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The study of floor and water sample analysis of various categories of dairy farms in and around Mumbai

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Abstract
The present study was conducted at the department of Livestock Production Management, Mumbai Veterinary College, Mumbai. For the present study twenty four floor swab samples and eighteen water sample from twenty four dairy farms in and around Mumbai were collected. The dairy farm were categorized as small (Animal less than 50), Medium (Animal more than 50 and less than 100) and Big (Animal more than 100). The floor sample and water sample were collected and were subjected to total viable count and most probable number analysis at the department of Veterinary Public Health, Mumbai Veterinary College, Mumbai. The results revealed non significant difference between the treatment groups for both floor swab and water analysis. The results pertaining to the presence of *Staphylococcus*, *E.coli*, Yeast and mould and *Salmonella* spp. showed the presence as 37.5,37.5 25 and 0% for big dairy farms whereas for the medium dairy it was 25,0,50 and 12.5% and that to for the small dairy farm it was 62.5,25,25 and 12.5% respectively. The water was also analysed for the MPN values, the result revealed that the big, medium and small dairy farm has 1, 3 and 1 potable water sample respectively which was good for drinking purpose whereas the other samples were non potable. There is a need to educate the dairy farmers to keep the floor and water hygiene to prevent the occurrence of disease at farms.

Keywords: Total viable count, most probable number, swab floor sample, water sample

Introduction
In Mumbai region of Maharashtra most of the dairy farms are having conventional/closed system of housing due the space constraint. In view of this the floor of the farms has important bearing as far as the health of the animals is concerned, since the floor of the housing is the place where the animal spends most of its time as compared to the other essentials of housing viz. sidewalls, roof etc. Therefore utmost care should be taken to maintain the floor hygiene of shed in order to avoid the chances of infection to the animal. Many scientist has suggested to keep the floor dry and clean to avoid the chances of infection to the animal. The humidity factor in Mumbai also plays a significant role in increasing the infection in shed.

Secondly the water is one the basic need of the animal and to have maximum production from dairy animal ample access to clean and potable water is essential. However it has been observed that the dairy animals are provided water which is highly contaminated, the reason may vary from unhygienic water tank, irregular washing and disinfection of water tank on regular basis, lack of knowledge on water hygiene etc. Usually the microbiological quality of water is assessed by checking non- pathogenic bacteria of fecal origin. *E. coli* and *Enterococcus* sps members are traditionally used as hygienic indicator bacteria [1]**.** Today, the water resources have been the most exploited natural system and there needs to be law in near future to stop the exploitation of water resources at will by the people.

In the present study the dairy farm were categorized as small (Animal less than 50), Medium (Animal more than 50 and less than 100) and Big (Animal more than 100) with an objective to know which farms are performing better in terms of economics and health status. Secondly due to population growth, urbanization, industrial effluents conveying directly in available water source the pollution of water bodies is increasing at an alarming rate. Therefore nowadays the water qualities studies needs to be given priority and various regulations on these aspects needs to be formulated before the situation goes out of hand [2].

In view of the important bearing of these two factors in spread of infection the present study

was planned to sensitized the dairy farmers of the region.

Materials and Methods

The present study/experiment was conducted in various category of dairy farms in and around Mumbai region. The various categories of dairy farm identified were Big (having animals more than 100), Medium (having animals more than 50 and less than 100) and small (having animals less than 50). The various dairy farms were visited and the floor swab was taken.

In all eight samples from each category of dairy farms were taken and given in the VPH Department of Mumbai Veterinary College, Mumbai and were analysed for total viable count. In the same way the water samples from the various categories of farms were collected in clean and disinfected plastic bottle of 500 ml. While taking the water sample the precaution was taken that the water from the middle of the tank was taken by discarding the other contamination source viz. Algae .The collected sample was airtight and fixed and it was given in the VPH Department of Mumbai Veterinary College, Mumbai and were analysed for Microbial count and MPN.

