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Comparative Efficacy of Commonly Used Disinfectants in Dairy Facilities

D. Divyalakshmi, N. Kumarvelu and Thanga. Thamil Vanan
Department of Livestock Production Management, Madras Veterinary College, Chennai-7
*Corresponding author e-mail: nandhi7121989@gmail.com

ABSTRACT
Disinfection of animal shed means making them free from disease producing organisms. An attempt has been made to assess the efficacy of disinfectants. The study was carried out in three different seasons of the year in six dairy facilities which housed indigenous and cross bred lactating cows. All the animal facilities were within the recommended dimensions. Five disinfectants namely, Dettol® (4.8 % chloroxylenol), chlorine dioxide, sodium hypochlorite, calcium hypochlorite and cow urine based disinfectant were used. The efficacy of the disinfectants was found out by dilution method. In dairy cattle shed the order of efficiency of disinfectants in descending order ranges from chlorine dioxide, Dettol®, calcium hypochlorite, cow urine based disinfectant and sodium hypochlorite in all the seasons of the year Hence, spraying with chlorine dioxide or using 4.8% chloroxylenol in dairy facilities was found to be effective in all seasons of the year.
***Key words**: Pig fattener sty, Farrowing pen, Disinfectants, Seasons.*

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INTRODUCTION

Intensive livestock farming provides optimum conditions for the concentration of pathogens and transmission. The crowding of animals in an enclosed environment is highly conducive for the transmission of diseases. Sanitation program in livestock sheds include adequate ventilation, drainage, regular cleaning and disinfection of farm premises [31]. Disinfection is one of the important activities in a commercial livestock farm to sustain the health of animals and quality of products obtained. Disinfectants are agents which are targeted against microorganisms and aimed at reducing their pathogenicity. Generally, a commercially available disinfectant should exhibit the ability to reduce microbial contamination by several orders of magnitude in a standard test method in order to be approved for use. In farms however, not all disinfectants exhibit the activity that one would expect on standard tests [14]. Therefore, the efficacy of disinfectants is based on its power of destroying or reducing inert disease causing germs. The main goal of disinfection activities is to interrupt the route of transmission of germs between the infection source and healthy subjects. Little is known about effectiveness of cleaning and disinfection procedures applied on small scale livestock rearing in our country. The purpose of this paper is to evaluate the efficacy of some commonly used disinfectants, considering the hygiene systems used with dairy cattle rearing and to provide a programme of standard disinfection protocol.

MATERIAL AND METHODS

Experimental location

The experiment was carried out at Livestock arm Complex, Madhavaram, Chennai-51 located between latitudes 12° 9′ and 13° 9′ N and longitudes 80° 12′ and 80 ° 19′ E with an altitude of 22 m above MSL. The laboratory works were carried out in Vaccine Research Centre – Bacterial Vaccines, TANUVAS. The experiment period comprised rainy (August – December), winter (January– February) and summer (March – April) seasons. Six conventional dairy sheds housing 14 cross bred cows with head to head system fed with recommended roughage and concentrate ration was selected. The sheds were provided

with full monitor king truss with natural ventilation. The dairy cows were provided with a floor space of 6m^2/ animal.

Selection of disinfectants

Dettol®, chlorine dioxide, sodium hypochlorite, calcium hypochlorite and a traditional cow urine based mixture were tested for their efficacy as disinfectant in diary facilities. Dettol® (4.8 % chloroxylenol) was used in 1:20 concentration as suggested by Olowe *et al*., [28] and Chinedu *et al.,* [7] and diluted with distilled water and splashed on the floor as disinfectant in dairy sheds. The stock solution of chlorine dioxide was prepared according to standard method [2]. Stock solution of chlorine dioxide was prepared with sodium chlorite and citric acid reagent which was diluted in distilled water. After 30 minutes, 5ml of the stock solution was diluted in one litre of distilled water and the gas liberated was sprayed over the livestock premises. Sodium hypochlorite (4% readymade laboratory reagent) was diluted with distilled water and the concentration was brought down to 2% and splashed directly on the floor of dairy premises. 30% Calcium hypochlorite (9% available chlorine) as readymade chemical was dusted directly on the floor. Cow urine based disinfectant was prepared by mixing 500 ml of cow urine (which was collected from an healthy indigenous cattle), with 250 gms of freshly ground neem leaves *(Azadirachta indica)*, 250 gms of tulsi leaves (*Ocimum tenuiflorum)* and 250 gms of ritha nuts *(Sapindus mukorosse)* along with 250 ml of commercially available pine oil *(Pinus palustris)*. The mixture was freshly prepared and swabbed in the floor of dairy facilities as suggested by Mandavgane *et al.* [24].

