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RESEARCH ARTICLE

Study on incidences, risk factors and bacterial populations involved in subclinical mastitis cases in the conventional *vis-à-vis* organic dairy farming under small holder system in Indian Sundarban region

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Abstract: Organic dairy farming is being promoted in Indian Sundarban region through the 'Women Milk Producers' Cooperative Society' under the aegis of Sundarban Milk Union of West Bengal. The present study was undertaken to investigate the prevalence of sub clinical mastitis (SCM) in dairy herds maintained by small holders in two production system (organic *vis-à-vis* conventional). Total numbers of 34 dairy farmers were selected for the study. Out of the 34 numbers of farmers, 50% farmers (n =17) had adopted organic milk production practices and the remaining (n = 17) farmers used to practice conventional dairy farming. California Mastitis Test (CMT) kit was used to evaluate incidences of SCM. A semi-structured questionnaire was used for analysis of associated management risk factors for SCM. Positive milk samples from organic growers were subjected to microbial analysis and antibiogram. Data was analyzed for descriptive statistics by SPSS program v. 20.0. Results revealed that the incidences of SCM is not significantly ($p<0.05$) different in organic (35.3%) and conventional (29.4%) dairy production system, indicating chronic and long standing infection in udders. Besides, no significant ($p<0.05$) difference between the two production system was observed in management risk factors associated with SCM. It was found that *Staphylococcus aureus* is the causative organism responsible for SCM under organic dairy production system in the study area. It can be concluded that to reduce high rate of SCM in the region, regular monitoring of udder health with holistic approach is necessary.

Keywords: Antibiogram, California Mastitis Test, Organic Dairy, Sub Clinical Mastitis

Introduction

Health-conscious metropolitan consumers are increasingly inclining to consume branded organic milks for obvious human health benefits (Butler and Stergiadis, 2020). Driven by consumer demand, organic milk market size in India has already reached INR 6,082 million in 2021 andexpected to further expand 5 times by 2027. Organic milk production system in general,based on feeding cows on feed ingredients grown organically (without using synthetic pesticides or chemical fertilizers) and avoiding using synthetic growth promoters, hormones, antibiotics and drugs in husbandry. Apart from the milk production with enhanced health benefits, one of the prime objects of organic dairy farming is the welfare of dairy animals (Chander et al. 2013).

From the production point of view, maintenance of udder health is one of the critically important aspect in dairy cows. Inflammation of udder is termed as 'mastitis' and in India alone this disease causes estimated economic losses to the extent of 7000 crore (Bansal and Gupta, 2009). Sub clinical mastitis (SCM), characterized by low level of persistent inflammation and reduced milk yield, is much more prevalent form than clinical mastitis (Birhanu et al. 2017; Sohidullah et al. 2023). SCM like tip of an iceberg, not only can endanger mammary tissue of the lactating cow by progressive fibrosis, but also may impact human health through possible zoonoses in the value chain. Although, incidences of SCM are reported from organic dairy herds in different parts of the globe (Villarand López-Alonso2015; Hansmann et al. 2019), little information is known regarding its incidences and microbial population involved in SCM cases in local cattle breeds reared under organic practices in coastal saline affected zone of Indian Sundarban. Present study was conducted to explore the prevalence of SCM in dairy cows maintained under organic certification regulations (NPOP 2005) and conventional husbandry practices by small holder dairy farmers in Indian Sundarban region.

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Materials and methods

Study area and sampling

Two villages namely Nafargunj and Masjidbati in Basanti block of Indian Sundarban region were purposively selected for the study during May-June 2022.Map of the studied area was shown in Fig 1 and the precise locations where the samples were collected are indicated by red dots.The map was prepared using QGISv. 2.2 software (QGIS Development Team 2013).

Randomly 34 numbers of dairy farmers who were the members of 'Women Milk Producers' Cooperative Society' under the aegis of Sundarban Milk Union, West Bengal, were selected for milk sampling and door to door survey. Out of the 34 numbers of farmers, 50% farmers (n =17) had adopted organic milk production practices and certified by Rajasthan State Organic Certification Agency (RSCOA) as per National Program for Organic Production (NPOP). Remaining 50% farmers (n =17) were rearing cows under conventional practices. A pre-tested and piloted semi-structured questionnaire was used for analysis of risk factors related to the development of Sub-clinical mastitis in dairy herds. The questionnaire was developed in local language (Bengali) with consultations from subject expert and previous research studies.

Milk Sample Collection

Cow milk samples (n = 34) from apparently healthy quarters were screened for detection of subclinical mastitis by California Mastitis Test Kit (De Laval, India) as per Dingwell et al.(2003). CMT scores (N, Trace, 1, 2 and 3) for the affected udder quarter were based on the degree of gel formation, where 'N' means no reaction and 'Trace', '1', '2' and '3' denote positive samples with increasing reaction.

