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BACTERIAL CONTAMINATION OF DAIRY YARDS AND CALF HOUSES AND ITS RESISTANCE TO SOME DISINFECTANTS

(With 8 Tables)

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التلوث البكتيري في مساكن الماشية المفتوحة وحظائر العجول ومدى مقاومته للمطهرات

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في هذه الدراسة تم عزل الملوثات البكتيرية من الحوائط قبل وبعد عمليات التنظيف والتطهير في خمس حظائر ماشية مفتوحة وخمس حظائر للعجول بمحافظة الغربية. وتم تقييم خمسة مطهرات في تلك المساكن ومعمليا. المركبات التي استخدمت هي الفينول و الكلور واليود ومركبات الامونيوم الرباعية والفورمالين. وقد أظهرت النتائج فاعلية المركبات الامونيوم الرباعية ثم والفورمالين على كل المطهرات تحت الدراسة.

SUMMARY

Five dairy yards and five calf houses from different sites at Gharbia Governorate were under study at February of 2003. The samples were taken from wall if shelter of yard of dairy farms. In addition to, walls of fattening calves. The samples were taken before and after cleansing and disinfection. Five classes of disinfectants used in veterinary practice were evaluated for their effectiveness against some bacterial contaminations. The obtained results revealed that Staphylococcus aureus was the most prevalent bacteria in dairy yards. Proteus species were dominant in calf houses. The most resistant bacterium was found to be Streptococcus. The tested disinfectants in vivo and vitro were phenol, quaternary ammonium compound, iodophor, chlorine and formaldehyde. Each product was diluted according to the manufacturers recommendation.

Key words: *Bacterial contamination, calf houses, disinfectants.*

INTRODUCTION

Biosecurity, which regularly includes cleaning and disinfecting, is one of the best methods used to reduce the level of pathogens in animal facilities. In general, a sanitation program should include safe and easy procedures outlining the correct application of detergents and disinfectants, proper use of application equipment, and an effective monitoring system (Ruano *et al.*, 2001). Quaternary ammonium compounds react with cell membrane, denature essential cell proteins and inactivate cellular damage of the cell membrane resulting in leakage of cell components. Chlorine also forms substitution products with proteins and amino acids (Earnshaw and Lawrence, 1998). Many factors must be considered when one chooses a disinfectant. The presence of organic material on or in the surface to be treated, organic material in the diluents, the quality of the water (including hardness, pH, nitrate content, and bacterial contamination), corrosiveness or toxicity of the product, the application method, temperature, the porosity of the surface being cleaned, the length of contact time, correct concentration, and the cost are factors with an impact on the selection of the most appropriate disinfectant. In addition, one must consider the infectious organism targeted and its susceptibility to various classes of disinfectants (Davison *et al.*, 1999).

Consequently, intensive environment bacteriological sampling is needed before and after cleanness and disinfection of cattle housing systems. The aim of this work is to compare between the efficacies of some disinfectants against some bacterial isolates that contaminate cattle environment.

MATERIALS and METHODS

This study was carried out in open yard system with dirty floor and calf pens in Gharbia governorate. Bacteriological samples (50 to 100) were taken before cleanness and disinfection (C&D) then repeated at the same places after C&D. They were made by swabbing the surfaces ($0.5m^2$/sample) of wall of dairy yards and calf houses. The swabs were put into jars containing 100 ml of buffered peptone water (BPW). (Valancony et al., 2001).

Ten percent of disinfectant neutralizer was added to the BPW taken after C&D swabs.

Disinfectants:

Five disinfectants were tested:

1- Sodium hypochlorite (Clorox) contains 52.5 g chlorine /liter. It was produced by The Egyptian Company of house cleaning agents under license from Clorox International Trading Ltd. It was used at a concentration of 5%.
2- Iodosan 30, (Ewabo, Germany), contains total of 300g /Lt. polyethoxyeminoethanol/nonylphenoxypolyethoxyethanol iodine complex (at 10% of active iodine) and 198 g/L phosphoric acid. The recommended dose is 1.5 ml/L (i.e. 15%).
3- Quaternary ammonium compound (QAC) Antec (Cairo Pharmaceutical and chemical industries under license of Antec A.H. International co. England) and was used at a concentration of 5%
4- Formaline (Kemeta Co., Egypt).It was used at a concentration of 5%
5- Commercial Phenol, It was used at a concentration of 5%

Neutralizers:

The neutralizers were used in recovery broth medium against each disinfectant according to (Reem-Dosoky et al. 2000).

