culture

The test bacteria, *B. ovis* (ATCC 25840), were obtained from National Veterinary Research & Quarantine Services (NVRQS, Korea). Cultures were grown on 5% sheep blood agar at 37˚C for 72 to 96 hours in CO_2 incubator and stored sealed at 4˚C. Prior to experimental use, broth culture with *Brucella* Broth (Becton Dickinson & Co., USA) w/ 5% inactivated horse serum was initiated from single colony as same condition as *Brucella* agar culture.

Disinfectants

An alkaline disinfectant solution and three commercial farm disinfectants were chosen for efficacy testing. The active ingredients for the tested alkaline disinfectant solution and commercial farm disinfectants (A, B, C) are presented Table 1. Alkaline disinfectant solution was provided by G.N.C Bio Co. (Korea) and three commercial farm disinfectants were provided from their manufacturer (KBNP. Inc. Korea) The disinfectant was stored in the dark in room temperature and prepared for dilution on the day of evaluation. Determination of the antimicrobial efficacy of disinfectants was based on NVRQS Regulation No. 30, Republic of Korea.

Table 1. Composition of alkaline disinfectant solution and commercial farm disinfectants (A, B, C) tested against *Brucella ovis*

Disinfectant	Active ingredient	Concentration
Alkaline disinfectant solution (pH 14)	Sodium meta silicate	46.0% (w/v)
	Potassium carbonate	68.0% (w/v)
	Potassium citrate	2.6% (w/v)
	Borex	1.36% (w/v)
	Silver nitrate	0.026% (w/v)
A	Octyl decyl dimethyl ammonium chloride	3.0% (w/v)
	Dioctyl dimethyl ammonium chloride	1.5% (w/v)
	Didecyl dimethyl ammonium chloride	1.5% (w/v)
	Alkyl benzyl dimethyl ammonium chloride	4.0% (w/v)
B	Sodium dichlorisocyanurate	38.5% (w/w)
C	Potassium monopersulphate	50.0% (w/w)
	Sodium dichlorisocyanurate	5.0% (w/w)
	Sulfamic Acid	15.0% (w/w)
	Sodium hexametaphosphate	24.0% (w/w)
	Sodium do-decylbenzene sulphonate	5.0% (w/w)

Table 2. Experimental design for determination of the bactericidal efficacy of disinfectant

Treatment condition	Contents according to treatment condition				
	Distilled water (DM)	Standard hard water (HW)	Organic matter (OM)	Disinfectant	***Pathogen
**DW condition	+*	−	−	+	+
HW condition	−	+	−	+	+
OM condition	−	−	+	+	+
Pathogen control	−	+	−	−	+
DW control	+	−	−	−	+

*presence, -absence; **DW: distilled water condition, HW: standard hard water condition, OM: organic matter condition; ***Pathogen (Brucella ovis) in each treatment condition was titrated to be at least 10^8 cfu/ml viable organism with the same diluent (DM, HW, or OM) of treatment condition

Diluents and treatment condition

Testing was based on bactericidal effects of disinfectant diluents in three treatment conditions (DW condition, HW condition, and OM condition), pathogen control (disinfectant negative control), and DW control (both disinfectant and pathogen negative control) in Table 2. Standard hard water, a ingredient of HW treatment condition, was made by adding anhydrous $CaCl_2$ 0.305g and $MgCl_2 \cdot 6H_2O$ 0.139g into distilled water. Organic suspension, a ingredient of OM treatment condition, is a solution of 5% (w/v) yeast extract in standard hard water. The test organism were prepared by titration of Brucella broth (Becton Dickinson & Co., USA) culture into at least 4×10^6/ml viable organism with the same kind of diluents of treatment condition.

