STUDIES ON THE BACTERIOLOGY OF RAW MILK FROM DAIRY HERDS ON THE ACCRA PLAINS

A Thesis Presented to the
Department of Animal Science
(Faculty of Agriculture)
University of Ghana, Legon.

In Partial Fulfilment of the
Requirements for the Degree of
Master of Science (M.Sc.)
(Animal Science)

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DECLARATION

I do hereby declare that except references to other people's work which have been duly acknowledged, this work is the result of my own original research, and that this thesis either in whole, or in part, has not been presented for another degree elsewhere.

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ACKNOWLEDGEMENT

Thanks are due, first of all, to the Department of Animal Science for providing the funds and facilities for this work, and to Dr. E.N.W. Oppong, Ag. Head of Department for his encouragement, suggestions and criticisms.

I am very heavily indebted to Dr. R.K.G. Assoku, for his supervision, encouragement, suggestions and correction of the manuscript.

I am also very grateful to Dr. R.E. Larsen for his suggestions and encouragement, Dr. L.O. Ngere, who helped in the analysis of the results, and Mr. C.A. Abrahams, formerly of the Animal Research Institute, for help in initial laboratory techniques.

Special thanks are also due to Messrs. S.K. Qurranttey, Jumah Mahama, E. Kwetso, and M. Issaka (of the University Hospital) for their help in the field and the laboratory, and to Dr. J.D. Corkish of the Veterinary Services Laboratory, Accra, for his help on Tuberculin Testing.

Mention must be made also of Mr. Laryea and his staff at the A.R.S. Nungua, Mr. Yeboah of the Amrahia Dairy Farm, and Mallam Sidow of Ashalley Botchway, for their help during the collection of milk samples.

My sincere thanks go to Miss Gladys Lartey and Mr. H.Y. Ababio, who typed the manuscript, and to all those who contributed in diverse ways to making this work a success.

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June, 1973.

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CHAPTER I

INTRODUCTION

Milk has been defined by the United States Public Health Services (1953) as the lacteal secretion practically free of colostrum, obtained by the complete milking of one or more healthy cows, which contains not less than 8.25% milk solid-non-fat (SNF), and not less than 3.25% milk fats.

Apart from the major constituents by which milk has been defined (Table 1), it contains low concentrations (about one part per million) of iron, iodine, and copper, as well as traces of cobalt. Phospho-lipids, sterols, citric acid, enzymes and vitamins are present in small quantities (Vanstone and Dougall, 1960). Because all the constituents of milk are very essential for life, milk has been referred to as the "most nearly perfect food elaborated by nature" (Foster, Nelson, Speck, Doetsch and Olson, 1958).

Milk is also an excellent medium for the growth and the cow's udder also provides a suitable habitat for a number of pathogenic micro-organisms.

TABLE 1

MAJOR CONSTITUENTS OF MILK

CONSTITUENT	RANGE %	AVERAGE %
Water	38.87-91.55	87.60
Total Solids	8.45-16.13	12.60
Fat	1.03-6.39	3.60
Solid-Non-Fat	7.42-9.74	8.80
Proteins	2.37-4.26	3.30
Lactose	4.41-5.00	4.75
Mineral Matter	0.62-0.78	0.75
Lime	0.104-0.291	0.16
Potash	0.148-0.223	0.19
Soda	.036-0.090	0.07
Magnesia	0.005-0.028	0.018
Phosphoric acid	0.146-0.310	0.23
Chloride	0.054-0.242	0.10

Source: Vanstone and Dougall (1960).

The sources of these pathogenic organisms, though usually varied, include the diseased cow, personnel, milking utensils and other equipment.

Salle (1967) reported that tuberculosis, food-poisoning, infantile diarrhea, poliomyelitis, septic tonsilitis, and typhoid fever, are among the numerous human diseases whose organisms survive in, and are transmitted by, milk. It is therefore absolutely necessary that good quality milk be produced for the consumer.

Dairying is a developing industry in Ghana. Until recently, milk production was the sole monopoly of the Fulanis. The Fulanis keep animals from different people, and there may be as many owners as there are cattle in the kraals.

The University of Ghana has a dairy herd at the Agricultural Research Station (A.R.S.), Nungua, for teaching purposes, while the Animal Husbandry Division of the Ministry of Agriculture has a Dairy Farm at Amrahia.

The herd at the A.R.S., Nungua, is mainly crossbreds (Jersey X Shorthorn; Jersey X Gudali; and Jersey X N'dama) and a few Gudalis and N'damas. Ngere (1972), reporting on the evaluation of the present status of the dairy crossbreeding performance at A.R.S. Nungua, found that the overall average production for crossbreds was 3,225 lbs; when the local breeds (N'dama and Gudali) were included, the average dropped to 2,742 lbs. Though production records are lacking on Fulani farms, the production of these predominantly Sanga herds could be put at a liberal figure of 1,500 lbs, based on production records of the local breeds - N'dama and Gudali - at the A.R.S., Nungua. The friesian herd at the Amrahia dairy farm produces an average of 1 - 2 gallons per day per cow for a lactation period of 308 days.

One of the most important problems facing the infant dairy industry in Ghana is the production of clean, healthy milk for human consumption. This problem has been successfully tackled by certain countries with well-developed dairy industries, by laying down certain regulations and standards for milk production and rigidly enforcing them. These regulations and standards are obviously not applicable to the Ghanaian conditions, and studies on the bacteriology of raw milk produced in this country are therefore urgently needed.

The present study, with special reference to ~~the~~ public health importance, was therefore undertaken to investigate the bacteriology and hygiene of raw milk produced under three different farming systems in the Accra Plains of Ghana.

CHAPTER II

LITERATURE REVIEW

The problem of producing clean, healthy, milk for human consumption has been realised long ago in countries of high milk production and consumption. Work has, therefore, been done to evaluate the bacteriological quality of milk, and regulations and standards have been laid down for production of milk for consumption (e.g. The "Milk Ordinance and Code" of the U.S. Public Health Services).

Sources of bacteria in milk are varied, and since the publication of works by Ward (1900), Von-Frendenreich (1903) and Barthel (1906), it has been established that, in many instances, the udder of the cow is a natural habitat for some of these bacteria.

Dorner (1930), working on the bacteriology of aseptically drawn milk, reported bacterial counts between 530 and 4,390 per ml. on standard agar plates, which he considered 'normal'.

But Bergey (1904) had earlier reported that 32% of 272 samples of milk drawn into sterile tubes contained no bacteria per ml., 48.8% contained less than 500 bacteria per ml., and only 10.3% contained more than 5,000 bacteria per ml.

An average bacterial count of 500 - 1,000 per ml., was reported by Foster, et al, (1958), while Seamen (1963) found that milk leaving the udder contained between 300 and 400 bacteria per ml. Verma, Zal, R. Kotharalla and Seshacharyulu, (1944) found that milk produced under different conditions showed differences in bacterial counts, though they did not say how significant these differences were. The farms with better milking conditions showed low counts. Similar observations have also been reported by Abraham and Laryea (1968) in Ghana. They found that the University of Ghana Agricultural Farm, Nungua, had a mean bacterial count of 19,658 per ml., while the Fulani kraals had a mean of approximately 3 million bacteria per ml.

Crowdy (1939), however, found that milk produced in barns with beddings gave a mean of 220,000 bacteria per ml., as against 54,000 bacteria per ml., for milking in surroundings free of beddings. This author, therefore, recommended that grooming, cleaning of udders or hind quarters, trimming of tails to prevent their trailing in dirt, milking healthy cows, healthy milkers, clean milkers' clothes, and holding milk at low temperatures were practices to help obtain low bacterial counts.