Isolation and identification of organism in the positive milk samples

The positive milk samples (Trace, 1, 2 and 3) were pooled from all the quarters and transported to ICAR-IVRI (Eastern Regional Station), Kolkata in an icebox for bacterial isolation and identification (Bhattacharyya et al. 2016). Briefly, 10 µl of each sample was incubated overnight in trypticase soy broth (BD, BBL) at 37°C and inoculated in Baird Parker agar (BD, BBL) with egg yolk emulsion and mannitol salt agar (MSA; HiMedia). Colonies surrounded by bright yellow zone in MSA or black, shiny, and convex colonies surrounded by a clear zone in Baird Parker agar were primarily selected as *Staphylococcus*. Single-isolated colonies were taken in nutrient agar (HiMedia) slant and were further processed for confirmation as *S. aureus* using standard tests such as Gram's staining, catalase, coagulase, oxidase, indole, methyl red, urease, Voges–Proskauer, lecithinase production, mannitol, and glucose fermentation.

Antimicrobial susceptibility

Antibiogram of the isolated organisms was also studied as per Bhattacharyya et al. 2016 by disc agar diffusion technique using commercially available discs (HiMedia) against the following antibiotics— *Chloramphenicol, Cefoxitin, Tetracycline, Penicillin, Oxacillin, Trimethoprim-sulfamethoxazole, Erythromycin, Gentamicin, Linezolid* and *Ciprofloxacin.* The zone of inhibition was measured after incubation with antibiotic discs at 37°C for 18–24 hours following CLSI (CLSI, 2018) guidelines

Statistical Analysis

The data was statistically analyzed by using SPSS program v. 20.0 (IBM Corp. NY, USA) as per method described by Snedecor and Cochran (1994).Possible association of udder health with milk production practices (Organic *vis-à-vis* Conventional) was determined by *chi* square test. Values were considered significant at $P<0.05$.

Results and Discussion

Socio-economic profiles of dairy farmers are presented in table1. Most of the dairy farmers are middle aged adults with 61.8% belong to more than 35 years of age group. Dairy farming is a

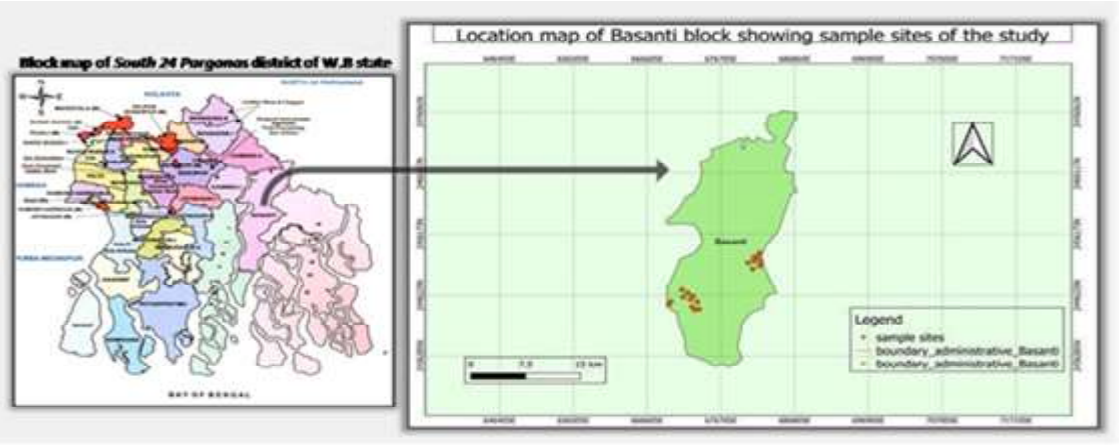

Fig. 1 Location map of milk sample collection

skilled job and thus may require experienced, physically capable and energetic work force. Education level is important for adoption of knowledge and skills. Results revealed that around 73.5% farmers are with secondary education background. This is in contrary to Das et al.(2017) who observed that majority of the dairy farmers under Sundarban Milk Union was only with primary educational background. Present study further revealed that majority (61.8%) of the dairy farmers belonged to middle class economy. Ahmed et al. (2020) similarly reported that majority (42.6%) of the dairy farm workers were from upper lower economic strata in the vicinity of Southwest Delhi, India. Most of the dairy farmers in our present study belonged to small and marginal farmers and thus their dairy practice level might be influenced by their socio economic status. Under the present study, majority (88.2%) respondents reported that they belonged to agricultural labour and 58.8% of respondents reported that they had 1-3 acre of land. This profile is typical to the Sundarban area and demonstrated that small holder dairy farming in this area is primarily for subsistence.

