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Research Article

Comparative efficacy of few disinfectants against bacterial load in pig facilities at livestock farm complex, Chennai

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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 during 2017-18 in pig fattener facilities and farrowing pen at Livestock Farm Complex, Madhavaram, Chennai. Four disinfectants namely, 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 pig fattener sty the efficacy of disinfectants in descending order were chlorine dioxide ($1.77x10^8$ ±1.10), sodium hypochlorite ($2.57x10^{11}$±1.15), cow urine based disinfectant ($1.68x10^{11}$±1.12) and calcium hypochlorite ($7.73x10^{11}$±1.06) in all the seasons of the year. In farrowing pen the order of efficacy of disinfectants were chlorine dioxide ($1.82x10^8$±1.17), sodium hypochlorite ($2.71x10^{11}$±1.15), calcium hypochlorite ($2.66x10^{11}$±1.17) and cow urine based disinfectant ($3.15x10^{11}$ ±1.07) in all the seasons of the year. Hence, spraying with chlorine dioxide in pig facilities was found to be effective in all seasons of the year.

Keywords: Disinfectants, Farrowing pen, Pig fattener sty, Seasons

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. Disinfection is one of the important activities in a commercial livestock farm to sustain the health of animals and quality of products obtained. Disinfection is a recommended disease-preventing measure that is commonly used in animal facilities. Chemical disinfectants (benzalkonium chloride, formaldehyde and glutaraldehyde) are widely used as a preventative or precautionary measure against bacterial infections in livestock animals such as cattle, swine and poultry (Lewis and McIndoe, 2004).

However, potentially toxic, corrosive or volatile problems have arisen because of the use of chemicals as disinfecting agents. The main goal of disinfection activities is to interrupt the route of transmission of germs between the infection source and healthy subjects (Graslund and Bengtsson, 2001). Little is known about the effectiveness of cleaning and disinfection procedures applied on livestock housing in our country. The purpose of this study is to consider the hygiene systems used with pig rearing and to provide a programme of cleaning and to evolve a standard disinfection protocol. Therefore, comparative efficacy of few disinfectants was investigated against bacterial load in pig facilities at Livestock farm complex, Chennai.

MATERIALS AND METHODS

The experiment was carried out at Livestock Farm Complex, Madhavaram, Chennai. The laboratory works were carried out in the Vaccine Research Centre – Bacterial Vaccines, Tamil Nadu Veterinary and Animal Sciences University. The experiment period comprised rainy (August – December), winter (January– February) and summer (March – April) seasons during 2017-18. Pig sty with four pens having conventional open run and pen system housing 10 - 13 piglets from the age of weaning till market age. Four conventional farrowing pens that housed one sow and 10 – 15 piglets in each pen were selected. The water used for mixing the disinfectants was tested for physical, chemical and microbial qualities (Table 1).

Concentration of disinfectants: The disinfectants *viz.*, chlorine dioxide, sodium hypochlorite, calcium hypochlorite and a cow urine based mixture were tested for their efficacy as disinfectant in swine facilities. Stock solution of chlorine dioxide was prepared by adding 40 gm of sodium chlorite in 50 gm of citric acid reagent which was already diluted in 50 ml water. After 30 minutes, 5ml of the stock solution was diluted in one litre of water and sprayed over the livestock premises. Sodium hypochlorite 4% was diluted with water and the concentration was brought down to 2% and splashed. Calcium hypochlorite 30% as readymade chemical was dusted. Cow urine based disinfectant equal quantities of the following ingredients viz., cow urine collected from indigenous cattle, freshly ground neem leaves *(Azadirachta indica)*, tulsi leaves (*Ocimum tenuiflorum)* and ritha nuts *(Sapindus mukorosse)* were mixed along with commercially available pine oil *(Pinus palustris)*. The mixture was prepared and sprinkled directly and mopped in the floor of livestock shed as suggested by Mandavgane *et al.* (2005).

Assessment of efficacy of disinfectants: 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 procedures mentioned by Prasad (1999). After application, floor swabs were taken after 1 hour, 8 hours and 24 hours post disinfection. 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.* (1999). The sample was transported aseptically from sampling site to the laboratory within one 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 (Hi-Media) with a composition of Tryptone- 5.0 g/lt, Yeast extract -2.500 g/lt, Dextrose-1.00 g/lt, Agar-15.00 g/lt having pH-7.0 ± 0.2. After solidification of agar, the plates were incubated in inverted position at 37^{o}C for 24 hours. After incubation, bacterial cells grew into distinct colonies, which were counted as CFU/ml with colony counter. All the procedures were done in Laminar air flow cabinet.

