



UNIVERSITI PUTRA MALAYSIA

**STUDIES ON BOVINE MASTITIS AND MILK QUALITY IN
SMALLHOLDER FARMS**

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MILK QUALITY IN SMALLHOLDER FARMS**

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ABSTRACT

Studies on bovine mastitis were carried out in 13 small-holder dairy farms while bulk milk from 12 of these farms were tested for milk quality. Although hygienic practises were comparatively poor, the level of subclinical mastitis was low with 19.6% of animals and 8.0% of quarters infected. No clinical cases of mastitis was observed. The predominant bacterial species was Staphylococcus aureus and was isolated from 43.3% of samples and 83% of herds. Antibiotic sensitivity tests on *S. aureus* isolates showed that 21%, 26.3% and 36.4% were resistant to penicillin, streptomycin and ampicillin, respectively. There was no resistance to chloramphenicol and tetracycline. The quarter infection rates among cows in the early and late lactation stages showed a tendency to increase. The effect of lactation number on subclinical mastitis showed a highly significant increase in infection rate from the fifth lactation onwards. Quality tests on bulk milk showed that the average fat content was 4.95% and total solids averaged 14.13%. The average total bacterial count was 55,316 per ml. milk. The resazurin reduction tests showed that all the milk samples tested were of acceptable quality. Storage of milk at 7°C for 48 hours did not produce any deteriorating effects.

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INTRODUCTION

Bovine mastitis can be of considerable economic importance to the dairy farmer. Financial losses result from reduced milk production, degrading of milk due to poor quality, veterinary fees and wastage of cows due to shortened productive life.

Although much work has been done overseas, little is known about mastitis in the local herds. With the exception of a few investigations carried out in government farms, no other work has been documented.

Dairying will be a very important component of the animal industry in the near future, and the target of the Veterinary Department's efforts in livestock development is the smallholder. It is therefore imperative that more is known about this disease, particularly in the smallholder farms.

It is also important to determine the quality of milk that the smallholders produce. This will provide more information on smallholder dairying as well as assuming importance from the public health aspect.

The objectives of this paper are to:

1. determine the prevalence of mastitis in smallholder farms,
2. study the causative bacteria of the disease,
3. evaluate the milking hygiene, management and other factors that can influence levels of mastitis, and
4. assess the milk quality from smallholder farms based on food value, degree of contamination and keeping quality.

It is hoped that this paper will serve to stimulate further work on mastitis, and on milk quality in the smallholder farms.

LITERATURE REVIEW

Koh and Joseph (1974) studied the local Indian Dairy (LID) and LID/Sindhi herd at Kluang comprising 226 animals, and reported subclinical mastitis in 54.9% of cows and 28.7% of quarters. Clinical cases were observed in 2.6% of cows and 0.7% of quarters. The major organisms isolated were Staphylococcus aureus and Streptococcus sp.

Analysis of isolates obtained from case submissions to the bacteriology laboratory at Universiti Pertanian Malaysia (both clinical and subclinical cases) revealed that S. aureus was the predominant isolate in 1976, 1977 and in 1978 accounting for 43.9%, 57% and 52.7% of total isolates, respectively (Shah and Nadzri, 1979). However, in 1979 S. epidermidis predominated and accounted for 37.9% of 219 milk samples.

Referring to the level of hygiene in relation to mastitis, Neave (1971) stated that the omission of all udder washing would result in a marked reduction in the spread of major mastitis organisms. ~~However,~~ he concluded that udder washing cannot be abandoned for aesthetic purposes and possibly to aid in the prevention of S. uberis and coliform infections.

Several workers demonstrated that effective hygiene did cause a dramatic decrease in infection rates in both experimental and commercial herds (Neave, 1971; Hoare, 1972; Philpot, 1975). Philpot (1975) concluded from data generated in four field studies that teat disinfection after milking was the most important of the hygienic measures, and that the use of sterile towels, udder and hand disinfectants have

little effect on the infection rate.

