Etlik Vet. Mikrob. Derg. 1989 6 (5)

EFFECTS OF DISINFECTANTS ON HAEMOPHILUS SOMNUS

K. Serdar DİKER (*) Jale ERDEĞER (*) Kazunori HASHIMOTO (**)

INTRODUCTION

The gram-negative, pleomorphic bacterium Haemophilus somnus is a bovine pathogen of worldwide distribution that is a significant cause of economic loss in feedlot cattle (7). H.somnus infections can occur as a wide range of clinical syndromes, including respiratory disease, arthritis, septicemia, reproductive disease, abortion and a severe and often fatal thromboembolic meningoencephalitis (2, 3, 9, 10). Not only is this organism an important pathogen, but also many apparently healthy cattle carry it on their nasal, vaginal or preputial mucosa (4, 5). Although the route of transmission in H.somnus infection has not been fully documanted, contaminated environment is most likely an important source in transmission of infection caused by this bacterium (6). Therefore, knowledge of the effects of common disinfectants against H.somnus is needed. These facts and paucity of published data about this subject prompted us to investigate the effect of disinfectants, commonly used in veterinary practice and in laboratories, against H.somnus under laboratory conditions.

(*) Department of Microbiology, Veterinary Faculty, Ankara University, 06110 Ankara, TURKEY.
(**) Central Research Laboratory, Nippon Zenyaku Kogyo Co.Ltd., Koriyama, Fukushima, JAPAN.

MATERIALS AND METHODS

Disinfectants: Brand names and active ingredients of common groups of disinfectants used for testing were as follows: Phenolic compound, Amphyl, phenylphenol 15% and amylphenol 6.3%; Iodophor group, Betadine, iodine polyvinylpyrrolidine 10% (1% available iodine); Quaternary ammonium compound, Zephirol, benzalkonium chloride 10%; Formaldehyde, formaldehyde solution 37%; Alcohol group, ethyl alcohol 95%. The concentrations of the disinfectants used in this study were based on the concentrations of the active ingredients given in formulation. Sterile triple-distilled water (pH. 7,0) was used as diluent.

Organisms: H.somnus strain 43826 and strain 805 were supplied from Dr. S.C. Groom, University of Guelph, Ontario, Canada. Brain - heart infusion (BHI) agar containing 0.5% yeast extract, thiamine monophosphate (1 mg/ml) and 7% defibrinated sheep blood was used for culturing the organisms.

Test procedure: The 48 h culture of H.somnus was suspended in sterile saline, centrifuged twice for 10 min at 5,000 rpm and the sediment was resuspended in Sorenson buffer (pH. 7,0). The bacterial count of suspension was carried out by the method of Miles and Misra (8). In the disinfectant assay, 0.2 ml of the bacterial suspension was added to 9.8 ml of the disinfectant solution to obtain the desired bacterial concentration (approximately 10^6 colony forming units-CFU per ml). Triple distilled water without disinfectant was used as test control. For all experiments, the period of contact ranged from 1 to 60 min, and all tests were performed at 20°C. Preliminary studies showed that neutralizer of disinfectants inhibited the growth of H.somnus and BHI broth containing 10% calf serum neutralized the tested concentrations of disinfectants. Therefore, BHI-serum broth was used as a neutralizer as well as a growth medium. To minimize the residual disinfectant activity, organisms in test agents were diluted 1:100 in Sorenson buffer before inoculation into BHI-serum broth. All broths were incubated at 37°C for 48 h. The broths were subcultured onto BHI-blood agar and incubated in an atmosphere of 10% CO_2 in air. The endpoint was defined as the concentration of disinfectant at which none of two strains survived. All tests were performed in duplicate.

RESULTS AND DISCUSSION

A restriction of this study was that we performed the tests in laboratory condition, not in natural settings. In natural conditions, many factors, including temperature and organic material affect the activity of disinfectants. For this study, however, we attemted to set up laboratory conditions close to those in the field.

Because some of the standard neutralizing agents (Tween 80, sodium thiosulfate) in recommended concentrations (1) inhibited the confluent growth of H.somnus strains, alternative methods were chosen for neutralization procedures. The Sorenson buffer was found to minimize disinfectant activity. Protein rich BHI-serum broth functioned both as neutralizer of residual disinfectant activity and as a growth medium.

Table 1 shows the killing effects of disinfectants used on the two strains of H.somnus. For each of the two strains, 0.078% phenolic compound, 70% ethyl alcohol and 10 ppm free iodine in iodophor killed the organisms within 1 min. Fifteen min was required for 2% formalin to kill the both strains. Quaternary ammonium compound at the concentration of 1:50.000 killed both strain within 15 min. For all disinfectants, lower concentrations took longer to kill. For each of two strains, 0.02% phenolic compound, 0.25% formalin, 1:200.000 benzalkonium chloride and 2.5 ppm free iodine in iodophor did not affect the survival of organisms within 1 h. All organisms in the control solutions showed growth. No difference was detected between the susceptibility of two strains to disinfectants. In general, the killing of H.somnus by common disinfectants we observed was somewhat higher than those of other gram negative organisms, perhaps due to its fastidious character.