Ayers, Cook and Clement (1918) found that four simple factors were essential for the production of milk with low bacterial content, namely, (a) sterilized utensils, (b) clean cows with clean udders and teats, (c) small-top pails and (d) a holding temperature of $10^{o}C$ or less. Conducting tests by varying one factor at a time, they observed that the average count of 65 samples of fresh milk was about 4,500 bacteria per ml., when only the udder and teats were not washed, but the other three conditions were satisfied. When all the four conditions were fulfilled, the mean count of the 65 samples was approximately

2,200 bacteria per ml. Preliminary statistics from the work of Covington, Egdell and Thomas, (1952) on 171 farms, to determine the influence of various factors related to production of high-quality milk, showed that factors concerned with milking methods and sanitation were of more significance, than were factors related to building or equipment, provided the buildings and equipment are clean.

De Filippis (1941) had argued that milk presented a characteristic total bacterial count, which was essentially independent of the method of production. He, however, conceded that "faults in milking procedure or laboratory techniques may affect the count temporarily". These opinions were based on the examination of bacterial counts of the outside of the udder, the milkers' hands, utensils, milk bottles, and the actual number of organisms added to milk from these sources.

This view has been supported by other workers. Brew (1949) reported that he had learned from the inspection of dairy barns (for over 42 years) that there was no descernible correlation between milk quality as determined in the laboratory and the 'score' of dairy barns. Kelly, Newman, and Hine (1917) and Hunter (1919) had agreed that, generally, bacterial content of milk was not a satisfactory index of sanitary production. McKenzie and Bowie (1946) observed that milk from certain farms, with seemingly unsatisfactory production conditions and methods, were consistently graded as satisfactory, whereas milk from other farms with visually observable good conditions and methods, often failed to make the standard. They attributed this partly to the fact that many of the poorer farms had minimal equipment to contaminate the milk. Heeres (1950) and Atherton (1959) also reported finding little relationship between production practices and bacterial test results.

Prucha and Weeter (1917), working with utensils thoroughly cleaned and sanitized, but with barns ranging from very clean to unclean, found that 54% of the milk samples had a count of less than 10,000 bacteria per ml., and only 14 out of 1,665 samples exceeded 50,000 bacteria per ml. It was therefore concluded that the condition of the barn exerted little measurable influence on the bacterial content of the milk; and in 1918, Prucha, Weeter and Chambers, showed that unsterilized untensils were largely responsible for excessive bacterial contamination of milk.

The important micro-organisms of milk and milk products are 'true' bacteria of the sub-order Eubacteriaceae, viruses of the order Virales, rickettsiae of the order Rickettsiales, yeasts and moulds (Foster et al;, 1958). These micro-organisms play a role either in the spoilage of milk (Walter, 1967), in diseases outbreaks, or in the manufacture of various dairy products (Pelczar and Reid, 1965; Stainer, Dondoroff and Adelberg, 1958).

Seaman (1963) had reported that the typical primary pathogens of milk were Corynebacterium pyogene, Streptococcus agalactiae and Staphylococcus aureus, while Mycobacterium tuberculosis and Brucella abortus might be excreted in milk when the body had been extensively invaded. Strep. mastitidis, B. abortus, Bacterium lipolyticum or related species and some micrococci had, however, usually been found in the udder (Dorner, 1930).

Cullen and Herbert (1967) examined bacteriologically milk samples, skin and teat canal swabs, throughout the lactation period and found that the organisms were mainly non-pathogenic, but the following were also found in relative proportions in the two sites; Coagulase negative - non-haemolytic staphylococci; Coagulase negative - slightly haemolytic staphylococci; Staph. aureus (producing alpha-and beta-haemolysis), Strep. uberis, Strep. viridans (alpha-haemolytic), Bacillus spp., Actinomyces spp., Pseudomonas spp., Proteus spp., and coliform organisms - mainly E. coli.

Entero-pathogenic coliform bacteria have been recovered from milk of healthy cows, as well as milk of cows with mastitis. Walter (1967) and King (1969) had found that examination of milk from cows with mastitis also implicated Strep. agalactiae, strep. dysgalactiae, Strep. uberis and staphylococci.

Abrahams and Laryea (1968) reported a predominance of Staph. albus, and micrococci in both the University Research Farm and the Fulani Kraals. The milk was cultured on Blood and MacConkey agar plates only.

Morrison and Hammer (1941), Sherman, Cameron and White (1941), Thomas and Chandra-Sekhar (1946), Thomas, Blodwen and Ellison (1949), Erdman and Thornton (1951) and Abdel-Malek and Gibson (1952) had all found that the bacteria in refrigerated milk belonged to one or more of the following genera: Achromobacter, Alcaligenes, Flavo bacterium, Pseudomonas, Aerobacter, Lactobacillus and Streptococcus.

Steck (1921), quoted by Dorner (1930), did not encounter any facultative anaerobes, and Dorner (1930), therefore, did not see any reason for searching for obligate anaerobes in milk. Weinberg, Nativelle and Prevot (1937), however, described reports of Clotridium perfringens being isolated from cheese and condensed milk. Renk (1962) also isolated this organism from 5 cases of fatal gangrenous mistitis of cattle in Germany. The scarcity of these reports is an indication of the rarity with which dairy products are associated with C. perfringens food poisoning (Walter, 1967).

CHAPTER III

MATERIAL AND METHODS

(1) DAIRY FARMS:

This study was carried out on three different farms: (i) The University of Ghana Agricultural Research Station (A.R.S.), Nungua; (ii) The Amrahia Dairy Farm of the Animal Husbandry Division; The Fulani Kraals (Ashaley Botchway).

These three farms were chosen because of the differences in management, milking procedures, and the level of hygiene on each farm.

(i) Agricultural Research Station (ARS), Nungua; Farm A:-

Located North-east of Accra, the A.R.S., Nungua, had a dairy herd of forty-nine (49) cows this season (1972-73), made up mostly of crossbreds. "The research efforts of the station are chiefly devoted to development of dairy cattle by crossing local breeds with Jersey and Fresians ...." (University of Ghana Calender, 1970-72).

The herd was kept in paddocks, and milked twice (3 a.m. - 6 a.m. and 1 - 3 p.m.) daily. At milking time, the cows were brought into the milking barn, and stood near the feeding troughs. A towel, dipped in 1% Lactosan (antiseptic), was used to wash the udder, which was then dried with a clean towel. A small amount of supplemental feed (usually wheat-bran) was given to each cow while being milked.

The hind-legs and tail of each cow were restrained by a milking rope. (Fig. 1). The hands of each milker were first washed in 1% Lactosan, rinsed with tap water, and then dried with a clean towel. The milkers, sitting on low stools (see Fig. 1) first stripped a small amount of milk from each functional teat into a strip cup for examination. In the absence of any abnormal appearance e.g. floccules, the cows were milked by hand into clean buckets. The hands were again washed before the next cow was milked.

(ii) <u>Amrahia Dairy Farm of the Animal Husbandry Division; Farm B</u>:-

The cows on this farm were kept in roofed, concrete pens, bedded on a mixture of saw-dust and straw. (Some of the animals were, however, left in the paddocks overnight, for lack of saw-dust and straw).

The animals were mostly imported Friesians, and a few Hereford X Friesian crosses.

The cows were milked twice (5.15 - 6.30 a.m. and 3 - 5 p.m.) daily. At milking time, the cows were driven into the milking palour, and stood at the 'milking stand' near the feed troughs. The udder of each cow was washed with a towel dipped in 1% Anti-Germ 50*, and dried with a clean towel. The cows were given

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* Anti-Germ 50 - Active Ingredients:

Alkyl (C^{14} 60%, C^{16} 30%, C^{12} 5%, C^{18} 5%) dimethyl benzyl-ammonium chloride	25%
n-alkyl (C^{12} 5%, C^{14} 30%, C^{16} 17%, C^{18} 3%) dimethyl ethyl-benzyl ammonium chloride	25
Isopropyl alcohol	30%
Inert ingredients	20%
	100%

Fig. 1

Hand Milking at the A.R.S. Nungua.