Total viable count

For evaluating total viable count (TVC), standard pour plate technique was followed. Tenfold dilution was prepared by transferring 1 ml of milk sample to 9 ml of Normal saline solution (NSS). Dilutions were standardized for further procedure. A quantity of 0.1 ml inoculums from 10^{-2} and 10^{-3} to 10-3 and 10^{-4} dilutions were used for pour plate technique to which plate count agar (Hi-media Laboratories, Mumbai) was poured and mixed thoroughly by rotating the plates. The plates were incubated for 24 hours at temperature of 37 ˚C. After 24 hours colonies were counted using bacteriological colony counter. TVC of water samples was expressed as log cfu/ml, TVC of swab samples was expressed as log 30 cfu/cm^2. Total viable counts were calculated by using standard formula given by AOAC (1997).

The bacterial colonies were counted with the help of the bacteriological colony counter and colony forming (CFU) was calculated by using the following formula.

$$\text{CFU/Ml} = \frac{\sum c}{[n1+(0.1\times n2)]\times d}$$

Where:

$\sum_c$ = Total no. of colonies developed on all the plates

n_1 =No. of plates retained in lower dilution

n_2 = No. of plates retained in higher dilution

d = Dilution factor corresponding to lower dilution

Determination of differential count

For the isolation of *Staphylococcus aureus*, *E. coli*, and Yeast and Moulds selective media were used. For the Isolation of *E. coli*, Staphylococcus aureus, and Yeast & Moulds was done as per the method described by Bacteriological Analytical Manual (BAM, 1998).

Isolation of *Staphylococcus aureus*

By using 0.1 ml inoculums of 10^{-2} and 10^{-3} dilution of sample on Baird parker Agar (BPA) (Hi-Media Laboratories, Mumbai). The inoculums was spread by means of L-shape spreader and plates were kept overnight at 37 ºC for incubation. Characteristic colonies of *Staphylococci* spp. showing typical black colonies were selected.

Isolation of *Escherichia coli*

A quantity of 0.1 ml of inoculums from dilutions 10^{-2} and 10^{-3} were used by spread plate technique on Levine's Eosin Methylene Blue Agar (EMB) (Hi-media Laboratories, Mumbai). Bluish purple coloured colonies with greenish metallic sheen were considered indicative of *E. coli*.

Isolation of yeast and moulds

By using 0.1 ml. Inoculums from 10^{-2} and 10^{-3} dilutions of sample on Sabouraud Dextrose Agar (SDA) (Hi-media Laboratories, Mumbai) by spread plate method. Incubation was done at 25^0C for 5 days. Colonies were analyzed for Yeast & Moulds isolation.

Recording and Handling of data

The water samples were analyzed for physico-chemical and microbial analysis. The qualitative physico-chemical analysis were estimated. The mean values of quantitative physico-chemical and microbial values of water samples were compared between groups.

The percentages of qualitative physical analysis were recorded. The mean quantitative chemical analysis of water in relation to moisture and acidity were compared between groups. The microbial analysis of water in relation to total TVC counts, total Staphylococcal count, total *E. coli* count and total Yeast and Moulds counts were analyzed within and amongst groups. The (ANOVA) Analysis of Variance was followed by comparison of means between treatment groups using WASP® software.

MPN procedure

Dilution of sample take water sample as it is. Other product make initial suspension with 25g sample+225ml peptone water (0.1%) 1/9 ratio (w/v).

MPN technique = Take three tubes of double-strength E.E Broth medium 10 ml +10 ml test sample to each tube. Take three tubes of single-strength E.E Broth medium 10 ml + 1 ml test sample to each. Take three more tubes of single-strength E.E Broth medium 10 ml + transfer 1ml of the first decimal. Then incubate these nine tubes at 35 °C or 37 °C for 24 h. for isolation Streak a Ioopfull from each of the nine incubated cultures on violet red bile glucose agar plates and incubate the plates at 35 °C or 37 °C for 24 h then count the positive tubes & calculate MPN number per ml or per gram, as per the table given. Typical pink to red colonies (with or without precipitation haloes) or colorless, mucoid colonies have developed, select at random five such colonies for biochemical confirmation. Then subculture it by streaking on nutrient agar plates and Incubate at 35 °C or 37 °C for 24 h ± 2 h. Select a well-isolated colony from each of the incubated plates for biochemical confirmation.

Biochemical confirmation

Oxidase reaction: test is negative if color of filter paper has not turned dark in 10 sec.