Ethyl alcohol is commonly used in veterinary practice to disinfect skin and instruments. Our results showed that in the tested concentrations, ethyl alcohol was effective against H.somnus. Iodophors, phenolic compounds and quaternary ammonium compounds are mainly used to disinfect skin and instruments. Iodophor and quaternary ammonium compounds can also be used as water sanitizers. In this study, all three disinfectants were effective against H.somnus at the concentrations below that recommended. Formalin (10%) is commonly used to fix and disinfect tissues and to inacti-

vate organisms when preparing whole cell antigen. In practice, tissues are preserved in 10% formalin for at least 8 h, thus this exposure should kill H.somnus completely. When inactivating organism in antigen preparation procedure, 1 h contact of 0.5% formalin with H.somnus should be sufficient to kill the organism.

These studies demonstrate that, under the conditions we tested (pH. 7,0 ; 20°C), the recommended standard concentrations of disinfecting agents are adequate to destroy H.somnus.

TABLE 1. Killing of two strains of H.somnus (10^6 CFU/ml) by common disinfectants.

Disinfectant	Conc. tested	Growth after the following times (min)				
		1	5	15	30	60
Phenolic (Amphyl)	0.15 %	—	—	—	—	—
	0.078%	—	—	—	—	—
	0.04 %	+	+	—	—	—
	0.02 %	+	+	+	+	+
Iodophor (Betadine)	10 ppm	—	—	—	—	—
	5 ppm	+	+	—	—	—
	2.5 ppm	+	+	+	+	+
Quaternary ammonium c. (Zephirol)	1: 50.000	+	—	—	—	—
	1:100.000	+	+	+	—	—
	1:200.000	+	+	+	+	+
Formalin (Formaldehyde sol.)	2 %	+	+	—	—	—
	1 %	+	+	+	—	—
	0.5 %	+	+	+	+	—
	0.25 %	+	+	+	+	+
Ethyl alcohol	70 %	—	—	—	—	—
	40 %	+	—	—	—	—
Controls		+	+	+	+	+

SUMMARY

The effect of common disinfectants at the different concentrations on two Haemophilus strains was investigated. At an inoculum size of 10^6 CFU/ml, 0.078% phenolic compound, 70% ethyl alcohol and 10 ppm free iodine in iodophor killed two strains within 1 min. Quaternary ammonium compound (1:50.000) and 2% formalin killed both strains within 15 min. Recommended standard concentrations of disinfecting agents were found adequate to destroy H.somnus under the conditions we tested (pH. 7,0 ; 20°C).

ÖZET

DEZENFEKTANLARIN HAEMOPHILUS SOMNUS ÜZERİNDEKİ ETKİLERİ

Farklı konsantrasyonlardaki genel dezenfektanların iki Haemophilus somnus suşu üzerindeki etkileri incelendi. % 0.078'lik fenolik bileşik, % 70'lik etil alkol ve iyodofordaki 10 ppm serbest iyot, 10^6 CFU/ml yoğunluğundaki organizmaları bir dakika içinde öldürdü. Kuaternar amonyum bileşiğinin 1:50.000'lik dilusyonu ve % 2 formalin her iki suşu 15 dakika içinde öldürdü. Test edilen koşullarda (pH. 7,0 ; 20°C), dezenfektanların önerilen standart konsantrasyonlarının H.somnus'u öldürmeye yeterli olduğu belirlendi.

REFERENCES

1 — BASS, G.K. (1977) : Method of testing disinfectants. p, 49-77. In: Disinfection, Sterilization and Preservation, 2nd ed., Ed: S.S. BLOCK, Lea and Febiger, Philadelphia.

2 — CHLADEK. D.W. (1975) : Bovine abortion associated with Haemophilus somnus. Am. J. Vet. Res., 36: 1041.

3 — CORBEIL, L.B., WIDDERS, P.R., GOGOLEWSKI, R., ARTHUR, J., INZANA, T. J., WARD, A.C.S. (1986) : Haemophilus somnus; bovine reproductive and respiratory disease. Can. Vet. J., 27: 90-93.

4 — CORBEL, M.J., BREWER, R.A., SMITH, R.A. (1986) : Isolation of bacteria of the Haemophilus-Histophilus group from the genital tracts of cattle. Vet. Rec., 118: 695-696.

5 — CORSTVET, R.E., PANCIERA, R.J., RINKER, H.B., STARKS, B.L., HOWARD, C. (1973) : Survey of tracheas of feedlot cattle for Haemophilus somnus and other selected bacteria. J. Am. Vet. Med. Assoc., 163: 870-873.

6 — DEWEY, K.J.. LITTLE, P.B. (1984) : Environmental survival of Haemophilus somnus and influence of secretion and excretions. Can. J. Comp. Med., 48: 23-26.

7 — HUMPHREY, J.D., STEPHENS, L.R. (1983) : Haemophilus somnus: a review. Vet. Bull., 53: 987-1003.

8 — MILES, A.A., MISRA, S.S. (1938) : The estimation of the bactericidal power of blood. J. Hyg., 38: 732-749.

9 — MILLER, R.B., LEIN, D.H., McENTEE, K.E., HALL, C.E., SHIN, S, (1983) : Haemophilus somnus infection of the reproductive tract of cattle: a review. J. Am. Vet. Med. Assoc., 182: 1390-1392.

10 — STEPHENS, L.R., LITTLE, P.B., WILKIE, B.N., BARNUM, D.A. (1981) : Infectious thromboembolic meningoencephalitis in cattle: a review. J. Am. Vet. Med. Assoc., 178: 378-384.