Note: (i) The Clean bucket, and concrete floor.
(ii) The Milker is sitting on a low stool.

supplemental feed (wheat-bran). A small amount of milk was stripped from each functional teat onto a strip cup for examination. In the absence of any abnormalities, a clean teat-cup was attached to each teat (Fig. 2) and the cows were machine milked in sets of eight, after which the teat-cups were again washed in 1% Anti-Germ 50, rinsed in clean tap water before being used again.

At the end of each milking session, the whole milking line was washed and aseptically cleaned, using both hot water and dilute formic acid. The floor of the milking palour was also washed and disinfected after the animals had been released for grazing.

iii) Fulani Kraal: Farm C:-

On this farm, the cattle were housed in kraals made of wooden stocks (inexpensive wood from the bush). Milking was done in these open kraals, which were heavily infested with house flies (*Musca domestica*). The calves were separated from the cows in the evening and early in the morning (about 5.30 a.m.), the cows were taken for grazing and returned at about 9 a.m. for milking. At milking time, the calves were released, one at a time (according to number of milkers available) from their separate compartment, into the main kraal to search for their dams. (Fig. 3).

Each calf was allowed to suckle the dam for a short period. The Fulanis exploit the finding that the natural stimulation for milk let-down is the sucking of the teats by the calf (Smith, 1959). The neck of the calf was then tied loosely to the foreleg of the cow (Figure 4).

Fig. 2

Machine Milking at Amrahia Dairy Farm.

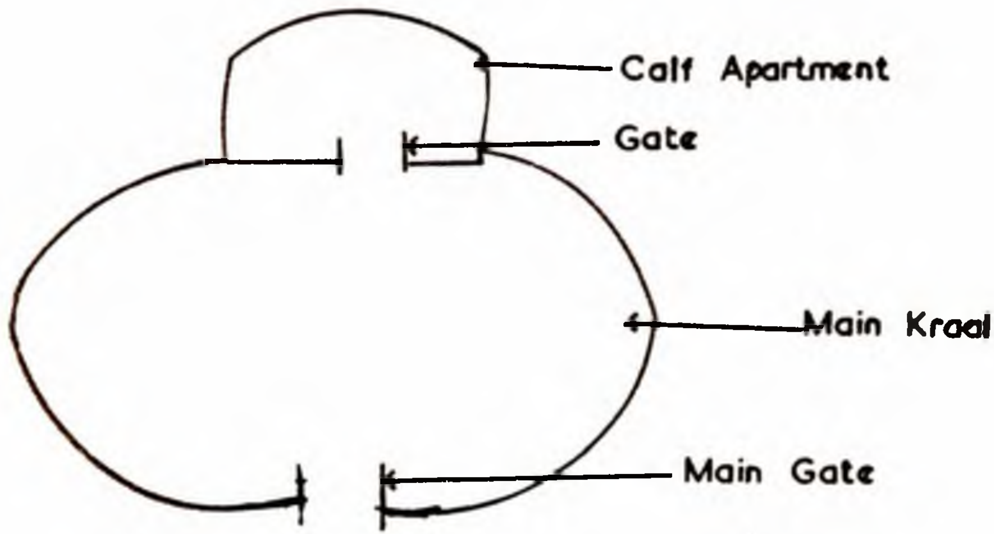

Fig. 3

FLOOR PLAN OF THE FULANI KRAAL

Fig. 4

Restraining a Calf during Milking at the Fulani Kraal

The hind legs and the tail of the cow were also restrained by a "milking rope", and the cow milked by hand into calabashes, or bowls, (Fig. 5) and then bulked in kerosine tins for transportation to the market.

(2) COLLECTION OF MILK SAMPLES:

Milk samples were taken from cows at three different stages of lactation:

(a) Early lactation samples were those collected from cows which had not been lactating for more than three and a half ($3\frac{1}{2}$) months (i.e. from date of calving).

(b) Mid. lactation samples were from cows which had been lactating for more than three and a half ($3\frac{1}{2}$) months but not more than six and a half ($6\frac{1}{2}$) months.

(c) Cows which had been lactating for more than six and a half ($6\frac{1}{2}$) months were sampled as late lactation cows.

The method of collection of milk samples depended very much on the method of milking. In the case of the A.R.S., Nungua, and the Fulani Kraal where hand-milking was practiced, the milk was mixed by gentle stirring, using the sampling dipper, before samples were taken into sterile, labelled MacCartney bottles with the dipper. At the Amrahia Dairy farm, however, a direct outlet from the milk containers was used to collect the milk samples into sterile, labelled MacCartney bottles. Cows to be sampled were milked first, and the number of samples taken at a time was limited to the number of containers

Fig. 5

Milking at the Fulani Kraals

Note: (i) The milking is done in the open Kraal, into a large calabash.

(ii) The milker is squatting.

(eight) available on the farm.

The udder or each cow sampled on any of the farms was inspected for any abnormalities; for example, for non-functional teats and hard, indurated udders. The calving date, feeding regime and the health records of each cow, where available, were recorded.

The milk samples in the MacCartney bottles were packed on ice in a thermos-flask and transported to the laboratory for examination.

(5) LABORATORY PROCEDURES:

(A) MEDIA:

(i) Blood Agar - 20 gms. of Bacto-Blood Agar Base, were weighed into 500 mls. of distilled water in a conical flask. The mixture was heated and stirring till the medium was completely dissolved. The medium was then autoclaved at 15 p.s.i. (121°C) for 15 minutes, and allowed to cool in a water bath to 44 - 46°C.

A sterile MacCartney bottle of sterile defibrinated blood* (approx. 25 mls.) was added aseptically, with thorough mixing and distributed with sterile precautions into sterile petri-dishes. Air bubbles were removed by quickly passing a bursen flame two or three times over the plates.

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* - Sterile defibrinated blood:- Sheep blood obtained aseptically into sterile universal bottles containing sterile glass beads.

The plates were covered and allowed to set.

This medium, which was routinely used in this study, has been recommended, as a base which might be used in the isolation and cultivation of many fastidious pathogenic micro-organisms (Difco Manual, 1965).

(ii) MacConkey Agar: - 25 gms of Bacto-MacConkey Agar, were weighed into 500 mls of distilled water in a conical flask. The mixture was stirred while heating till the medium was completely dissolved. It was then autoclaved at 15 p.s.i. (121°C) for 15 minutes and distributed into sterile petri-dishes as described for the blood aga

This medium is a differential plating medium recommended for use in the detection and isolation of all types of dysentery, typhoid and para-typhoid bacteria (Difco Mannual, 1965).

(iii) Nutrient Agar:- This agar has also been recommended as a general culture medium for the cultivation of the majority of less fastidious micro-organisms (Difco 1965). 12.5 gms of Bacto-Nutrient Agar were weighed into 500 mls of distilled water in a conical flask heated to dissolve, and bottled in MacCartney bottles with stirring till the medium completely dissolved in alliquots of 10 mls. and autoclaved at 15 p.s.i. (121°C) for 15 minutes.

(iv) Nutrient Broth: - This liquid medium has been recommended for general laboratory use for the cultivation of micro-organisms that are not exacting in food requirements (Difco, 1965). 4 gms of Bacto-Nutrient Agar were dissolved in 500 mls. of distilled water, in conical flasks by heating and stirring, distributed into MacCartney bottles and autoclaved at 15 p.s.i. (121°C) for 15 minutes, cooled and stored in the refrigerator for use.