Fermentation test: yellow color develops throughout the contains of tube and most strains produce gas then the reaction is positive

Result expression

If at least 80% of the selected typical colonies are oxidase-negative and glucose-positive and thus confirmed as presence of organisms.

Calculation of the most probable number (MPN)

1. Count the number of tubes giving a positive reaction for each dilution.
2. Using the MPN table (given below), determine from the number of positive tubes in the different dilutions, MPN index.
3. In the case of liquid products, the number of organisms per millilitre is calculated by dividing the MPN index by 10. In the case of other products for which initial suspensions are prepared, the number per gram is equal to the MPN index.

Results

Water quality is important from public health point of view as it is vehicle for biological and microbial hazardous substances. Source of water play an important role in determining its quality. It is impossible to prevent all pollution but minimum standards can be achieved by various means. WHO (1993) recommended the guidelines for potable water based on acceptability aspects, microbial aspects, chemical aspects and radiological aspects [3]

Water quality plays significant role in improving animal performance and also nutrition and health [4]. Today, efforts are being made to improve water quality and its resources [5 6], since livestock requires large quantum of clean water everyday [7].

The status and safety of drinking milk cannot be determine without microbiological analysis of water which is very essential as far as safeguard to human health is concerned [8 9]. This study will educate the dairy farmers to improve their rearing practice thereby improving dairy production. This study will serve as an educational tool for the farmers, to change their breeding technology, looking forward to improve their dairy production [10].

The results of the floor swab sample are presented in table 1.

Table 1: The Microbial analysis of Floor swab sample of different categories of Farm

Sr. No	Types of dairy farms/Category	Parameter				
		TVC Log CFU/10cm^2	Staph aures	E.Coli	Yeast & Mould	Salmonella spp.
A	Big	5.15	Present	Present	Present	Absent
		5.17	Present	Present	Present	Absent
		4.96	Absent	Absent	Absent	Absent
		5.26	Absent	Absent	Absent	Absent
		5.2	Absent	Absent	Absent	Absent
		4.8	Absent	Absent	Absent	Absent
		5.14	present	Present	Absent	Absent
		5.03	Absent	Absent	Absent	Absent
		Avg.5.089 ± 0.05	3/8= 37.5	3/8=37.5	2/8=25	0/8
		SE=.05313				
		SD=0.667				
B	Medium	4.61	present	Absent	present	Absent
		4	Absent	Absent	Absent	present
		5.06	Absent	Absent	present	Absent
		4.7	Absent	Absent	Absent	Absent
		4.98	Absent	Absent	present	Absent
		5.07	Absent	Absent	Absent	Absent
		5.18	present	Absent	present	Absent
		5.07	Absent	Absent	Absent	Absent
		Avg. 4.834	2/8=25	0/6-0	6/3=50	1/6=12.5
		SE=.13805				
		SD=0.390				
C	Small	5.20	Absent	Absent	Absent	Absent
		5.14	present	present	Absent	Absent
		5.15	Present	Present	Absent	Absent
		3.6	Absent	Absent	Absent	present
		4.58	present	Absent	Absent	Absent
		5.18	present	Absent	present	Absent
		5.11	Present	Absent	Absent	Absent
		4.78	Absent	Absent	Absent	Absent
		Avg. 4.844	5/8=62.5	2/8=25	1/8=12.5	1/8=12.5
		SE=.19458				
		SD=0.549				

Groups	1	2	3	4	5	6	7	8	Average± SE
I	5.15	5.17	4.96	5.26	5.2	4.8	5.14	5.03	.05313
II	4.61	4	5.06	4.7	4.98	5.07	5.18	5.07	.13805
III	5.21	5.14	5.15	3.6	4.58	5.18	5.11	4.78	.19458

Analysis for TVC farm floor swabs

Treatment means	
S.No	Average
Treatment A	5.089 ± 0.05
Treatment B	4.834
Treatment C	4.844

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	7	0.801	0.114	0.629	0.725
Treatments	2	0.334	0.167	0.918	0.422
Error	14	2.545	0.182	-	-
Total	23	-	-	-	-

Coefficient of Variation = 8.662

Treatments found to be Non Significant The floor swab sample of different categories were analysed and there was non significant difference between them (8 samples from each category were analysed). The results revealed that there was non-significant difference between the treatment groups viz. Big, medium and small dairy farms. It was observed that the small dairy farms were maintaining good hygiene as compared to medium and big dairy farms, but statistically no significant difference was seen amongst them. This may be probably due to the overall macro picture of the floor showing hygiene and cleanliness however the micro picture was totally different showing contamination of floor.