RESULTS AND DISCUSSION

Effect of chlorine dioxide in pig fattener sty and farrowing pen during different seasons against floor microbial load in pig fattener sty and farrowing pen during rainy, winter and summer is provided in Table 2. It is evident, that there was no significant difference (P<0.05) in the efficacy of chlorine dioxide in pig fattener sty between seasons, but highly significant (P<0.01) reduction in floor microbial load was observed in post disinfection assessment in all seasons. It was noted that in farrowing pen, the floor microbial load did not differ statistically before water wash in the morning. After the application of chlorine dioxide, the reduction in microbial load did not differ significantly between seasons in one hour and eight-hour post disinfection. Highly significant (P<0.01) reduction in microbial load was observed after chlorine dioxide application in all the seasons. The disinfectant effect of chlorine dioxide was reported by earlier workers in poultry and pig facilities (Luyckx *et al.,*

Table 1. Quality 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

Table 2. Effect of chlorine dioxide on bacterial load of pig facilities at Livestock Farm Complex.

Treatment		Bacterial load CFU/ml		
		Rain	Winter	Summer
Before wash	Pig fattener	1.08x10^{12Db}±1.24	1.21x10^{12Db}±1.19	6.21x10^{11Da}±1.11
	Farrowing	1.23x10$^{12\ D}$±1.21	9.38x10$^{11\ D}$±1.28	7.92x10$^{11\ D}$±1.11
After wash	Pig fattener	5.86x10^{11Cb}±1.09	5.89x10^{11Cb}±1.19	3.53x10^{11Ca}±1.07
	Farrowing	7.31x10^{11Cb}±1.05	4.31x10^{11Ca}±1.20	3.58x10^{11Ca}±1.12
1 hr PD	Pig fattener	2.72x10$^{6\ A}$ ±1.37	1.83x10$^{6\ A}$ ±1.30	1.83x10$^{6\ A}$ ±1.30
	Farrowing	1.6x10$^{6\ A}$ ±1.15	1.86x10$^{6\ A}$ ±1.04	2.34x10$^{6\ A}$ ±1.33
8 hr PD	Pig fattener	1.77x10$^{8\ B}$ ±1.10	1.70x10$^{8\ B}$ ±1.31	2.27x10$^{8\ B}$ ±1.33
	Farrowing	1.82x10$^{8\ B}$ ±1.17	1.94x10$^{8\ B}$ ±1.12	1.72x10$^{8\ B}$ ±1.13
24 hr PD	Pig fattener	1.12x10$^{12\ D}$±1.19	1.12x10$^{12\ D}$±1.14	7.38x10$^{11\ D}$±1.11
	Farrowing	1.01x10$^{12\ D}$±1.08	1.06x10$^{12\ D}$±1.18	7.54x10$^{11\ D}$±1.08

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

Table 3. Effect of sodium hypochlorite on bacterial load of pig facilities at Livestock Farm Complex.

Treatment		Bacterial load CFU/ml		
		Rain	Winter	Summer
Before wash	Pig fattener	1.17x10^{12Db}±1.15	7.92x10^{11Ca}±1.11	6.45x10^{11Da}±1.09
	Farrowing	9.71x10^{11Db}±1.07	1.68x10$^{12\ Dc}$±1.20	4.12x10$^{11\ a}$±1.11
After wash	Pig fattener	6.28x10^{11Cb}±1.14	3.58x10^{11Ba}±1.12	3.23x10^{11Ca}±1.11
	Farrowing	5.17x10^{11Cb}±1.08	6.87x10^{11BCb}±1.09	1.85x10$^{11\ a}$±1.37
1 hr PD	Pig fattener	4.26x10^{11Bb}±1.41	3.18x10^{11Bb}±1.12	1.57x10^{11Ba}±1.13
	Farrowing	3.67x10 ^{11B}±1.12	5.12x10$^{11\ B}$ ±1.18	3.16x10^{10}±31.68
8 hr PD	Pig fattener	2.57x10^{11Ab}±1.15	1.72x10^{11Ab}±1.13	8.54x10^{10Aa}±1.26
	Farrowing	2.71x10$^{11\ A}$±1.15	2.82x10$^{11\ A}$ ±1.17	1.82x10^{11}±1.15
24 hr PD	Pig fattener	8.38x10$^{11\ C}$±1.05	7.54x10^{11C}±1.08	6.6x10 $^{11\ D}$ ±1.10
	Farrowing	1.08x10$^{12\ D}$±1.03	1.12x10 ^{12CD}±1.24	7.67x10 ^{11}±1.09

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

Table 4. Effect of calcium hypochlorite on bacterial load of pig facilities at Livestock Farm. Complex.