1- Lecithin (0.3%) and Tween 80(3%) for phenol and formalin.
2- Letheen broth [Letheen broth (2.07%) and Tween 80 (0.05%)] for Antec (QAC).
3- Sodium thiosulphate (0.5%) for iodosan-30.
4- Sodium sulphite for chlorine neutralization (Taghi-Kilani et al., 1996).

Cleanness and disinfection of houses:

Contract cleaners carried out the following process. The litter was removed. The walls and floors were then mechanically brushed and all surfaces sprayed with sodium hydroxide 5% to loosen adherent fecal material. The surfaces were then cleaned by power washing using plain water. The clean surfaces were sprayed with different disinfectants at various locations.

1- Evaluation of disinfectants in vivo:

Each house was visited on two occasions, the first after removal of the litter and dry cleanness but before any washing, and the second 3-4 days after completion of the whole cleansing operation and starting of disinfection. Samples were taken before and after cleaning, after washing and after drying the disinfectants. Wall surfaces were sampled; a surface area of 0.5 m 2 was vigorously swabbed after sampling and

returned to laboratory for incubation within 6 h. The samples were placed directly into buffered peptone water with neutralizer.
Retained bacterial samples were plated on tryptic soy agar and grown for 24 hr at 36 C under normal atmosphere. Pure fresh culture was inoculated into 10 ml of 1% peptone broth at a sufficient quantity (modified from Davies and Wray, 1996).

2- Evaluation of disinfectants in vitro:

Saline solutions containing specific microorganism (10^{5}/ml) were used. Every solution (0.1ml) was inoculated to 10 ml of each disinfectant dilution. After 5, 10, 15and 20 minutes, inoculum was transferred from the inoculated disinfectant to 4 ml of 1%peptone broth (subculture tubes with neutralizer). Subculture tubes were incubated for 48 hr at 36 C under normal atmosphere and checked for growth. Growth was determined by visual observation of cloudiness of the broth or pellet formation at bottom of tube. Samples showing growth in any tube were repeated in duplicate. Samples showing growth in multiple dilution levels of one or more disinfectant type were identified (modified from AOAC, 1980).

3- Identification of microorganisms:

Swabs were inoculated on peptone water and incubated at 37C for 24 hours, then streaked on nutrient, blood, chocolate and MacConkey's agar plates and incubated at 37oC for 24-48 hours. The isolated colonies were identified morphologically, culturally and biochemical (Kreig and Holt, 1984; Quinn, et al., 1994).

RESULTS

Table (1) showed the bacterial species that were isolated from the walls of different cattle farms. Gram- positive bacterial species (mainly Staphylococcus species) were isolated mainly from dairy farms. On contrary, in calf houses the gram- negative species (mainly Proteus species) were more predominant. The effect of different disinfectants on bacterial species in vivo were illustrated in Tables (2 to 7) and in vitro in Table (8). The cleaning process make a reduction in the prevalence of *Klebsiella* , *Proteus rettegri* and *Staphylococcus aureus* contamination but this not statistically significant (Table 2). No *Citrobacter* isolated from walls of calf house (Table 2) or walls of dairy yard (Table 6) after cleaning. Cleaning showing a significant reduction in contamination before application especially Iodosan 30 (Tables 3). However, only a few products were able to disinfect walls without cleaning. The Washing