A direct relationship between the age of the cow and the incidence of mastitis has been observed by several workers (Hughes, 1960; Narayanan and Iya, 1953; Plastridge, 1958). Bagadi (1974) found a significant and positive correlation between the advancement of lactation and the prevalence of mastitis caused by staphylococci and streptococci but not by coliforms and corynebacteria in Sudanese dairy cows.

Milk Quality

There is no established standard for milk quality in Malaysia. A proposal for Revised Food Law has been drafted, but is yet to be legislated.

Milk quality can be of great significance from the public health aspect. Tolle (1972) concluded from literature and work at the German Federal Streptococcal Centre that group B streptococci infections are transmitted relatively frequently to man via cow's milk and that horizontal and vertical transmission could then occur in human populations. It was therefore felt that mastitis control programmes should give priority to elimination of S. agalactiae.

Enterotoxigenic strains of S. aureus have been isolated from raw milk samples collected in Karnal city, India (Gosh and Laxminarayana, 1972). Raw market milk of cows and buffaloes that contained high proportions of S. aureus were considered a high risk to consumers of contracting acute gastroenteritis (Garg, Bhargava and Narayan, 1975).

MATERIALS AND METHODS

Field Test

A total of 148 animals from 13 different farms were tested for clinical and subclinical mastitis. The animals were mainly of LID type. Hand milking was practised and calves were allowed to suckle their dams prior to milking. Quarter milk samples were then collected and first examined for any gross abnormalities. The samples were then subjected to the California Mastitis Test (CMT).

Quarter milk samples yielding CMT scores of 2+ or 3+ were collected aseptically into sterile McCartney bottles, placed in a thermos flask with ice and brought to the laboratory. Udders and teats were also examined for gross abnormalities.

Bacteriology

Bacteriological investigation consisted of isolation and identification of pathogens and sensitivity tests.

Samples were incubated at 37°C for one hour, and then the contents of each bottle thoroughly mixed. Each sample was then streaked on to plates of blood agar and MacConkey's medium. Plates were incubated at 37°C and examined 24 hours later. Standard laboratory procedures were used to identify isolated bacteria. Plates that did not show any growth after 48 hours incubation were discarded.

Antibiotic sensitivity testing was carried out on isolated cultures of Staphylococcus aureus.

Milk Quality

Duplicate bulk milk samples from 12 different farms were tested

for milk quality. In each case, after thorough mixing of the milk using a long handed dipper approximately 300 mls. was transferred into a sterile bottle and capped with a sterile stopper. Samples were transported in ice to the UHM Dairy Unit Laboratory.

Analyses of milk samples included determination of Specific Gravity, pH, clot on boiling tests, alcohol precipitation tests (using 68% ethyl alcohol), fat percentage (Gerber's method) and the one hour resazurin reduction test. As an additional test to check on the keeping quality, samples were stored at 7°C and retested 48 hours later using the one hour resazurin reduction test. The surface culture method was employed for the bacteriological examination of bulk milk samples.

To complete the study, a questionnaire was used to obtain additional information which covered various aspects of dairy management. Wherever possible, results were statistically tested using chi-square analysis.

Difficulties encountered during the course of investigation

One main problem was the difficulty in obtaining precise information. This was due to lack of proper record keeping. Whatever information that was obtainable came from memory, and therefore carried the probability of a certain degree of error.

The other problem involved the sampling of quarter milk for bacteriology. In a few cases, it was impossible for the author to collect samples due to difficulties in handling the animals. There was no choice but to allow the farmer to do it, instead. Hence, a sampling error could have been introduced as a result of possible breakdown of aseptic technique associated with sample collection.

RESULTS

Mastitis:

A case of subclinical mastitis is defined as one in which the CMT reaction was graded 2+ or 3+ and which produced positive bacteriology results. Table 1. shows the levels of subclinical mastitis in the farms tested.