(v) Lowenstein-Jensen Medium:

7.5 gms of Lowenstein-Jensen medium base were weighed into 120 mls of distilled water in a conical. The mixture was heated with constant agitation till it boiled for about one minute, autoclaved at 121°C (15 lbs. steam pressure) for 15 minutes, and allowed to cool to 50°C.

Fresh eggs (not more than two days in storage) were washed in water, wiped with a clean towel, dipped in 75% alcohol wiped clean. 200 mls. of whole egg were aseptically collected. The base was then gently mixed with the whole egg till a uniform mixture was obtained. The mixture was then distributed into sterile screw-capped tubes, arranged in slanted positions in an oven, and coagulated and inspissated at 85°C - 90°C for 45 minutes.

Lowenstein-Jensen is most popularly used for the isolation and cultivation of Mycobacteria (Baltimore Biological Laboratory Manual B.B.L.; 1968).

(vi) Trypticase Soy Agar (TSA):- 40 gms T.S.A. powder were suspended in 1,000 mls. distilled water in a conical flask, heated with frequent agitation till it boiled for one minute.

The solution was then autoclaved at 121°C (15 lbs. steam pressure) for 15 minutes. The agar was then cooled to 45°C in a water bath and .007 gms. of crystal violet added and poured into plates with sterile precautionary measures. B.B.L. (1968) recommending that Brucella may be isolated from milk specimen on this medium.

Sterility Test:

All plates and MacCartney bottles of agar media and bottles of broths were incubated at 37°C for 18 - 24 hours (over-night). Contaminated plates showed colonial growth, while contaminated broths showed turbidity. These were discarded, and those plates and broths found sterile were stored in the refrigerator and used when required.

(3) STANDARD PLATE COUNTS FOR BACTERIA

(i) Dilutions:-

The milk samples in the MacCartney bottles were rotated to mix thoroughly. 5 mls. of each samples pipetted using sterile pipette* into 45 mls. of sterile distilled water. This gave an initial dilution of 1:10, and subsequently ten-fold serial dilutions were made, with a final dilution of 1:1000 for each sample.

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* All glass wares and distilled water were sterilised by autoclavint at 121°C (15 p.s.i.) for 15 minutes.

(ii) Plates:-

1.0 ml. of 1:100 and 1:1,000 dilutions of each sample from Farms A and B, and 0.1 ml. of each of 1:100 and 1:1,000 dilutions of each sample from Farm C, (Limitations were determined from preliminary studies) were pipetted into sterile 4-in petri-dishes and covered up. Then 10-12 ml. of molten Nutrient agar held at (44-46°C) in a water bath, were poured into each petri-dish, taking sterile precautions. The mixture was allowed to set and was incubated at 37°C for 48 hours, in the inverted position.

After incubation, the number of colonies on each plate was counted, using the Gallenkamp Colony Counter+.

The number of bacteria in 1 ml. of the original milk sample was calculated as:-*

$Q \times 10^{b}$ = Number of bacteria/ml. (when 1 ml. of each dilution was used for plating, as for Farms A and B).

$Q \times 10 \times 10^{b}$ = Number of bacteria/ml. (when 0.1 ml. of each dilution was used for plating, as for Farm C).

where Q = Number of colonies counted in the plate

b = The dilution of sample plated.

+ Gallenkamp and Co. Ltd. Technico House, London

* Source: Crowley, et al, (1969).

(C) ISOLATION AND IDENTIFICATION OF BACTERIA:

(a) Staining:

(i) Direct:

The MacConkey bottles containing the raw milk samples were mixed by rotating and shaking several times. A loopful of each sample was taken aseptically onto 2 microscopic slides, and a thin film produced by spreading the drop with the edge of another slide. The smear was air dried and fixed by heat, and stained by (a) Gram's Stain and (b) Methylene-Blue Stain (Appendixes 4 and 7 respectively) and examined microscopically.

(ii) Cream/Sediment:

Sterile centrifuge tubes were balanced in pairs with raw milk (10-15ml.) from each sample and centrifuged at 3,000 r.p.m. for an hour. The cream and sediment were mixed on an agglutination plate with a sterile loop. Three smears were made on microscopic slides from each sample, air dried and fixed by heat.

Slide 1: Stained by Ziehl-Neelsen Method (appendix 5) for acid-fast organisms e.g. *Mycobacterium tuberculosis*.

Slide 2: Stained by the Modified Ziehl-Neelsen Method Appendix 6) for Brucella.

Slide 3: Stained by the Grams Stained Method (Appendix 4).

Each slide was carefully examined under the microscope.

(b) Culturing:

(i) The milk sample in each MacCartney bottle was mixed, and loopful of the sample taken aseptically and streaked on Blood agar and MacConkey agar.

(ii) Each sample was also inoculated into Nutrient broth. All the plates and broths were incubated at 37°C for 24 hours.

The size, form, colour and the effect of the colonies on agar (e.g. haemolysis in Blood agar) were recorded. Identification of the organisms were confirmed by staining.

(iii) Cream/Sediment Mixture:

Samples of the cream/sediment mixture were inoculated onto Lowenstein-Jensen medium, and Trypticase Soy agar.

The streaking out on all plates was as described by Sirockin and Cullimore (1969).

The Lowenstein-Jensen agar (tubed) and Trypticase Soy agar were incubated in carbon dioxide at 37°C, and inspected every two days.

(iv) Plating of Nutrient Broth Culture on Blood and MacConkey Agars:

After incubating the Nutrient broth for 24 hours, stained slide examination often showed mixed growth of organisms. To obtain pure culture for staining and identification, a sterile loopful of the broth culture was sub-cultured onto Blood and MacConkey agar plates and incubated for 24 hours.

Single, well-described colonies were then picked, stained and examined.

(c) Serological (Immunological) Tests:

(i) Brucella Agglutination Test:- Whenever Brucella organisms were suspected from stained slides and culture plates, sterile serum samples were obtained from the suspected cows and agglutination test(s) performed on these, using a 1:10 dilution of a Brucella stained antigen*. Two-fold serial saline dilutions were used, and the method was as described by Oppong (1966).

(ii) The Single Intradermal Comparative Tuberculin Test:-

This test was conducted by the Veterinary Services Division.

(d) Biochemical Tests:- Where coliforms and other members of the Enterobacteriaceae were suspected, biochemical tests were performed to identify and differentiate between them.

(i) Bile Salt (EIJKMAN TEST):- This test was used to ascertain whether the coliforms detected were *Escherichia coli*; it depended on the ability of *E. coli* to produce gas when growing in bile-salt lactose peptone water; atypical coliforms are unable to do this (Cruickshank, 1970).

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* Donated by Ministry of Food and Agriculture Laboratories, Weybridge, New Haw.

(ii) Indole production test: This test demonstrates the ability of certain bacteria to decompose the amino-acid, tryptophane, to indole, which accumulates in the medium (Cruickshank, 1970). The method employed was as described by Cruickshank, (1970).

(iii) Triple Sugar Iron (T.S.I.) Agar:- Triple Sugar Iron agar has been recommended as a medium for use in the identification of Gram-negative enteric pathogens, particularly members of the Salmonella-Shigella groups (Difco Manual, 1965). These organisms have ability of fermenting lactose, saccharose, dextrose (with formation of acid and gas) and also to produce hydrogen sulphide.

T.S.I. (Difco Manual, 1965) tubes were inoculated with suspicious colonies from primary media, and incubated at 37°C for 24 hours.