On farm disinfectant efficacy study

To study the efficacy of the disinfectants, the microbial load in the floor of animal sheds, before and after water wash was taken in the animal sheds. The disinfectants were applied according to the recommended procedure as mentioned above. After application, floor swabs were taken after 1 hour, 8 hours and 24 hours post disinfection.

Collection of samples

The samples were taken in three different places (feeding area, standing area and dunging area) inside the shed with individual sterile cotton tipped swabs by swabbing within 10 cm^2 area as suggested by Gibson *et al.,*[13]. The sample was transported aseptically from sampling site to the laboratory within half an hour and the test was carried on. The efficacy study of the disinfectants was carried out by dilution method where serial dilutions were done. An aliquot of 1 ml was taken from dilution and poured in sterile petri plates in triplicate and mixed with 20 ml of liquefied sterilized plate count agar. After solidification of agar, the plates were incubated in inverted position at 37^{o}C for 24 hours. After incubation, bacterial cells grow into distinct colonies, which were counted with colony counter. All the procedures were done in laminar air flow cabinet.

RESULTS AND DISCUSSION

Effect of Dettol® as disinfectant in dairy shed during different seasons is presented in Table 2. The mean ± S.E of floor microbial load in dairy shed during rainy, winter and summer before water wash were $8.55x10^{11}$±1.034, $8.43x10^{11}$±1.11 and $5.78x10^{11}$±1.10 ; after water wash were $5.17x10^{11}$±1.14, $5.23x10^{11}$±1.06 and $3.29x10^{11}$±1.15; one hour post disinfection were$2.31x10^{7}$±1.22, $9.18x10^{7}$±1.53 and $3.24x10^{6}$±1.35; eight hour post disinfection were $5.14x10^{9}$±1.69, $1.99x10^{9}$±1.26 and$5.02x10^{8}$±1.34; and 24 hour post disinfection were $7.69x10^{11}$±1.07, $8.63x10^{11}$±1.02 and $6.55x10^{11}$±1.12, respectively. Statistically high significant (P<0.01) difference in microbial load before and after disinfection is observed with Dettol application between seasons in dairy shed. The microbial load highly reduced (P<0.01) in dairy shed after disinfection with Dettol in all seasons. Singh [34], Olowe *et al.* [28], Rutula *et al*. [32], Maes *et al.* [23], Okesola *et al.* [26], Chima et *al.* [6], Olorode and Okpokwasli [27] and Chinedu *et al.* [7] also have reported the efficiency of Dettol as a potential disinfectant in different livestock sheds. The contact time of phenolic compounds is 10-30 minutes as suggested by Fotheringham [11], the floor microbial load started to decrease since phenolic compounds have action even in the presence of organic matter, but after 24 hrs the microbial load started to increase.

Effect of chlorine dioxide as disinfectant agent in dairy during different seasons is presented in Table 3. The mean ± S.E of floor microbial load in dairy shed during rainy, winter and summer before water wash were $9.22x10^{11}$±1.11, $7.07x10^{11}$±1.14 and $6.29x10^{11}$±1.051; after water wash were $4.12x10^{11}$±1.19, $4.03x10^{11}$±1.12 and $2.73x10^{11}$±1.12; one hour post disinfection were $2.65x10^{6}$±1.34, $2.10x10^{6}$±1.36 and $4.02x10^{6}$±1.28; eight hour post disinfection were $3.95x10^{7}$±1.35, $1.74x10^{7}$±1.21and $2.94x10^{7}$±1.36; and 24 hour post disinfection were $1.01x10^{12}$±1.23, $8.02x10^{11}$±1.19 and $9.02x10^{11}$±1.15, respectively. It is observed from the Table 3, that the season does not have any influence in the efficacy of chlorine dioxide in dairy shed between seasons. The floor microbial load reduced significantly (P<0.01) post disinfection with chlorine dioxide in all the seasons in dairy shed. The disinfectant effect of chlorine dioxide was reported by earlier workers [3, 9, 38,33, 12, 22, 23, 25]. When chlorine dioxide reacts and decays, chlorite

and chlorate are formed both of these compounds have bactericidal effect [38]. After the contact time, the bacterial load started to increase due to accelerated decomposition of chlorine dioxide, which could be anticipated to occur either through reductive reactions or photolysis. Light induced decomposition can be ruled out as a significant source of gas loss [22].