Treatment		Bacterial load CFU/ml		
		Rain	Winter	Summer
Before wash	Pig fattener	1.36x10^{12Bd}±1.07	1.46x10^{12Cb} ±1.16	7.25x10^{11Da}±1.07
	Farrowing	1.61x10^{12Db}±1.41	1.69x10^{12Cb}±1.16	5.87x10^{11Da}±1.10
After wash	Pig fattener	2.51x10^{11Ba}±1.15	7.69x10^{11Bb} ±1.06	3.5x10$^{11\ Ca}$ ±1.14
	Farrowing	5.17x10^{11Bb}±1.08	7.83x10^{11Bc}±1.08	3.47x10^{11Ca}±1.51
1 hr PD	Pig fattener	1.69x10^{11Aa}±1.12	5x10^{11Ab} ±1.17	1.62x10^{11Ba}±1.11
	Farrowing	3.87x10^{11AB}±1.14	6.80x10^{11Bc}±1.08	1.62x10^{11Ba}±1.25
8 hr PD	Pig fattener	7.73x10^{11Cc}±1.06	3.16x10$^{11\ Ab}$±1.17	8.83x10^{10Aa}±1.22
	Farrowing	2.66x10^{11Ab}±1.17	3.69x10^{11Ab}±1.16	7.9 x10$^{10\ Aa}$±1.06
24 hr PD	Pig fattener	1.08x10^{12Db}±1.05	1.13x10^{12BCb}±1.24	5.82x10^{11Da}±1.09
	Farrowing	9.12x10$^{11\ Ca}$±1.05	9.51x10^{11Ba}±1.47	7.45x10^{11Da}±1.06

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

Table 5. Effect of cow urine based disinfectant on bacterial load of pig facilities at Livestock Farm Complex.

Treatment		Bacterial load CFU/ml		
		Rain	Winter	Summer
Before wash	Pig fattener	9.3x10$^{11\ C}$ ±1.17	8.09x10$^{11\ C}$±1.05	8.2x10 $^{11\ D}$ ±1.10
	Farrowing	1.74x10^{12Db}±1.37	2.91x10^{12Cb}±1.45	6.17x10^{11Ca}±1.13
After wash	Pig fattener	5.27x10$^{11\ B}$ ±1.07	5.22x10$^{11\ B}$±1.06	5.2x10$^{11\ C}$ ± 1.11
	Farrowing	6.1x10$^{11\ Bb}$ ±1.07	6.82x10^{11Bb}±1.23	2.39x10^{11}±1.10^{Ba}
1 hr PD	Pig fattener	3.84x10^{11Bb}±1.06	4.64x10^{11Bb}±1.06	2.06x10^{11Ba}±1.12
	Farrowing	4.86x10^{11ABb}±1.08	5.39x10^{11Bb}±1.16	1.77x10^{11Aa}±1.04
8 hr PD	Pig fattener	1.68x10^{11Aa}±1.12	3.19x10^{11Ab}±1.10	1.37x10^{11Aa}±1.16
	Farrowing	3.15x10$^{11\ Ab}$ ±1.07	2.7x10$^{11\ Ab}$ ±1.16	1.36x10^{11Aa}±1.14
24 hr PD	Pig fattener	9.51x10$^{11\ C}$ ±1.09	8.15x10$^{11\ C}$±1.03	8.5x10$^{11\ D}$ ±1.02
	Farrowing	9.84x10$^{11\ Cb}$ ±1.06	7.94x10^{11Bb}±1.11	6.19x10^{11Ca}±1.06

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

Table 6. Correlation between seasons and treatments on microbial load in pig fattener sty.