Table 1. Animal and quarter infection rates in the farms tested

Farm	No. animals tested	No. quarters tested	Animal infection rate (%)	Quarter infection rate (%)
A	14	56	28.6	14.3
B	9	36	22.2	5.5
C	13	52	0	0
D	9	36	22.2	8.5
E	5	20	40.0	15.0
F	13	51	0	0
G	10	40	0	0
H	15	60	33.3	13.3
I	5	20	20.0	15.0
J	23	87	39.1	16.3
K	6	24	33.3	12.5
L	17	68	5.9	1.5
M	9	35	11.1	2.8
Total	148	585	19.6	8.0

The animal infection rate ranged from 0% to 40.0% with an average of 19.6% while the quarter infection rate ranged from 0% to 16.3% with an average of 8.0%.

No clinical cases of mastitis was encountered in the farms. However, seven cases of obstructed teats were found.

Bacteriology

The bacteriology results of 60 quarter milk samples that produced CMT reactions of grade 2+ or 3+ are presented in table 2. The results of antibiotic sensitivity tests on S. aureus isolates are shown in table 3.

Table 2. Frequency of various bacterial isolates from milk samples

Bacterial isolate	No. of samples	% total samples	No. of farms	% total farms
<u>S. aureus</u>	26	43.3	11	84.6
<u>S. epidermidis</u>	14*	23.3	10	77.0
Streptococcus sp.	8	13.3	1	7.7
<u>Corynebacterium pyogenes</u>	4	6.6	1	7.7
Bacillus sp.	4	6.6	2	15.4
Klebsiella sp.	1	1.6	1	7.7
No growth	13	21.6		
Mixed infections	10	16.6		

*out of the 14 isolates, 8 were involved in mixed infections and only 6 samples produced pure isolates of S. epidermidis.

Table 3. Results of antibiotic sensitivity tests on S. aureus

Antibiotic	No. tested	No. sensitive	No. resistant	No. intermediate
Penicillin G (10 U)	19	12 (63%)	4 (21%)	3 (16%)
Streptomycin (10 ug)	19	10 (52.6%)	5 (26.3%)	4 (21.2%)
Tetracycline (30 ug)	19	17 (89.5%)	-	2 (10.5%)
Chloramphenicol (30 ug)	19	19 (100%)	-	-
Ampicillin (10 ug)	11	6 (54.4%)	4 (36.4%)	1 (9.1%)

Milking hygiene and management

Hygienic practises in the farms were simple. Udders were cleaned only if they appeared dirty. Inspection of udders showed that they appeared clean in most farms visited.

However, when there was visible contamination on the udder cleaning was by splashing water (either tap or well water) and wiping with a cloth. No soap or disinfectant was used and the same udder cloth may be used for the entire herd.

Hands were not cleaned with soap or disinfectant prior to milking and disinfectant teat dipping was not practised. Milking utensils were washed with tap or well water and allowed to dry in the sun.

Another common feature in all farms was that milkers were never or only seldom changed.

Other factors influencing mastitis

The effect of the stage of lactation and lactation number on levels of subclinical mastitis were analysed and the results are presented in tables 4 and 5, respectively.

Table 4. Quarter infection rate among cows in different stages of lactation

Stage of lactation	No. animals tested	No. quarters tested	No. quarters infected	% quarters infected
Early (0-2 months)	45	178	17	9.6 N.S.
Middle (>2-6 months)	51	202	18	8.9 N.S.
Late (<u>></u> 6 months)	33	130	12	9.2 N.S.
Total	129	510	47	

The increased infection rates during early and late lactation stages were not significant at $P = 0.05$.

Table 5. Quarter infection rates among cows in different lactations

Lactation number	No. animals tested	No. quarters tested	No. quarters infected	% quarters infected
1	18	72	2	2.77
2	24	95	1	1.05
3	12	47	0	0
4	20	80	3	3.75
5	11	44	6	13.44**
6	7	28	4	14.28**
7 and above	7	28	4	14.28**
Total	99	394	20	

** The increased quarter infection rates from the 5th lactation and above were found to be highly significant ($P = 0.001$)

Milk quality

The various tests on bulk milk samples are divided into those that represent food value and those which measure degree of contamination and keeping quality. The results of these tests are presented in tables 6 and 7, respectively. Average values of similar tests from various other sources are included in the tables for comparison.