(iv) Sugar Reactions: Lactose, Glucose, Maltose, Mannitol, Sucrose and Trehalose:-

5 mls. of Andrades indicator (Cruickshank 1970) were added to 500 mls. of Peptone-Water base (Cruickshank 1970) and 5 mls. of the Peptone water base (plus indicator) were then pipetted into screw-capped tubes. (Glass Durham tubes were inserted before the caps were loosely screwed). The tubes were sterilised and 0.25 ml. of sterile 10% solutions of each sugar was added. The tubes were inspected and those showing bubbles after storage (in refrigerator) were discarded.

Each tube was then inoculated aseptically, labelled and incubated at 37°C for 24 hours. Tubes which gave negative results were re-incubated for another 18 - 24 hours.

CHAPTER IV

RESULTS AND DISCUSSION

A total of 225 raw milk samples from the University of Ghana Agricultural Research farm, the Amrahia farm and the Fulani Kraals, all on the Accra Plains, were examined to provide some information on the bacteriology of raw milk on the Accra Plains.

A. STANDARD PLATE (BACTERIAL) COUNTS OF RAW MILK:

Table 2 shows that the total bacterial counts of samples of raw milk from the Fulani Kraals were the highest of the three farms.

The A.R.S. (Nungua) had a bacterial count ranging from 3,000 to 227,000 per ml., whilst the Amrahia Dairy Farm was between 3,000 and 272,000 bacteria per ml. The range for the Fulani Kraals was between 3,000 and 1,100,000 bacteria per ml. (Appendix 1, 2, 3).

The Amrahia Dairy Farm, where machine-milking was practised, had the least overall mean bacterial count of 27,160 bacteria per ml.; the A.R.S. (Nungua) had 61,466 bacteria per ml., and at the Fulani Kraals, the mean bacterial count of 345,413 per ml. was the highest (Table 2). At the Amrahia Farm the udder of the cows were routinely washed with Anti-Germ 50 (an antiseptic) and dried before the animals were machine-milked; the milk was then conveyed through sterilised pipe lines into a cooling vat. This high level of hygiene and milking practice obviously accounted for the low overall bacterial count obtained on this farm.

Though hand-milking was practised at the A.R.S. (Nungua), the level of hygiene was relatively high. The udder of the cows and the hands of the milker were washed with Lactosan (an antiseptic) dried with a clean towel, and the cows were milked into clean milking buckets. The degree of bacterial contamination at the A.R.S. (Nungua) was, however, higher than that at the Amrahia Dairy Farm, probably because the hands were not clean enough, and the milking buckets not completely free of contaminating bacteria. The milk could also have been contaminated by the often humid, dust-ridden milking barn.

The poor bacteriological quality of the milk from the Fulani Kraals might have been due to the poor milking hygiene, lack of aseptic precautions and primitive milking practices.

The early stage of lactation for the Amrahia Farm had a low mean of 24,600 bacteria/ml., the A.R.S. (Nungua), 65,120 bacteria/ml., and the Fulani Kraal had a high mean of 203,760 bacteria/ml. This pattern of low mean counts for Amrahia Dairy Farm and high for the Fulani Kraals was repeated on both farms during the mid and late stages of lactation (Table 2).

This shows that the level of hygiene, and milking practices, had the same effect on the bacterial count on the farms irrespective of the stage of lactation (i.e. farms of good hygiene had low bacterial counts at every stage of lactation).

The mean bacterial counts for the Amrahia Dairy Farm and A.R.S. (Nungua), where the levels of hygiene and milking practices were generally high, showed very little fluctuations of differences between the stages of lactation and there was no definite pattern of increase, but the mean bacterial count for the Fulani Kraals increased with the duration of lactation (Table 2). On this particular farm, milking was continued for well over 8 months, and weaning time was correspondingly higher. The demand, therefore, for more milk by the calf, the long duration of milking and the forced extraction of milk from the animals, all predispose the udder to infection, as evidenced by the numerous teat wounds and damaged teat canals (Appendix 3).

According to the United States Public Health Services "Milk Ordinance and Code" (1953) Grade A raw milk for pasteurization should not exceed 100,000 bacteria per ml., prior to mixing with other producer milk, while Grade A pasteurized milk and milk products should not exceed 20,000 bacteria per ml. Based on these requirements, Table 3, shows the grading of the raw milk from each of the three farms.

59 samples, or 78.67% of milk, examined from the A.R.S., Nungua, was Grade A; only 16 samples, or 21.33% of the milk, was below the standard. 72 samples, or 96% of the raw milk from the Amrahia Dairy Farm, was Grade A, while at the Fulani Kraals, only 13.33% (10 samples) of the raw milk conformed with the "A" grade.

TABLE 3

Grading of Samples of Raw Milk According to "Milk Ordinance and Code" 1953, of the U.S. Public Health Services

	A.R.S. NUNGUA			AMRAHIA DAIRY FARM			FULANI KRAALS		
	EARLY	MID LACTATIONS	LATE	EARLY	MID LACTATIONS	LATE	EARLY	MID LACTATIONS	LATE
GRADE A Raw Milk NOT exceeding 100,000 bacteria per ml.	(19)* 76%	(22) 88%	(18) 72%	(24) 96%	(24) 96%	(24) 96%	(6) 24%	(2) 8%	(2) 8%
Total (75 samples)		(59) 78.67%			(72) 96%			(10) 13.33%	
Raw Milk exceeding 10,000 bacteria/ml.	(6)* 24%	(2) 12%	(7) 28%	(1) 4%	(1) 4%	(1) 4%	(19) 76%	(23) 92%	(23) 92%
Total (75 samples)		(16) 21.33%			(3) 4%			(65 86.67%	

* 25 samples were examined for each stage of lactation for each farm.

The differences in grades of milk on the various farms were clearly a reflection of the different milking practices on each of these farms. De Filippis (1941), who reported that milk presents a characteristic total bacterial count essentially independent of method of production, nenevertheless, conceded that faults in milking procedure might affect the count. The differences in the grades obtained in this study might, therefore, be due to the faults and differences in the milking procedures. This observation clearly is in contrast with the findings of Heeres (1950) and Atherton (1959), that there is no relationship between production practices and bacterial test results.

At the Amrahia Dairy Farm, the udder of the cows was washed with 1% Anti-Germ 50, dried with a clean towel before being machine-milked. The only possible sources of milk contamination were probably improper cleaning of udder, milking of cows with sub-clinical mastitis, and using improperly sterilised milking-machine. Contamination from these sources on this farm was apparently very small, as evidenced by the low mean bacterial count (Table 2).

At the A.R.S. Nungua, the udders of the cows were washed with 1% Lactosan, dried with a clean towel, but the animals were milked by hand. Here, the contamination was only moderately high (Tables 2 and 3). At the Fulani Kraals, where milking was done in the fly-infested, open kraals, without prior washing of the udders, the degree of bacterial contamination was extraordinary: more than 86% of the raw milk (65 samples) did not conform to the grade "A" classification.

Abrahams and Laryea (1958) had reported that 100% of the raw milk produced at the University Farm (A.R.S., Nungua) was Grade A, and that only 10% of the milk from the Native Kraal (Fulani Kraals) was Grade C; 90% of this milk was Grade D. This grading was based on a United States Public Health Services Milk Ordinance classification which required Grade A raw milk not to exceed 50,000 bacteria per ml. This type of classification cannot be found anywhere, even from the literature.

The differences in these mean bacterial counts from these farms were also analysed statistically, using Snedecor and Cochran methods (1967).

As shown in Table 5, the results of the analysis of variance do not show any significant differences of the Standard Plate (Bacterial) Counts at 37°C within either of the three farms, or the three stages of lactation.