effect denoted by (W) showed the significance of washing before the application of disinfectant (Table 7). So, the least affected disinfectants by organic matter were found to be QAC (Antec). The prevalence of bacterial species on wall surfaces was relatively high before cleaning process and disinfection. Following disinfection, bacteria were found in five wall surfaces (3 dairy yards and 2 calf houses) from 10 wall surfaces under study. The yard circuit was the most contaminated before C&D. (Not all bacteria were exposed to all of the disinfectants). For this reason in vitro test was the answer for evaluating all disinfectants in clean environment. This experiment revealed that the most effective disinfectant was found to be QAC. In addition, the most resistant organism was Streptococcus faecalis, which was killed after 15 min. Formalin, was found to be the second potent disinfectant, the most resistant organisms were found to be B.anthracoid and Streptococcus faecalis (were killed after 15 min.). Commercial phenol failed to kill Streptococcus faecalis in 20 minutes as did iodophor and sodium hypochlorite. The latter failed to kill Klebsiella. The most resistant bacteria were found to be Streptococcus, which was killed, by QAC and formalin. The second was found to be B.anthracoid that resists the halogens (iodophor and sodium hypochlorite

Table 1: Bacterial species isolated from five walls of different dairy yards and 5wallsof different calf houses.

Bacterial species	Dairy yards	Calf houses
Citrobacter amaloniticus	1	1
E.coli	0	1
Klebsiella pneumoniae	3	2
Proteus mirabilis	0	4
Proteus rettegri	0	2
Bacillus anthracoid	3	1
Staphylococcus aureus	5	2
Staphylococcus	1	0
Streptococcus faecalis	2	0

Table 2: Efficacy of sodium hypochlorite against bacteria isolated from wall of dairy yards (D) and calves (C) houses.

Bacterial species	Before Any Application	After Cleanness only	After Disinfection only	After Cleanness and Disinfection
Citrobacter amaloniticus	C	NI	NI	NI
Klebsiella pneumoniae	D	D	D	D
Proteus mirabilis	C	C	C	NI
Proteus rettegri	C	NI	NI	NI
Bacillus anthracoid.	D	D	D	D
Staphylococcus aureus	D	NI	NI	NI
Streptococcus faecalis	D	D	D	D

NI = Not isolated after 1 hr. after application

Table 3: Efficacy of Iodosan 30 against bacteria isolated from wall of dairy yards (D) and calves (C) houses.

Bacterial species	Before Any Application	After Cleanness only	After Disinfection only	After Cleanness and Disinfection
Klebsiella pneumoniae	D	D	D	NI
Proteus spp.	C	C	C	NI
Bacillus anthracoid.	DC	DC	DC	DC
Staphylococcus aureus	D	D	D	NI
Staphylococcus epidemicus	D	D	D	NI

NI = Not isolated after 1 hr. after application

Table 4: Efficacy of Antec against bacteria isolated from wall of dairy yards (D) and calves (C) houses.

Bacterial species	Before Any Application	After Cleanness only	After Disinfection only	After Cleanness and Disinfection
E.coli	C	C	NI	NI
Klebsiella pneumoniae	C	C	NI	NI
Staphylococcus aureus	DC	D	NI	NI

NI = Not isolated after 1 hr. after application.

Table 5: Efficacy of Formalin against bacteria isolated from wall of dairy yards (D) and calves (C) houses.

Bacterial species	Before Any Application	After Cleanness only	After Disinfection only	After Cleanness and Disinfection
Proteus mirabilis	C	C	NI	NI
Staphylococcus aureus	D	D	D	NI
Streptococcus faecalis	D	D	NI	NI

NI = Not isolated after 1 hr. after application

Table 6: Efficacy of Commercial Phenol against bacteria isolated from wall of dairy yards (D) and calves (C) houses.