All samples tested were negative for the clot on boiling tests and alcohol precipitation tests.

Table 6. Results of tests on bulk milk - food value

Farm	Specific gravity	% Fat	% Total solids*
A.	1.031	5.0	14.49
B	1.032	4.8	14.49
C	1.030	3.9	12.89
D	1.028	5.2	13.98
E	1.030	4.7	14.37
F	1.032	4.6	14.25
G	1.031	4.8	14.24
H	1.029	6.3	15.58
I	1.028	6.5	15.57
J	1.025	4.1	11.89
K	1.030	4.5	13.63
L	1.030	5.0	14.24
Range	1.025 - 1.032	3.9 - 6.5	11.89 - 15.58
Average	1.0296	4.95	14.13
Institut Haiwan Kluang**	1.031		14.50
Smallholder, Kluang**	1.029		13.80
Smallholder, Chaah**	1.029		13.80
Smallholder, Jasin**	1.029		12.30
Smallholder, Seremban**	1.029		13.10

* % total solids was calculated using Richmond's formula,

$$T = \frac{G}{4} + 1.22F + 0.14, \text{ where } G = \text{lactometer reading at } 60^{\circ}\text{C,}$$

F = fat %, and

T = total solids

** Source: Kumaravel (1976,1977)

Table 7. Results of tests on bulk milk - degree of contamination and keeping quality

Farm	pH	Estimated total bacteria count per ml. milk	Estimated total coliform count per ml. milk	One hour resazurin reduction test
A	6.90	108,000	2,000	5.75 (5.75)*
B	6.80	68,100	2,600	5.5 (5.5)
C	6.75	8,150	0	6.0 (6.0)
D	6.80	54,900	2,100	5.5 (5.25)
E	6.75	59,200	1,700	5.25 (5.25)
F	6.80	48,000	0	6.0 (6.0)
G	6.75	61,000	3,100	5.75 (5.75)
H	6.80	52,600	0	6.0 (5.75)
I	6.70	19,050	550	6.0 (6.0)
J	6.70	112,500	3,950	5.5 (5.5)
K	6.50	28,250	300	6.0 (6.0)
L	6.70	43,250	1,650	5.75 (5.75)
Average	6.74	55,316	1,445	5.74 (5.48)
Institut Haiwan, Kluang**	6.64	28,118	35	
Padang hijau, Majuternak**	6.58	281,538	11,111	
UPM dairy unit	6.60	6,489	2,626	

* Figures in parentheses are values obtained after storing samples for 48 hours at 7°C

**Source: Kumaravel (1976, 1977)

DISCUSSION

The level of subclinical mastitis was found to be low when compared to results obtained by Koh and Joseph (1974) and other reports where no mastitis control programme was practised; Kingwill et al. (1970) reported 55% cows and 28% quarters infected in 1,422 cows, while Pearson et al. (1971) reported 52% cows and 31% quarters infected in 1,344 cows.

Clinical cases of mastitis were found to be rare. Analysis of case records from the Department of Clinical Studies, UPM showed that clinical mastitis was observed in only 16 cases during 1976, 1977 and 1978 (Wong, 1980).

S. aureus was the most prevalent bacterial isolate. This was in agreement with findings by Koh and Joseph (1974) who reported S. aureus in 26.2% of samples, and by Narayanan and Iya (1953) who isolated beta hemolytic staphylococci from 58% of cows tested. In both these studies hand milking was practised. This finding is of some concern as response to treatment in staphylococcal infections is comparatively poor and satisfactory methods of eradication from herds have yet to be devised (Blood and Henderson, 1974).

S. epidermidis ranked as the second most common isolate in this study. This organism has been isolated by Hoare (1972) who found 83% of herds and 19.4% of quarters infected with it. S. epidermidis has been disregarded as a mammary pathogen (Blood and Henderson, 1974) but Forbes and Herbert, (1968) consider it significant when pure isolates are recovered from quarters with high cell counts. In this study, 6 isolates or 10% of samples conformed to the latter interpretation.