Table1: Socio-economic profile of dairy farmers

	Characteristics	N	Frequency (%)
Education	Illiterate	4	11.8
	Primary	2	5.9
	Secondary	25	73.5
	College	3	8.8
Family member	<3	4	11.8
	3-5	21	61.8
	>5	9	26.5
Economic status	Upper Middle Class	1	2.9
	Middle class	21	61.8
	Poor	12	35.3
Age	<20	0	0
	20-35	13	38.2
	>35	21	61.8
Occupation	Housewife	1	2.9
	Agricultural labour	30	88.2
	Non-agricultural labour	2	5.9
	Job	1	2.9
Land	<1 acre	11	32.4
	1-3 acre	20	58.8
	>3 acre	3	8.8

Table 2:Udder Health condition as denoted by California Mastitis Test (CMT) scores in two different milk production types

Variables	CMT Scores	N	Frequency (%)
Organic Production	N	11	64.7
	T	4	23.5
	1	1	5.9
	2	1	5.9
	3	0	0
Conventional Production	N	12	70.6
	T	2	11.8
	1	3	17.6
	2	0	0
	3	0	0

(N: No reaction; T: Trace; 1: Distinct precipitate but does not gel; 2: Distinct gel formation; 3: Strong gel formation)

In the present study, 34 cows (local cattle breed) were screened for SCM using CMT kit and 11 cows were detected positive for SCM (Table 2). Around 64.7% and 70.6% cows respectively from organic and conventional dairy system gave negative test in CMT. Only 5.9% of milk samples from organic dairy production were scored 2 in CMT test, whereas only 17.6% samples from conventional dairy production reached only score 1. This indicates incidence of SCM is well under control in local cattle breeds in both the production system.

Table 3 depicts number of udder quarters affected with SCM in organic and conventional production systems. Under the organic production system, two quarters showed higher incidence (50%) of SCM infection followed by single quarter (33.33%) and three quarters (16.67%). Under conventional system of rearing, single quarter infection was mostly prevalent (80%) followed by two quarters infection. Overall, the results indicate that quarter level prevalence is lower in conventional farming system than organic farming system. This may be due to use of antibiotics in conventional farming system.

The association between production type (organic *vis-à-vis*conventional) and incidence of SCM is presented in Table 4. SCM was observed in 35.3% and 29.4% of cows respectively in organic and conventional dairy farming system. No significant ($p>0.05$) difference was found between two farming system in regards to udder health. This shows that SCM is a matter of concern in both organic and conventional dairy practices. In agreement to our findings, Kouøimská et al. (2014) observed that the somatic cell count (SCC) in milk samples did not vary in organic and conventional dairy farms. It is evident that SCM is more about maintaining udder health and hygiene in individual dairy cows than the farming system itself.

Management level risk factors associated with SCM were found to be at par ($p>0.05$) in both conventional and organic production systems (Table 5) in the study area. Present study revealed that 94.1% and 76.5% farmers under conventional and organic regime respectively adopted good practices like *'Use of disinfectants for cleaning of milking utensils and cow shed*' and *'Washing of udder and hands before and after milking'*. However, dairy farmers in both the production system showed the reluctance in following the other critical udder management practices like *'Teat dipping'* or *'Dry cow therapy*'. It is obvious from the present study that small holder dairy farmers irrespective of the production system, needs to be exposed in more intensive training on the importance of maintaining the udder health. Bhakat et al. (2022) indicated that the risk factors associated with udder health are more in the small holder dairy farmers (with 2-3 dairy cows) in tropical climate. Poor sanitation and hygiene is the foremost cause of sub clinical mastitis in dairy animals (Sinha et al. 2014). Our present study clearly showed that the both conventional and organic dairy production system managed by the small holder dairy farmers of the coastal zone of Sundarban faces prominent threats of sub clinical mastitis.The region may become epitope of chronic mastitis infection if potential management risk factors cannot be mitigated to a certain level. Risk factors for SCM are also associated with socio economic status of the dairy farmers (Ahmed et al. 2020). Thus multiple factors are intriguingly related to management risks associated with bovine sub clinical mastitis. Further scientific study needs to be carried out to identify the knowledge and practice gap of small holder dairy farmers in that area.