The results therefore agree with previous reports of Brew (1949), Kelly, et al. (1971), Hunter (1919), and De Filippis (1941) that generally there was very little relationship between production methods and bacterial counts.

TABLE 4

Summary of Standard Plate (Bacterial) Counts at 37°C for Analysis of Variance (From Appendix 1, 2 and 3

	A.R.S. NUNGUA	AMRAHIA DAIRY FARM	FULANI KRAALS	GRAND TOTAL
Total count for farms (per ml.) (X 10^{-4})	461.0	203.7	2,590.6	3255.3
Total count for Lactation stages (per ml.) (X 10^{-4})	733.7	871.6	1,650.0	3255.3

TABLE 5

Analysis of Variance of Standard Plate Counts

Source of variations	df	SS	MS	Observed F	Required F	
					5%	1%
Farms	2	45,771.96	22,885.98	1.8981	6.94	18.00
Stages of Lactation	2	6,509.01	3,254.51	0.2699	6.94	18.00
Error	4	48,229.02	12,057.26	-		
Total	8	100,509.99	-	-		

B. ISOLATION OF BACTERIA OF PUBLIC HEALTH IMPORTANCE

(a) Mycobacterium tuberculosis: Four suspected cases of Myco. tuberculosis (one from the Amrahia Dairy Farm, and the other three from the Fulani Kraals) were identified from stained smears. Milk samples from these cows, when cultured on Lowenstein-Jensen medium at 37°C for four weeks, and for 8 weeks on Dorset egg agar medium, however, did not show any growth.

The Single Intradermal Comparative Tuberculin Test on the doubtful case at the Amrahia Dairy Farm was also negative (Corkish, 1973; Personal communication).

The non-isolation of Myco. tuberculosis, during the period of this research does not necessarily conclude its absence in the raw milk from these farms at all times.

(b) Brucella spp.: Attempts were also made to determine the incidence of brucellosis in this study. Seven suspected cases of brucellosis were diagnosed from the Fulani Kraals, and eight cases from the Amrahia Dairy Farm; none were detected at A.R.S. Nungua (Table 6). The organism was detected in stained smears of milk sediments and cultures on T.S.A. medium.

TABLE 6

Results of Brucella Agglutination Test, and Trypticase Soy Agar (T.S.A.) Culture for Brucella spp.

TITRE		$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$1/16$	$1/32$	$1/64$	$1/128$	$1/256$	Trypticase Soy Agar
Amrahia Dairy Farm	X21	++	±							+
	1	++	±							+
	30	++	±							+
	46	++	±							+
	71	-	-	-	-	-				-
	107	++	±							+
	120	-	-	-	-	-				-
	163	++	±							+
Standard Serum of known Titre		+++	+++	+++	+++	+++	++	±		
Fulani Kraals	1	++	±							+
	2	+++	++	±						+
	3	+++	++	±						+
	4	+++	++	±						+
	5	+++	++	±						+
	6	++	±							+
	7	+++	++	±						+
Standard Serum of known Titre		+++	+++	+++	+++	+++	++	±		

Confirmatory serum agglutination test, performed on a random sample of animals and the suspects from these farms showed an infection rate of 13.64% (44 animals sampled) at the Amrahia Farm, and 18.45% (38 animals sampled) at the Fulani Kraals (Table 6).

Though these results at Amrahia Dairy Farm, and at the Fulani Kraals were lower than the 23.47% infection rate reported by Oppong (1966) in a survey of bovine brucellosis in Southern Ghana, it is useful to note that the agglutination test was carried out on a fewer number of animals than Oppong (1966) and only as a confirmatory test on suspected cases.

These findings suggest that a sizeable proportion of raw milk produced by some cows on the Accra Plains is infected with the brucella organism, and the epidemiology of the incidence of human undulant fever in the Accra region should be examined in relation to the consumption of unpasteurized or unboiled milk by farmers and other workers.

The non-isolation of brucella organism from milk samples from the A.R.S., Nungua is due to the fact that since 1963 (when the disease was diagnosed on the farm), the dairy herd and all heifers on this farm have been routinely vaccinated every year against brucellosis.

C. INSOLATION OF OTHER IMPORTANT BACTERIA (Other than *M. tuberculosis* and *Brucella spp*.):

These other types of bacterial are also of great importance to human health, particularly members of the enterobacteriaceae, which have been implicated in certain types of food poisoning and infantile diarrhoea.

Tables 7 and 8 summarize and clearly show the bacterial flora of all the raw milk samples - other than *Myco tuberculosis* and *Brucella spp*. - isolated from the different farms. It is evident from the results that the isolation of the different types of bacteria are in larger number in the Fulani Kraal samples than the other two farms.

(i) *Bacillus spp*.: Anthrax bacillus is the only Gram-positive and variable spore-bearing aerobic rod that is pathogenic to man and animals; the other members of the group are non-pathogenic, but are important from the point of view of bacteriological studies (Mahanta, 1965).

Two species of the genus *Bacillus*, were isolated, namely: *B. cereus*, and *B. subtilis*.

Both were indole-negative, and produced acid in glucose and sucrose. *B. subtilis* produced acid in mannitol and arabinose, while *B. cereus*, did not.

B. subtilis and *B. cereus*, including closely related strains, are generally encountered in raw milk (Foster *et al*. 1958) and milk has been reported as being one of the habitats

TABLE 7

Key for recognition of Bacteria isolated from raw milk

	Bacillus cereus	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus spp.*	*Streptococcus spp.*	*Corynebacterium pyogenes*	*Corynebacterium diphtheria*	*Lactobacillus spp.*	*Pseudomonas aeruginosa*	*Aerobacter aerogenes*	*Alcaligenes viscolactis*	*Pasteurella spp.*
Gram stain	+	+	-	+	+	+	+	+	-	-	-	-
Microscopic morphology	B x	B	B	C xx	C	B (Pleo)	B	B	B	B	B	B
Spores	+	+				-	-	-				
Lactose fermentation			+									
Indole (44oC)	-	-	+			-	-		-	+	-	
Gas from MacConkey (Bile Salt) broth 44oC			+									
Mannitol	-	+	$\underline{+}$			-			-	$\underline{+}$	$\underline{+}$	
Glucose	+	+	$\underline{+}$			+	+		+	$\underline{+}$	-	
Lactose	-	-	$\underline{+}$			+	+			$\underline{+}$	-	
Maltose	-	-	$\underline{+}$			+				-	-	
Sucrose	+	+	-			+				-	-	
T.S.I.(H_2S)			-							-	-	
Arabinose	-	+							-			

Note: $\underline{+}$ Acid plus gas
B x - Bacilli
C xx - Cocci
Pleo - pleomorphic

TABLE 8

Distribution of The Different Types of Bacteria (Other than Brucella spp. and Mycobacterium tuberculosis

FARMS	A.R.S. (NUNGUA)						AMRAHIA DAIRY FARM						FULANI KRAALS					
STAGES OF LACTATION	EARLY		MID LACTATIONS		LATE		EARLY		MID LACTATIONS		LATE		EARLY		MID LACTATIONS		LATE	
BACTERIA	No. +ve	%	No. +ve	%	No. +ve	%	No. +ve	%	No. +ve	%	No. +ve	%	No. +ve	%	No. +ve	%	No. +ve	%
Bacillus cereus	2*	8	-	-	-	-	1	4	5	20	1	4	3	12	3	12	1	4
B. subtilis	1	4	-	-	-	-	4	16	-	-	3	12	5	20	4	16	3	12
E. coli.	3	12	7	28	8	32	1	4	5	20	3	12	15	60	11	44	12	48
Staphylococcus spp.	9	36	11	44	13	52	10	40	12	48	17	68	13	52	15	60	19	76
Streptococcus spp.	1	4	1	4	3	12	6	24	5	20	8	32	7	28	10	40	16	54
C. pyogenes	1	4	2	8	2	8	5	20	2	8	6	24	3	12	5	20	6	24
C. diphtheria	-	-	-	-	1	4	3	12	1	4	2	8	3	12	4	16	5	20
Lactobacillus spp.	2	8	-	-	2	8	3	12	5	20	4	16	6	24	7	28	7	28
Pasteurella spp.	-	-	-	-	-	-	+	-	-	-	-	-	3	12	3	12	2	8
Ps. pyocyanea	1	4	-	-	1	4	1	4	-	-	2	8	2	8	4	16	3	12
Aerobacter aerogenes	-	-	2	8	-	-	2	8	1	8	-	-	1	4	3	12	4	16
Alc. viscolactus	-	-	-	-	1	4	1	4	3	12	6	24	8	32	5	20	4	16
Fungus: *Saccharomyces spp.*	-	-	2	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* 25 samples were examined for each stage of lactation for each farm.

of B. cereus, and as a source of B. subtilis (Breed, Murray and Smith, 1959).