Although Streptococcus sp. especially S. agalactiae have long been

regarded as major mastitis pathogens (Kingwill et al., 1970; Tolle, 1975; Blood and Henderson, 1974), only one farm was found to be infected with this group.

C. pyogenes accounted for 6.6% of samples, and were all isolated from one farm. Kurek (1973) found subclinical infections caused by this organism in 19.1% of 988 animals tested.

Bacillus and Kliebsiella sp. are widely distributed in the environment of the dairy cow, and have been reported to cause mastitis (Jasper et. al. 1972; Blood and Henderson, 1974).

Antibiotic sensitivity tests on S. aureus showed that the efficiency of drugs decreased in the order; chloramphenicol, tetracycline, penicillin, streptomycin and ampicillin. Penicillin resistant strains were not common and were found in 21% of isolates. This agreed closely with results obtained by Koh and Joseph (1974) who reported 22% of such isolates.

Hygienic practises were found to be inferior when compared to UPM standards, where udder and hand disinfection and post milking teat dipping were routinely done. However, in spite of this low level of hygiene the level of subclinical mastitis was found to be low. It is possible that there existed one or more overriding factors that nullified or reduced the contributory effects of lower levels of hygiene towards mastitis. It is suggested that some of these factors were: 1. the suckling effect of calves might have a cleansing action so as to remove pathogenic bacteria from the udder, 2. the practice of hand milking had less traumatic effect on the teats, and hence decreased the predisposition to mastitis when compared to machine milked herds. The actions of the milking machine as a traumatic factor causing external lesions on teats are well established (Thomas and Thiel, 1971).

3. an inherent immunity in the animal that resisted infection, and 4. the fact the LID cows were low producing animals and hence less susceptible to mastitis. Kingwill, Dodd and Neave (1971) stated that mastitis occurs in all animals but more frequent in cows kept for commercial production. LID cows would not qualify as commercial producers in this sense due to their very low production when compared to breeds like the Friesian and Jersey.

Analysis of quarter infection rates among cows in different stages of lactation showed a tendency to increase in the early and late lactation stages although it was not significant. Oliver et al., (1956) and Munch Peterson (1968) reported that infection of the mammary gland occurs most readily shortly after calving.

Analysis of the effect of lactation number on mastitis showed a highly significant increase in infection rate after the fifth lactation. It has been suggested by Murphy (1945) that the older the udder the more susceptible is the udder tissue for infection. However, factors of greater importance were the degree and duration of exposure to infection and the hygienic measures (Stableforth; 1959).

Milk Quality

The average specific gravity of 1.0296 compared favourably with values from various smallholder areas elsewhere but was lower than the average obtained from Institut Haiwan, Kluang.

The average fat percentage of 4.95% was found to be high on a comparative basis though still remaining within the normal limits for LID animals. The variability in fat percentage among the farms ranging from 3.9 to 6.5% was to be expected. Fat content was the most variable among all the components in milk, and variability up to 1% had been observed

due to the individuality of the animal (Johnson; 1974).

The total solids content averaged 14.13% and compared favourably with 14.04% quoted for tropical cows by Johnson (1974). On a comparative basis, the percentage total solids was found to be higher than other smallholder areas, though it was less than the average for Institut Haiwan, Kluang.

The results of the reduction tests show that all the milk samples were of acceptable quality with regards to degree of contamination. Storage of milk at 7°C for 48 hours did not have a deteriorating effect.

The average total bacterial count of 55,316 per ml. milk was found to be higher when compared to Institut Haiwan, Kluang and UPM indicating that hygienic measures in smallholder farms are less stringent. However, the figure falls within acceptable limits for Grade A raw milk following American standards which limit total bacterial count per ml. to 100,000.

It can thus be concluded that milk from smallholders was of acceptable quality with respect to food value, degree of contamination and keeping quality. However, it must be emphasized that this applied only to ex-farm milk samples, and that milk which the consumer eventually received might be very different due to effects of poor storage, transportation, possible adulteration, etc. It would appear that any damage done to the milk was not the fault of the farmer but rather the work of the middle-man through which milk was marketed.

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