Table1. Physical, chemical and microbial qualities of water samples used with disinfectants

Physical examination	
Appearance	Clear
Turbidity	Nil
Smell	Nil
Chemical examination	
Parameters	**Value**
Ammonia	absent
Chloride	150 ppm
Sulphate	+
Sulphide	absent
Nitrate	+ 20 mg/ L
Nitrite	0.2 mg/L
Phosphate	absent
Fluoride	absent
Residual chlorine	absent
Iron	0 mg/L
Copper	absent
Lead	absent
Zinc	absent
p^H	6.8
Alkalinity	100 ppm
Hardness	120 ppm
TDS	510 ppm
Microbiological examination of water	
Total Viable Count/ml	1.2 $X10^1$/ml
E.coli	not detected

Effect of sodium hypochlorite as disinfectant agent in dairy shed during different seasons is presented in Table 4. The mean ± S.E of floor microbial load in dairy shed during rainy, winter and summer before water wash were $9.17x10^{11}$±1.24, $1.25x10^{12}$±1.26 and $6.76x10^{11}$±1.08; after water wash were $4.96x10^{11}$±1.17, $6.7x10^{11}$±1.17 and $3.22x10^{11}$±1.11; one hour post disinfection were $3.5x10^{11}$±1.10, $5.25x10^{11}$±1.12 and $2x10^{11}$±1.11; eight hour post disinfection were $2.6x10^{11}$±1.11, $3.36x10^{11}$±1.13 and $1.32x10^{11}$±1.17; and 24 hour post disinfection were $9.73x10^{11}$±1.13, $1.14x10^{12}$±1.23 and $6.71x10^{11}$±1.08, respectively. From Table 4 it is observed that the basal microbial load of the floor before wash was almost similar in all the seasons in the dairy shed. There was significant reduction ($P<0.05$) with water wash and highly significant reduction ($P<0.01$) in post disinfection load in all the seasons in dairy shed. . The reduction in floor microbial load post disinfection was noted to be highly significant ($P<0.01$) in all the livestock sheds. The antiseptic effect of sodium hypochlorite were discussed by Owen *et al.* (1995), Fotheringham [11], Rutala *et al.* [32], Benedictis *et al.* [4], Jordan *et al.* [16], Maes *et al.* [23], Kim *et al.* [19], Eterpi *et al.* [10], Kaoud *et al.* [17] and Taharaguchi *et al.* [36]. The decreased bactericidal action of sodium hypochlorite in farm premises may be due to the inactivation of sodium hypochlorite by the presence of organic soiling and the instability of the compound in warm and sunny conditions as suggested by Fotheringham [11]. Thomas and Sastry (2012) insisted the use of sodium hypochlorite for udder washing and utensil rinsing.

Effect of calcium hypochlorite as disinfectant agent in dairy shed during different seasons is presented in Table 5. The mean ± S.E of floor microbial load in dairy shed during rainy, winter and summer before water wash were $1.16x10^{12}$±1.22, $6.48x10^{11}$±1.11 and $6.12x10^{11}$±1.09; after water wash were

$5.99x10^{11}$±1.14, $4.18x10^{11}$±1.11 and $3.07x10^{11}$±1.09; one hour post disinfection were $3.32x10^{11}$±1.15, $3.28x10^{11}$±1.15 and $1.48x10^{11}$±1.10; eight hour post disinfection were $2.17x10^{11}$± 1.13, $1.86x10^{11}$±1.33; and $7.25x10^{10}$±1.16 and 24 hour post disinfection were $7x10^{11}$ ±1.45, $1.08x10^{12}$ ±1.09; and $6.41x10^{11}$± 1.10, respectively. From the Table 5 it is noted that there exists highly significant (P<0.01) difference in the efficacy of calcium hypochlorite in reducing the floor microbial load in dairy shed between seasons and post disinfection counts. The application of calcium hypochlorite as floor disinfectant reduced the floor microbial level in livestock sheds significantly. Fotheringham [11], Islam *et al.* [15] and Thomas and Sastry [37] have mentioned the use of bleaching powder in livestock premises. Action of bleaching powder is influenced by temperature, p^H, and presence of organic substance in floor of livestock premises [21] since the water used for cleaning the sheds had nearly a neutral acidity the effect of bleaching powder may be decreased.