Bacterial species	Before Any Application	After Cleanness only	After Disinfection only	After Cleanness and Disinfection
Citrobacter amaloniticus	D	NI	NI	NI
Klebsiella pneumoniae	DC	C	C	C
Proteus mirabilis	C	C	NI	NI
Bacillus anthracoid.	D	D	NI	NI
Staphylococcus aureus	DC	DC	DC	NI

NI = Not isolated after 1 hr. after application

Table 7: Efficacy of tested disinfectants on bacteria isolated from different cattle farms in vivo

Bacterial species	Chlorine	Iodophor	Quaternary ammonium	Formalin	Phenol
E.coli	NA	NA	S	NA	NA
Klebsiella pneumoniae	R	W	S	NA	R
Proteus mirabilis	W	W	NA	S	S
Proteus rettegri	NA	W	NA	NA	NA
Bacillus anthracoid	R	R	NA	NA	S
Staphylococcus aureus	NA	W	S	W	W*
Staphylococcus epidermidis	NA	W	NA	NA	NA
Streptococcus faecalis	R	NA	NA	S	NA

NA= Not available
W= Washing must be applied to kill
R= Resistant to disinfectant after 1 hr.
S= Sensitive to disinfectant after 1 hr.

Table 8: Efficacy of tested disinfectants on bacteria isolated from different cattle farms in vitro for 20 minutes

Bacterial species	Chlorine	Iodophor	Quaternary ammonium	Formalin	Phenol
E.coli	10 min	5 min.	5min.	5min.	10min.
Klebsiella pneumoniae	20 min.	20 min	5min.	5min.	20min.
Proteus mirabilis	15 min.	15 min.	5min.	5min.	5min.
Proteus rettegri	15 min.	10 min.	5min.	5min.	5min.
Bacillus anthracoid	Resist	Resist	10min.	15min.	10min.
Staphylococcus aureus	Resist	20min.	10min.	10min.	15min.
Staphylococcus epidermidis	Resist	20 min.	10min.	10min.	15min.
Streptococcus faecalis	Resist	Resist	15 min.	15min.	Resist

DISCUSSION

Surfaces that look may might still harbor high levels of contamination, even after cleaning. Moreover, successful disinfection can be achieved even when cleaning has been suboptimal as in case of QAC. These observations agree with those made by Gelinas and Goulet (1983); Davies and Wray (1995). They showed that QAC and phenolic compounds are not affected by the presence of organic matter. Contrary results were reported by Linton et al. (1987) who found that, the organic matter adversely affect on disinfection potency of QAC. Ineffective disinfection may actually increase the contamination rate by encouraging growth of microorganisms on surfaces made moist even by sublethal levels of disinfectant (Kradel and Miller, 1991). For this reason, errors such as over-dilution of disinfectant because of faulty metering devices or application to wet surfaces should be avoided. We have found that *Klebsiella* to be resistant to phenol (in vivo only) and sodium hypochlorite, *Bacillus spp.* resistant to Iodosan 30 and sodium hypochlorite and *Streptococcus faecalis* sensitive to QAC and formalin. These results indicate that, gram-negative bacteria were highly sensitive

to the action of disinfectants under test. While gram-positive bacteria showed less sensitivity. This is coincided with (Reem-Dosoky *et al.*, 2000). Concerning Iodophors supports other observations of the increases in resistance of gram-positive bacteria to iodophors (Zorawski and Shwarek, 1984). Staphylococcus aureus showed a greater resistance to phenolic compounds (in vivo) than did gram-ve bacteria (Reem – Dosoky *et al.*, 2000).

In general, QAC, Formalin and Phenols were best suited for use because they were not as susceptible to deactivation by organic material greatly, and QAC was relatively non-corrosive to equipment. In this study, the most effective was QAC followed by the formalin and phenolic preparations this in accord to the findings reported by (Berchieri and Barrow, 1996). The apparent efficacy of formaldehyde treatment may result from improved penetration of organic matter and biofilms in field disinfection situations.

Quaternary ammonium compounds (QAC) were found to be effective against gram – positive and most gram –negative organisms. Low concentrations of chlorine were found to be adversely affected by organic matter not completely removed from surfaces. Because chlorines have oxidizing action, resulting in metal corrosion, repeated disinfection of animal houses with chlorines should be avoided (Shirai *et al.*, 1994). The aldehydes, especially formaldehyde, commonly used to disinfect animal houses, have been evaluated in the United States by the (Department of Labor Federal Register 1987) for their harmful effects on the human body. The cleanness is the most easily and inexpensively method to reduce bacterial contamination by mechanically removing them through sanitary practices such as washing housing, equipment, hands, boots, and clothing with soap and water. These practices should, where appropriate, be followed by applying a disinfectant according to manufacturer directions and be regularly rotated to prevent resistance development. Organic matter should always be removed from target surfaces prior to applying any disinfectant, since its presence can neutralize or limit the –cidal effect of even the most efficacious compounds.