Source	Type III Sum of Squares	d.f	Mean Square	F	Significance
Corrected Model	218.377[a]	59	3.701	35.827	0
Intercept	258634.278	1	258634.278	2503474.548	0
season	17.597	2	8.798	85.165	0
Treatment	3.766	3	1.255	12.152	0
Time	156.725	4	39.181	379.259	0
Season * Treatment	6.734	6	1.122	10.864	0
Season * Time	4.375	8	0.547	5.293	0
Treatment * Time	12.864	12	1.072	10.377	0
season * Treatment * Time	16.316	24	0.68	6.58	0
Error	30.993	300	0.103		
Total	258883.648	360			
Corrected Total	249.37	359			

Table 7. Correlation between seasons and treatments on microbial load in farrowing pen.

Source	Type III Sum of Squares	d.f	Mean Square	F	Sig.
Corrected Model	259.158[a]	59	4.393	3.291	0
Intercept	260046.934	1	260046.934	194857.393	0
Season	53.158	2	26.579	19.916	0
Treatment	4.479	3	1.493	1.119	0.342
Time	159.405	4	39.851	29.861	0
Season * Treatment	8.572	6	1.429	1.071	0.38
Season * Time	9.477	8	1.185	0.888	0.527
Treatment * Time	11.344	12	0.945	0.708	0.743
Season * Treatment * Time	12.724	24	0.53	0.397	0.996
Error	400.365	300	1.335		
Total	260706.456	360			
Corrected Total	659.523	359			

2016, Gosling *et al.* 2018 and Jiang *et al.* 2018). When chlorine dioxide reacts and decays, chlorite and chlorate are formed both of these compounds have bactericidal effect (Volk, 2002). After the contact time, the bacterial load started to increase due to accelerated decomposition of chlorine dioxide, which could be anticipated to occur either hrough reductive reactions or photolysis.

Effect of sodium hypochlorite pig fattener sty and farrowing pen during different seasons are presented in Table 3. It is noted that the efficacy of sodium hypochlorite in the floor of pig fattener sty was highly significant ($P<0.01$) between seasons. It is evident that the action of sodium hypochlorite as a disinfectant did not differ significantly between seasons, whereas reduction in floor microbial load, post disinfection was highly significant ($P<0.01$) during rainy and winter season. The antiseptic effect of sodium hypochlorite in the present study is in agreement with the findings of Aarnisalo *et al.* (2007) and Kaoud *et al.* (2013). 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 (1995).

Effect of calcium hypochlorite pig fattener sty and farrowing pen during different seasons are presented in Table 4, which depicts that season and post disinfection count significantly ($P<0.01$) are influenced by the application of calcium hypochlorite on the floor in fattener pig sty. Action of bleaching powder is influenced by temperature, p^H, and presence of organic substance in floor of livestock premises (Linton, 1940). Since the water used for cleaning the sheds had nearly a neutral acidity, which may decrease the effect of bleaching powder.

Effect of cow urine based disinfectant in pig fattener sty and farrowing pen during different seasons are presented in Table 5. It is observed that there was a highly significant ($P<0.01$) reduction in floor microbial load of pig fattener sty and farrowing pen in all the seasons by the application of cow urine based disinfectant. Cow's urine is an effective natural agent in inhibiting bacteria and fungi, and also has a high potential lipase activity (Kumar, 2013). 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 (Mandavgane *et al.* 2005). 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.* (2012) and Kayastha (2014). In pig fattener sty the order of efficacy of disinfectants were chlorine dioxide > sodium hypochlorite > cow urine based disinfectant > calcium hypochlorite in all the seasons of the year, respectively. In farrowing pen the order of efficacy of disinfectants were chlorine dioxide > sodium hypochlorite > calcium hypochlorite > cow urine based disinfectant in all the seasons of the year, respectively. The seasonal effect of the disinfectants (Table 6 and 7) 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. Hence, it is concluded that spraying with chlorine dioxide reduced the floor microbial load significantly than other disinfectants used.

Conclusion

A study was conducted to assess and compare the disinfection efficacy. Chlorine dioxide, sodium hypochlorite, calcium hypochlorite and a cow urine based mixture were tested for their efficacy as disinfectant in swine farrowing and fattening facilities. The disinfectants were applied by recommended protocols. The efficacy was determined by dilution method. The study illustrated the order of disinfectants in decreasing order as follows chlorine dioxide > sodium hypochlorite > cow urine based disinfectant > calcium hypochlorite in all the seasons of the year, respectively. In farrowing pen the order of efficacy of disinfectants were chlorine dioxide > sodium hypochlorite > calcium hypochlorite > cow urine based disinfectant in all the seasons of the year, respectively.

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