The highest number of these bacilli was isolated from the Fulani Kraals, followed by the Amrahia Dairy Farm; the A.R.S. Nungua had the least bacilli count (Table 8).

The number of B. subtilis organism gradually declined with the increase in the length of lactation at the Fulani Kraals. B. cereus contamination of milk was also found to be quite high at the Amrahia farm during the mid-lactation period.

The high incidence of these thermoduric, spore-bearing bacilli at the Fulani Kraals might be due to the general uncleanliness of the milking equipment used, and the milking of animals with unclean udders on uncemented, open, dusty milking kraals. This conclusion is in fact in agreement with the observations of Foster and colleagues (Foster et al.; 1958) that a high incidence of the genus Bacillus in milk was invariably the result of the dusty coat of cows, unclean udders and milking in dusty, open air.

The high incidence of these aerobic bacilli at the Amrahia Farm during the mid lactation period, was also most probably due to accumulation of heat-resistant, spore-bearing bacteria in the milking line, owing probably to faulty cleaning after each milking session (Bryan, Bryan and Mason, 1946).

The relatively low number of these bacteria at the A.R.S. Nungua, was probably because the milking buckets were washed regularly, dried and sterilised after each milking session.

(ii) Escherichia coli: Gram-negative rods of varying forms, occurring singly, sometimes in pairs and short chains, E. coli produces, at 44°C, gas from MacConkey bile-salt broth and indole. Production of acid and gas at 44°C has been found to be a satisfactory evidence for E. coli and other faecal coliforms (Collins and Lyne, 1970). E. coli, a common micro-organism of the intestinal tract of various domestic animals, including cow (Mahanta, 1965; Foster et al., 1958; Cruickshank, 1970), is commonly found in cow dung, and therefore an easy contaminant of milk.

The Fulani Kraals, with poor milking hygiene, and where hand milking was routinely practised in open kraals, showed the highest (44 - 60%) E. coli isolations, followed by the A.R.S. Nungua (12 - 32%), where hand milking was also practised but the standard of milking hygiene was considerably higher.

The Amrahia Dairy Farm, where machine-milking was practised, showed the lowest percentage (4 - 20) of E. coli isolations, This was probably due to the high level of hygiene, and the relatively, low E. coli., contamination could only be due to faulty cleaning of udder, improperly sterilised teat cups or animals with sub-clinical E. coli mastitis. E. coli is ordinarily non-pathogenic, when confined to the intestinal tract of young calves (Mahanta, 1965) but has been associated with mastitis in cows (Salle, 1967), and abortion in ewes (Cruickshank, 1970).

The presence of E. coli in some of the raw milk could also be viewed with some interest. E. coli has also been accepted as a normal and probably beneficial inhabitant of the human bowel, but it has been implicated in some cases of appendicitis, gastroenteritis and urinary infections (Taylor, 1966).

(iii) Staphylococcus spp.: It is evident from Table 8 that the number of isolations of the species of this genus was the largest of all the types of bacteria isolated from the various farms.

The Fulani Kraals showed a high incidence (76%) of staphylococcal contamination, the degree of contamination, increasing on each farm with the prolongation of milking (Table 8). This high level of contamination is likely to be dangerous. This high staphylococcal contamination at the Fulani Kraals could be explained by the observation that the majority of cases of bovine mastitis on this farm was caused by staphylococcus species (Appendix 3) and ranged from chronic to per acute.

Some species of staphylococcus (i.e. Staph. aureus) have been found on the udders of cows and on human skin and may easily gain access into the raw milk; the organism may even be present in milk obtained under the most hygienic conditions (Foster, et al. 1958). Staphylococci have been incriminated in abortion in livestock (Stableforth and Galloway, 1959) mastitis in most domestic animals (Mahanta, 1965; Salle, 1967; Seaman, 1963) and osteomyelitis, broncho-pneumonia septicaemia and localised abscesses in humans (Cruikshank, 1970). The enterotoxin produced by this organism during growth in milk and milk products has been responsible for certain human cases of food poisoning (Salle, 1967).

(iv) Streptococcus spp.: The highest number of streptococci was isolated from the Fulani Kraals (Table 8), and about 80% of the milking cows in the Fulani Kraals had some from of defect or the other, of the small udders (Appendix 3).

Most of the animals on this farm had either two or three functional teats, and sometimes had open wounds on the teats. The loss of one or more quarters of the udders of these cows was invariably due to mastitis, and this generally accounted for the higher incidence of streptococci, staphylococci and *Escherichia coli* on this particular farm.

Streptococci have been isolated from the mouth, nose, throat, genital tract, and faeces of healthy animals and man, and also from milk and milk products (Mahanta, 1965). Several species (*Strep. pyogenes*, *Strep. agalactiae*, *Strep. dysgalactiae*, *Strep. uberis* and *Strep. zooepidemicus*) are known to cause mastitis, which may lead to the loss of one or more quarters of the udder, and some species (e.g. *Strep. pyogenes*) are infectious to man, causing scarlet-fever and septic sore-throat (Mahanta, 1965; Salle, 1967; Foster, *et al*, 1958).

(v) *Corynebacterium spp.*: Two species of this genus, *C. diphtheria* and *C. pyogenes*, were isolated from the three farms. *Corynebacterium spp.*, like the other genera isolated, showed a tendency to increase in number with increasing stage of lactation, on all the farms (Table 8). It is interesting to note that *C. diphtheria* was practically absent from A.R.S. Nungua, whereas the Fulani Kraals, as usual, showed a high incidence.

C. diphtheria, has been implicated in epidemics of diphtheria among drinkers of raw milk (Foster, *et al*., 1958), while *C. pyogenes* is the cause of the so-called bovine 'summer-mastitis' — a fulminating, per-acute mastis of either dry cows, milkers or heifers (Seaman, 1963).

(vi) Lactobacillus spp.: The highest percentage of Lactobacilli (28) was isolated from milk obtained at the Fulani Kraals. These organisms were also regularly isolated from the Amrahia Farm. Very few numbers of these organisms were, however, isolated from A.R.S., Nungua, and this is difficult to explain.

Lactobacilli are invariably found in milk and milk products, and *Lactobacillus acidophilus* has been used for the preparation of sour milk drinks used in hospitals (Mahanta 1965; Briggs and Briggs, 1954; Orla-Jensen, 1919; Seaman, 1963).

This group of micro-organisms, strictly speaking, cannot be called pathogenic, but their intimate association with dental-caries makes them important as a public health hazard (Mahanta, 1965).