REFERENCES

AOAC (1980): Official methods of analysis 13th Ed. Washington.

Berchieri Jr, A. and Barrow, P.A. (1996): The antibacterial effects for Salmonella enteritidis phage type 4 of different chemical disinfectants and cleaning agents tested under different conditions. Avian Pathology 25: 663-673.

Davies, R. H., and Wray, C. (1995): Observation on disinfection regimes used on Salmonella enteritidis infected poultry units. Poult. Sci. 74: 638-647.

Davies, R.H. and Wray, C. (1996): Studies of contamination of three broiler breeder houses with Salmonella enteritidis before and after cleansing and disinfection. Avian Diseases 40:626-633.

Davison, S., Benson, C.E., Ziegler, A.F. and Eckroade, R.J. (1999): Evaluation of disinfectants with the addition of antifreezing compounds against nonpathogenic H7N2 avian influenza virus. Avian Diseases 43: 533-537.

Department of Labor Federal Register (1987): Occupational exposure to formaldehyde; Final Rule. U.S. Govt. Printing Office.

Earnshaw, A.M.; and Lawrence, L.M. (1998): Sensitivity to commercial disinfectants, and the occurrence of plasmids within various Listeria monocytogenes genotypes isolated from poultry products and the poultry processing environment. Journal of Applied Microbiology. 84:642-648.

Gelinas, P. and Goulet, J. (1983): Neutralization of the activity of eight disinfectants by organic matter. J.Appl.Bact. 54: 243-247.

Kradel, D.C. and Miller, W.L. (1991): Salmonella enteritidis observations in field related problems. In Proceedings of the 40 Th Western Poultry Disease Conference, Acapulco, 24-27 April. Pp. 146-150.

Kreig, N.R. and Holt, J.G. (1984): Bergey s Manual of systemic Bacteriology. Vol.1. Williams and Wilking.

Linton, A.H.; Hugo, W.B. and Russel, A.D. (1987): Disinfection in vet. and farm animals practice. Black well scientific Publications.

Quinn, P.J.; Carter, M.E.; Markey, B.K. and Carter, G.R. (1994): Clinical Veterinary Microbiology, Mosby yearbook Europe Limited.

Reem-Dosoky, M., Hafez, A.H., Sotohy, A.S. and Hosnia-Swaify, A. (2000): Evaluation of some commercial disinfectants against some pathogens in presence of interfering substances. Assiut Vet. Med. J. (43) 86:147-155.

Ruano, M.; El-Attrache, J. and Villegas, P. (2001): Efficacy comparisons of disinfectants used by the commercial poultry industry. Avian Diseases. 45: 972-977.

Shirai, J., Seki, R., Kamimura, R. and Mitsubayashi, S. (1994): Effects of invert soap with 0.05% sodium hydroxide on infectious bursal disease virus. Avian Diseases 38: 240-243.

Taghi-Kilani, R.; Gyurek, L.L.; Millard, P.J.; Finch, G.R. and Belosevic, M. (1996): Nucleic acid stains as indicators of Giardia muris viability following cyst inactivation. International Journal for Parasitology 26:637-646.

Valancony, H.; Fournier, G.; Drouin, P.; Danguy, R.; Toux, J.Y. and Balaine, L. (2001): Cleaning and disinfection of cage layer houses against Salmonella enteritidis: 1st part/evaluation of the bacteriological samplings. Sciences & Techniques Avicoles. 36:5-14.

Zorawski, C. and Skwarek, P. (1984): Bactericidal activity of Jodasept, Pollena Jodk and Biocide-30.Medycyna Weterynaryjna, 40(7): 413-4150.