The result (Table 6) obtained from bacteriological analysisof the organic milk samples revealed that *Staphylococcus aureus* is the only causative organism responsible for SCM (35.3%) in the study area. The milk samples from conventional production system was not analysed for bacteriology. *S. aureus* is the most common

Table 3: Number of quarters affected with SCM in two production types

Organic production			Conventional production		
No. of quartersaffected	No. of animals	Prevalence	No. of quarters affected	No. of animals	Prevalence
1	2	33.33%	1	4	80%
2	3	50%	2	1	20%
3	1	16.67%	3	0	0
4	0	0	4	0	0

Table 4: Association between the production type and incidence of SCM

	Responses	Production Type n, (%)		χ^2	*p* - value
		Organic	Conventional		
Incidence of SCM	Positive	6 (35.3%)	5 (29.4%)	0.134	0.714
	Negative	11 (64.7%)	12 (70.6%)		

Table 5: Management risk factors related to development of SCM

Variables	Production Types	Characteristics	N	Frequency (%)	χ^2	*p* - value
Milking Method	Conventional	Full hand milking	3	17.6	0.234	0.5
		Stripping	14	82.4		
	Organic	Full hand milking	2	11.8		
		Stripping	15	88.2		
Use of disinfectants for cleaning of milking utensils and cow shed	Conventional	Yes	16	94.1	2.110	0.33
		No	1	5.9		
	Organic	Yes	13	76.5		
		No	4	23.5		
Teat dipping	Conventional	Yes	1	5.9	1.030	0.5
		No	16	94.1		
	Organic	Yes	0	0		
		No	17	100		
Dry cow therapy	Conventional	Yes	1	5.9	0.366	0.5
		No	16	94.1		
	Organic	Yes	2	11.8		
		No	15	88.2		
Washing of udder and hands before and after milking	Conventional	Yes	16	94.1	1.030	0.5
		No	1	5.9		
	Organic	Yes	17	100		
		No	0	0		

Table 6: Bacterial species isolated from subclinical mastitis cases observed under organic production

Bacterial Species	Total number of isolates	Prevalence (%)
Staphylococcus aureus	6	35.3%
Negative	11	67.7%
Total	17	100%

pathogen associated with sub clinical mastitis and one of the principal causes of the food borne diseases in humans (Campos et al. 2022). Absence of teat dipping practices during milking, continued adoption of stripping milking methods, poor hygiene in the shed and maintenance of chronically affected cows in the herd might be linked with high proportion of SCM cases with presence of *Staphylococcus aureus.* The antibiogram (Table 7) showed 100% sensitivity to *Chloramphenicol, Cefoxitin, Tetracycline, Oxacillin, Trimethoprim-sulfamethoxazole, Gentamicin, Linezolid* and *Ciprofloxacin*. However, isolates showed intermediately resistant to *erythromycin* (33.33%) and resistant to *Penicillin* (33.33%). Amoafo et al. (2021) indicated that the prevalence of resistant microbial population is higher in the conventional dairy farms than organic dairy farms. In the present research, the bacterial isolates from organic growers were sensitive to most of the common antibiotics and this might be due to the prevalent organic dairy farming practices like eliminating antibiotic usages, natural farming practices for fodder cultivation, use of herbal products to cure diseases etc. The growing emergence of AMR bacteria might be limited by adopting such organic practices in small dairy farms in the coming days.

Conclusion

Table 7: Antibiogram of isolates (n =6)

Name of Antibiotics	Resistant n(%)	Intermediate n(%)	Sensitive n(%)
Chloramphenicol	0 (0%)	0 (0%)	6 (100%)
Erythromycin	0 (0%)	2 (33.33%)	4 (66.67%)
Cefoxitin	0 (0%)	0 (0%)	6 (100%)
Penicillin	2 (33.33%)	0 (0%)	4 (66.67%)
Tetracycline	0 (0%)	0 (0%)	6 (100%)
Oxacillin	0 (0%)	0 (0%)	6 (100%)
Trimethoprim -sulfamethoxazole	0 (0%)	0 (0%)	6 (100%)
Gentamicin	0 (0%)	0 (0%)	6 (100%)
Linezolid	0 (0%)	0 (0%)	6 (100%)
Ciprofloxacin	0 (0%)	0 (0%)	6 (100%)

It was concluded that the prevalence of sub clinical mastitis in dairy herds is high among small holder dairy farmers in Indian Sundarban region. Production type (organic *vis-à-vis* conventional) did not significantly affect the incidences of sub clinical mastitis. Substantial management risks associated with SCM were observed in both the production type. Awareness and training by the milk unions might be the driver of change to reduce the risk factors present in the prevalent management of dairy farming. Microbial analysis from milk samples from organic growers revealed that *S. aureus* is the responsible organism for SCM in the organic dairy herds. In the backdrop of global emergence of AMR bacterial population, the more detailed study on SCM in different production system with more number of animals is the need of the time. The study strongly recommends more strict adherence to regulations of organic dairy farming and regular monitoring of udder health with CMT kit in the field to eliminate the threats of resistant infections.

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