Effect of cow urine based disinfectant in dairy shed during different seasons is presented in Table 6. The mean ± S.E of floor microbial load in dairy shed during rainy, winter and summer before water wash were $6.94x10^{11}$±1.08, $8.46x10^{11}$±1.08 and $8.12x10^{11}$±1.06; after water wash were $4.14x10^{11}$±1.10, $7.14x10^{11}$±1.04 and $4.46x10^{11}$±1.12; one hour post disinfection were $2.94x10^{11}$±1.15, $6.8x10^{11}$±1.05 and $2.34x10^{11}$±1.08; eight hour post disinfection were $1.92x10^{11}$±1.18, $1.82x10^{11}$±1.57 and $1.32x10^{11}$±1.77; and 24 hour post disinfection were $8.47x10^{11}$±1.03, $9.32x10^{11}$±1.14 and $8.38x10^{11}$±1.04, respectively. The action of cow urine based disinfectant did not differ significantly between seasons in 8 and 24 hours post disinfection count, whereas the reduction in floor microbial count was highly significant (P<0.01) within a season by the application of the cow urine based disinfectant (Table 6). The action of cow urine based disinfectant was highly significant (P<0.01) within a particular season in all livestock sheds.cow urine based disinfectant with different herbal extracts as a disinfectant agent have been reported by various workers in their findings [24, 5, 35, 8, 19, 20]. Cow's urine is an effective natural agent in inhibiting bacteria and fungi, and also has a high potential lipase activity [20]. Neem oil was found to contain different chemical substances viz., azadirachtin, meliantrol and salanin which were responsible for the pesticidal, larvicidal and insecticidal activities. The main constituent of cow urine that showed disinfectant activity was due to carbolic acid, which is a mixture of phenol and cresol [22]. The decreased activity of the cow urine based disinfectant used in the present study may be due to the decreased concentration of *Ocimum tenuiflorum* leaf extract used, since 500 – 600 mg/l of leaf extract with a contact time of 15-16 hours was required for inactivating *E.coli* and other harmful organisms as suggested by Sundaramurthi *et al.* [35] and Kayastha *et al.* [18]. This was tried because of the traditional use of cow urine and other constituents as disinfectants.

The season, dairy housing and efficiency of disinfectants were statistically analysed using three way analysis of variance to find out the most efficient disinfectant agent during various seasons and are presented in Table 7. The seasonal effect of the disinfectants may be due to the influence of temperature, humidity (both absolute and relative), sunlight (ultraviolet light) exposure and even atmospheric pollutants. These factors will affect the various bacterial organisms in different ways and degrees, and it is sometimes difficult to make generalizations.

Table 2. Effect of Dettol® in dairy shed

Treatment	Rain	Winter	Summer
Before wash	$8.55x10^{11Bb}$±1.034	$8.43x10^{11Cb}$±1.11	$5.78x10^{11Ca}$±1.10
After wash	$5.17x10^{11\ Bb}$ ±1.14	$5.23x10^{11Bb}$±1.06	$3.29x10^{11Ba}$±1.15
1 hr PD	$2.31x10^{7\ A}$ ±1.22	$9.18x10^{7\ A}$ ±1.53	$3.24x10^{6\ A}$ ±1.35
8 hr PD	$5.14\ x10^{9\ A}$ ±1.69	$1.99x10^{9\ A}$ ±1.26	$5.02x10^{8\ A}$ ±1.34
24 hr PD	$7.69x10^{11\ B}$±1.07	$8.63x10^{11Cb}$±1.02	$6.55x10^{11Ca}$±1.12

PD: Post Disinfection

Means bearing different superscript in the same row and column differ significantly

Table 3. Effect of chlorine dioxide in dairy shed

Treatment	Rain	Winter	Summer
Before wash	$9.22x10^{11Cb}$±1.11	$7.07x10^{11Dab}$±1.14	$6.29x10^{11Da}$±1.05
After wash	$4.12x10^{11B}$±1.19	$4.03x10^{11C}$ ±1.12	$2.73x10^{11}$ ±1.12
1 hr PD	$2.65x10^{6A}$ ±1.34	$2.10x10^{6A}$ ±1.36	$4.02x10^{6A}$ ±1.28
8 hr PD	$3.95x10^{7A}$ ±1.35	$1.74x10^{7B}$ ±1.21	$2.94x10^{7B}$ ±1.36
24 hr PD	$1.01x10^{12C}$±1.23	$8.02x10^{11D}$ ±1.19	$9.02x10^{11D}$±1.15