Test procedures

To verify the lowest effective dilution of the disinfectant, five serial dilutions of each disinfect were prepared and placed at 4˚C prior to test reaction. 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, the mixture was neutralized by 1:10 dilution of Nutrient broth (Becton Dickinson & Co., USA) w/5% inactivated horse serum at 37˚C. 0.1ml of the neutralized reaction mixture was subcultured into 10ml of recovery Brucella broth w/5% inactivated horse serum 37˚C for 72 to 96 hours in CO_2 incubator. The valid dilution was deter-mined if the greatest dilution showing no growth in two or more in the five replicates were confirmed. The final valid dilution 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

According to the final valid dilution of alkaline disinfect solution and three commercial disinfectants (Table 3), the antibacterial activities of all disinfectants after exposure 30min contact completely inactivated *B. ovis* with at least 100 fold dilution on all conditions. When the bactericidal effect on OM condition was evaluated, most disinfects showed lowered efficacy against *B. ovis* compared with DM or HW conditions. A bactericidal effect against *B. ovis* with same potency on OM condition compared with DW or HW conditions was reached only by commercial farm disinfectant A, a quaternary ammonium compound. When comparing the results of four disinfectants tested in this study, bacterial efficacy of alkaline disinfectants solution was not comparable with the average results of three commercial farm disinfectants but treatment of up to 1:20 dilution of alka-line disinfectant solution was sufficient to exert bactericidal activity against *B. ovis* on OM condition.

Table 3. Final valid dilution of alkaline disinfectant solution and three commercial disinfectants (A, B, C) tested against *Brucella ovis*

	Disinfectant	Treatment condition		
		DW	HW	OM
Brucella ovis	Alkaline disinfectant solution	220	220	20
	A	5000	5000	5000
	B	4000	4000	500
	C	4000	4000	200

DISCUSSION

Brucellosis in food animal can be effectively controlled with proper vaccination, quarantine programs, surveillance of individual and flock prevalence, and the presence of adequate veterinary services but high cost of this control program have significant influence on farm animal economy (Boschiroli et al, 2001; Sawyer, 1996). Use of disinfectants and were identified as key factors for effective Brucella control programs in cows, sheep, goats and camels (Al-Majali et al, 2009; Al-Majali et al, 2008; Al-Talafhah et al, 2003). Most of *Brucella* infection between animals or animal-humans were mediated by contact with infected tissue, discharge or contaminated materials. Thus, importance of sanitary strategies

such as, proper disposal of aborted materials and highly hygienic procedures are extremely important steps in any successful *Brucella* control program.

In our study, alkaline disinfectant solution was demonstrated to inactivate *Brucella* after 30 min exposure in DW, HW, and OM condition. Limited reports on antibiotic efficacy of alkaline solutions displayed varying activity results depending on the environmental conditions and microbial species (Brändle et al, 2008; Chavez de Paz et al, 2007; Kwon et al, 2003). Alkaline disinfectant solution similar to our disinfectant with same ingredients displayed acceptable virucidal effects on several types of avian influenza virus (Seo et al, 2007). Despite its strong alkalinity (above pH 12), these compounds in active dilution state was reported to be safe in application for poultry (Seo et al, 2007). In our preliminary study, the alkaline disinfectant solution with 10 fold dilution was also demonstrated to be non toxic and safe in direct contact to mammary skin and teat end in dairy cows (data no shown). Generally, other chemical disinfectant such as, glutaraldehydes, phenolic compounds and chlorine based disinfectants, are not recommended for directly application to the body of animals due to potential skin and mucosal toxicity (McDonnell and Russell, 1999). However, alkaline disinfectant solution could be safely used for not only environmental sanitation but the disinfection of contaminated animal body surface. Although the efficacy of alkaline disinfectant solution was less potent against *Brucella* compared to commercial farm disinfectants, advantage of safe application on animal body surface could empower the control measures for protection of *Brucella* spread in dairy cow and beef cow industry.

Disinfectant efficacy of alkaline disinfectant solution in this study has limitation that the results are based on in vitro test. Organic material in suspension (OM condition) could not represent all possible parameters of *Brucella* contaminated farm environments particularly in concern that *Brucella* spp. are intracellular organism. Now that the efficacy of alkine disinfectant solution against *brucella* was demonstrated *in vitro*, a controlled field trial are required to determine whether use of alkaline disinfectant solution will lead to reduce new *Brucella* infection ratio in endemic farm area.

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