The results pertaining to the presence of *Staphylococcus*, *E. coli*, Yeast and mould and *Salmonella* spp. showed the presence as 37.5,37.5 25 and 0% for big dairy farms whereas for the medium dairy it was 25,0,50 and 12.5% and that to for the small dairy farm it was 62.5,25,25 and 12.5% respectively.

The similar findings were reported by [11] who reported that the slower water replacement and available standing water leads to a greater pathogen load. Similarly, higher microbial load was observed in sheep pen by [12]. The higher microbial count in medium and big dairy farms in dunging area may be due to the accumulation of more quantity of faecal matter and the presence of moisture which favours the multiplication of bacteria at a faster rate and the contrast finding was reported by [13] they reported that there was highly significant difference ($P<0.01$)in microbial load between before and after water wash of the floors in both rubber and slatted floors. There was highly significant difference ($P<0.01$) between the sampling areas within a floor type. This indicated that the microbial load was lower in both rubber and polyurethane slatted floor when compared to concrete floor. This concurs with the findings of [14] who compared the microbial load between concrete and slatted flooring goat pen.

The results for the microbial water sample are presented in table. 2.

Table 2: The Microbial analysis of Water sample of different categories of Farm

Sr. No	**Types of Dairy farms/Category**	**Farm category and Farms**		**Parameters**				
			TVC Log CFU/ml	T VC C.FU/ml	Staph aures/ml	E.Coli/ml	Yeast & Mould/gm	Salmonella species/25
A	Big	LPM 1	5.11	1.29×10^5	Absent	Absent	Absent	Absent
		LPM 2	5.12	1.34×10^5	Present	Absent	Absent	Absent
		LPM 3	4.17	1.48×10^4	Absent	Absent	Absent	Absent
		LPM 4	5.10	$1.28X10^5$	present	present	present	present
		LPM 6	3.90	7.95×10^3	$1.98X10^2$	Absent	Absent	Absent
		LPM 7	3.90	7.98×10^3	Absent	Absent	Absent	Absent
		Average	4.550		6(3) =50%	16.66%	16.66%	16.66%
		S. D.	0.621					
		S. E.	0.254					
B	Medium	LPM 5	2.13	$1.36 X10^2$	Absent	absent	absent	absent
		LPM 8	3.93	$8.59 X10^3$	$3.33X10^2$	absent	$8.73X10^2$	absent
		LPM 9	4.07	$1.18 X10^4$	$2.93X10^2$	$2.53X10^2$	$3.80X10^2$	present
		LPM 10	3.14	1.40×10^3	Absent	Absent	Absent	absent
		LPM 13	3.17	$1.5 X10^3$	Absent	absent	Absent	absent
		LPM 15	6.93	$8.52X 10^6$	$3.33X10^3$	$3.87X10^2$	$3.40X10^2$	present
		Average	3.895		50%	66.66%	50%	33.33%
		S. D.	1.64					
		S. E.	0.67					
		LPM 11	4.24	$1.77X10^4$	$6.45X10^2$	absent	6.78×10^2	absent
C	Small	LPM 12	4.25	$1.79X10^4$	$9.39x10^2$	Absent	$4.67X10^2$	absent
		LPM 14	4.44	$2.77X10^4$	$2.63x10^2$	Absent	$1.23X10^2$	absent
		LPM 16	4.41	$2.63X10^4$	$8.34X10^2$	$9.12X10^2$	$2.34x10^2$	present
		LPM 17	3.05	$1.13X10^3$	Absent	Absent	Absent	Absent
		LPM 19	4.23	$1.72 X10^4$	$7.68X10^3$	$1.23X10^2$	$3.31X10^2$	present
		Average	4.103		6(5)=83.33%	6(2)=33.33%	6(5)=83.33%	6(2)=33.33%
		S. D.	0.524					
		S. E.	0.214					

Treatment means	
S. No	**Average**
Treatment A	4.550
Treatment B	3.895
Treatment C	4.103

Anova Table					
Source of variation	**Degrees of freedom**	**Sum of squares**	**Mean sum of squares**	**F cal**	**F prob**
Replications	5	4.646	0.929	0.767	0.594
Treatments	2	1.344	0.672	0.555	0.591
Error	10	12.111	1.211	-	-
Total	17	-	-	-	-

Coefficient of variation = 26.310

Treatments found to be Non-Significant The results revealed non-significant difference between the different categories of farms for the Microbial analysis of water.