PD: Post Disinfection
Means bearing different superscript in the same row and column differ significantly

Table 4. Effect of sodium hypochlorite in dairy shed

Treatment	Rain	Winter	Summer
Before wash	$9.17x10^{11Cab}$±1.24	$1.25x10^{12Cb}$ ±1.26	$6.76x10^{11Da}$±1.08
After wash	$4.96x10^{11Bab}$±1.17	$6.7x10^{11Bb}$ ±1.17	$3.22x10^{11Ca}$±1.11
1 hr PD	$3.5x10^{11ABb}$±1.10	$5.25x10^{11ABc}$±1.12	$2x10^{11Ba}$ ±1.11
8 hr PD	$2.6x10^{11Ab}$ ±1.11	$3.36x10^{11Ab}$±1.13	$1.32x10^{11Aa}$±1.17
24 hr PD	$9.73x10^{11Cab}$±1.13	$1.14x10^{12Cb}$ ±1.23	$6.71x10^{11Da}$±1.08

PD: Post Disinfection
Means bearing different superscript in the same row and column differ significantly

Table 5. Effect of calcium hypochlorite in dairy shed

Treatment	Rain	Winter	Summer
Before wash	$1.16x10^{12Cb}$ ±1.22	$6.48x10^{11BCa}$±1.11	$6.12x10^{11Da}$±1.09
After wash	$5.99x10^{11BCb}$±1.14	$4.18x10^{11ABb}$±1.11	$3.07x10^{11Ca}$±1.09
1 hr PD	$3.32x10^{11ABb}$±1.15	$3.28x10^{11Aa}$ ±1.15	$1.48x10^{11Ba}$±1.10
8 hr PD	$2.17x10^{11Ab}$ ±1.13	$1.86x10^{11Ab}$ ±1.33	$7.25x10^{10Aa}$±1.16
24 hr PD	$7x10^{11C}$ ±1.45	$1.08x10^{12C}$ ±1.09	$6.41x10^{11D}$±1.10

PD: Post Disinfection
Means bearing different superscript in the same row and column differ significantly

Table 6. Effect of cow urine based disinfectant in dairy shed

Treatment	Rain	Winter	Summer
Before wash	$6.94x10^{11D}$±1.08	$8.46x10^{11B}$±1.08	$8.12x10^{11D}$±1.06
After wash	$4.14x10^{11Ca}$±1.10	$7.14x10^{11Bb}$±1.04	$4.46x10^{11Ca}$±1.12
1 hr PD	$2.94x10^{11Ba}$±1.15	$6.8x10^{11Bb}$ ±1.05	$2.34x10^{11Ba}$±1.08
8 hr PD	$1.92x10^{11A}$±1.18	$1.82x10^{11A}$±1.57	$1.32x10^{11A}$±1.77
24 hr PD	$8.47x10^{11D}$±1.03	$9.32x10^{11B}$±1.14	$8.38x10^{11D}$±1.04

PD: Post Disinfection
Means bearing different superscript in the same row and column differ significant

Table7. Effect of season and treatment on microbial load in dairy shed

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	302.870[a]	74	4.093	28.685	0
Intercept	320173.84	1	320173.84	2243949.527	0
Season	18.583	2	9.291	65.12	0
Treatment	18.216	4	4.554	31.916	0
Time	227.087	4	56.772	397.887	0
Season * Treatment	4.963	8	0.62	4.348	0
Season * Time	3.784	8	0.473	3.315	0.001
Treatment * Time	25.666	16	1.604	11.242	0
Season * Treatment * Time	4.572	32	0.143	1.001	0.469
Error	53.506	375	0.143		
Total	320530.216	450			
Corrected Total	356.376	449			

CONCLUSION

In dairy cattle shed the order of efficiency of disinfectants in descending order ranges from chlorine dioxide, Dettol, calcium hypochlorite, cow urine based disinfectant and sodium hypochlorite in all the seasons of the year

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