(Six samples from each category were analysed It is evident from the result that there was non-significant difference between different dairy farms for the microbial water analysis.

The probable reason for the non-significant difference amongst treatment group may the common source of water i.e. Powai lake water which is supplying water to the different dairy farms of the region.

The results pertaining to the presence of *Staphylococuss*, *E. coli*, and *Salmonella* spp. showed the presence in water from big dairy farms, medium dairy, small dairy farms.

The similar findings were reported by [15] they reported that *Salmonella* spp. were isolated from 2/235 (0.8%) livestock drinking water troughs and shigatoxigenic-*E. coli* O157 was recovered from 6/473 (1.3%) troughs. The degree of *E. coli* contamination was positively associated with the proximity of the water through to the feed bank, protection of the trough from direct sunlight and [11] reported that the contaminated drinking water was the most important pathway of *E. coli* O157:H7 transmission to cattle and seasonal variation in E. coli O157:H7 prevalence in cattle. [16] Reported that all the water samples from lake, pond and Municipal water were contaminated with coliforms suggestive of sewage seepage to groundwater. The contrast finding was reported by [16] they observed a significant difference in microbial load ($p<0.01$) between different sources and in two different seasons, respectively. The Ground water and municipality water supply had CFU/mL of water in acceptable limits.[17] reported that the analysis of variance of SPC $\log_{10}$ values of water from household sources, public places and packaged water differed highly significantly ($p<0.01$).

The water was also analysed for the MPN Values and are presented in table 3. and from the result it is seen that the big, medium and small dairy farm has 1,3 and 1 potable sample respectively which is good for drinking purpose whereas the other samples were non potable.

Table 3: The MPN Values of different categories of Farm

Sr. No	Types of farms/Category	Sample	MPN/100 ml
1	Big	LPM 1	14
		LPM 2	14
		LPM 3	<2
		LPM 4	17
		LPM 6	7
		LPM 7	7
			1/6
2	Medium	LPM 5	<2
		LPM 8	9
		LPM 9	17
		LPM 10	<2
		LPM 13	2
		LPM 15	110
			3/6
		LPM 11	26
C	Small	LPM 12	27
		LPM 14	33
		LPM 16	31
		LPM 17	2
		LPM 19	42
			1/6

The MPN values for the different categories of farm is 1/6, 3/6 & 1/6 for Big, Medium and small dairy farms.

Similar findings were reported by [17] they reported that the MPN levels shows that high level contamination in ponds and lake followed by municipality water supply and were least in open well and bore well. The surface waters such as pond and lake had higher coliforms than groundwater sources. Similar results were obtained by [18] in their study on quality assessment of drinking water in Mumbai, India. The risk of contamination was found to be greatest in surface waters that were directly accessible by livestock or contaminated due to run off or drainage from a manure source but ground water had low level of bacterial contamination [4, 20] reported that out of ten total coliform counts for seven river samples, exceeded standard for coliform bacteria in water. All the ten water samples exceed the WHO [21] standard limit and the contrast finding was reported by [20] they reported that the mean total bacteria counts of river water ($\log_{10}$ cfu mL^{-1}) for different sites were significantly different ($p< 0.05$).

Conclusion

It can be concluded that there was no significant difference

between the treatment group both for the floor swab sample and water sample in various categories of dairy farm viz. big, medium and small with varying degrees of presence of *Staphylococcus*, *E. coli*, yeast and mould and *salmonella* spp. All the categories of farms were not maintaining the hygiene norms to provide clean potable water and also their floor hygiene was also not up to the normal standards

However, attempts should be made to educate dairy farmers by conducting various extension programme regarding the importance floor and water hygiene in order to minimize the infection and occurrence of disease at various categories of dairy farms.

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