These isolations of Lactobacilli cannot be directly related to the health of the animals, but it is important to note that since they occur in the intestine of mammalian animals, and in faeces and saliva of certain species (Cruickshank, 1970), any management and milking systems which easily expose the milk to faeces, dust and saliva as occurs regularly in the Fulani Kraals, are liable to be easily contaminated.

(vii) Pasteurella spp.: This organism was isolated only from the Fulani Kraals (Table 8). Since these organisms are usually found in the upper respiratory tract of normal calves, and the udder of the cow was not cleaned before milking at the Fulani Kraals, it was probable that the udder became contaminated by the suckling calf; the organisms eventually finding their way into the milk.

Milk-borne Pasteurella infection of human beings has not been reported, probably because pasteurization easily destroys these bacteria (Walter, 1967).

Organisms of the Pasteurella group are however, widely distributed in nature, and are frequently found in the upper air passages of normal cattle (Mahanta, 1965). Bovine mastitis has been reported by Barum (1954), Packer (1946), Rude, Johnson, and O'Conner, (1961), Schlotthauer (1944) and Tucker (1953) as having been caused by this organism.

(viii) Pseudomonas aeruginosa: Small numbers of Pseudomonas aeruginosa were isolated from milk during the early and late stages of lactation at A.R.S., Nungua and at the Amrahia Farm; the organism was, however, isolated from all the stages of lactation at the Fulani Kraals (Table 8). The organism which resists drying for several weeks when contained in pus or exudates (Mahanta, 1965), easily contaminates milk when cows with teat and udder wounds are milked indiscriminately, a practices prevalent at the Fulani Kraals. *Ps. aeruginosa* is also frequently present, although in small numbers, in normal intestinal flora of man and animals, and has been implicated in certain human urinary tract infections (Cruickshank, 1970).

The presence of Pseudomonas in milk is also very undesirable, for they are versatile spoilage agents with pronounced biochemical activity, especially on protein and fats (Foster *et al.*, 1958).

(ix) *Aerobacter aerogenes*: Again, there was a gradual increase in *A. aerogenes* isolations relative to stage of lactation at the Fulani Kraals, but at both the A.R.S., Nungua and the Amrahia Dairy Farms, only a few isolations were recorded during the early part of the lactation period (Table 8).

A. aerogenes has been found in milk and milk products and is one of the main causes of ropiness of milk, which is of great economic importance to the milk processor (Foster *et al.*, 1958; Seaman, 1963).

(x) *Alcaligenes viscolactis*: The Fulani Kraal had the highest percentage isolations of *Alcaligenes viscolactis*, and a few of these organisms were isolated from the Amrahia Farm. No isolations were recorded at the A.R.S. Nungua farm.

Foster and colleagues (Foster, et. al., 1958) had reported that A. viscolactis, usually found in soils, manure and dairy utensils, had been implicated in outbreaks of ropiness of milk and cream.

Fungus; Saccharomyces spp.: Only two milk samples from A.R.S. Nungua (Table 8) showed these large, ovoid cells in clusters, and they are relatively non-pathogenic.

It is interesting, finally, to note that despite the presence of coliforms of all kinds in samples from all the farms, no salmonellae was isolated, however, S. enteritidis, S. new port, S. thompson and S. typhi-murium have all been implicated in milk-borne outbreaks of human salmonellosis (Parry, 1966).

CHAPTER V

CONCLUSIONS AND SUMMARY

(A) CONCLUSIONS

Very elaborate equipment is not a pre-requisite for producing milk of low bacterial count, or of the best grade; simple milking hygiene and decent storage methods are very essential and necessary. The Fulani farmers should be encouraged to adopt simple sanitary precautions, like washing the udders of the cows and hands of milkers with soap before milking, and also keeping the calabashes, bowls and kerosine containers clean and dry.

The absence of positive cases of *Mycobacterium tuberculosis*, in the present study, should not lead to a wrong assumption that milk produced on these farms is tuberculosis-free, for except at A.R.S., Nungua, none of the herds are tuberculin tested yearly. Even attested herds should be tested yearly (Stableforth and Galloway, 1959).

Regular bacteriological examination and serological testing of all milking herds will be necessary to ensure good quality milk.

Milk produced on all these farms is sold unpasteurised to consumers and this, therefore, calls for effective measures to protect the health of the public from the hazards of milk-borne diseases.

It is therefore recommended that:

(a) A central milk depot be set up to buy all raw milk from the producing sources, pasteurise and sell to the public.

(b) Public Health inspectors should periodically visit these farms to advise on, and to enforce the maintenance of proper hygienic milking conditions.

(c) A legislation should be passed to regulate the conditions under which all cows should be milked and this should be vigorously enforced.

(d) The present bacteriological data could be the basis by which the National Standards Board might use to advise the Government on a 'Milk Ordinance Code'.

(B) SUMMARY

The bacteriological quality of raw milk, and the distribution of the various types of bacteria, from three farms (A.R.S. Nungua, Amrahia Dairy Farm and the Fulani Kraals) of different management, hygiene and milking practices, were determined at three different stages of lactation — Early-lactation: 1 - 3.5 months; Mid-lactation: 3.5 - 6.5 months, and Late-lactation: over 6.5 months.

The overall results indicate that raw milk produced by cows at the Ministry of Agriculture Farm (Amrahia) was by far hygienically superior to milk produced at A.R.S. (Nungua) and the Fulani Kraals. The standard of hygiene at the two former farms compared highly favourably with that of well established dairy farm elsewhere.

The Amrahia Dairy Farm had the least overall mean bacterial count of 27,000 per ml.; the A.R.S., Nungua, had 61,000 per ml., and the Fulani Kraals gave a mean total of 345,000 per ml. There were, however, no statistically significant differences between stages of lactation and mean bacterial count.

The Amrahia Dairy Farm produced 96.00% Grade A raw milk, whilst only 13.33% of the raw milk produced at the Fulani Kraals came within Grade A. 78.67% of the total number of raw milk samples from A.R.S., Nungua conformed with the Grade A.

B. cereus, *B. subtilis*, *E. coli*, *Staph. spp.*, *Strep. spp.*, *C. pyogenes*, *C. diphtheria*, *Lactobacillus spp.*, *Pasteurella spp.*,

Pseudomonas aeruginosa, Aerobacter aerogenes, and Alcaligenes viscolactis, were isolated from milk produced at the three farms. There was predominance of Staphlococci on all three farms.

Six cases of brucellosis from the Amrahia Dairy Farm, and seven from the Fulani Kraals were isolated. None was isolated from the A.R.S. Nungua.

Four cases of suspected tuberculosis (one from the Amrahia Dairy Farm and three from the Fulani Kraals) were investigated, but all proved negative after culturing, and tuberculin testing.

It is interesting to note that despite the high percentage of coliforms isolated from milk samples from the Fulani Kraals, and indeed from the other farms, no salmonellae were isolated in any of the samples.

The public health importance of the bacteria isolated was discussed, and a few suggestions as to how to protect the public from milk-borne diseases were offered.

REFERENCES

1. Abdel-Malek, Y. and Gibson, T. (1952). Studies in the Bacteriology of Milk. IV. The Gram Negative Rods in Milk. J. Dairy Res. 19, 294-301.

2. Abrahams, C.A. and Laryea, A.M. (1968). A preliminary investigation of the bacteriology of raw milk produced by cows on the Accra Plains. Ghana Med. J. 7, 100-103.

3. Andrey, J. Jr., and Frazier, W.C. (1959). Psychrophiles in milk held two days in farm bulk cooling tanks. J. Dairy Sci. 42, 1781-1784.

4. Atherton, H.V. (1959). A field study of the sanitary care of milking equipment on dairy farms. J. Milk Food Technol. 22, 273-278.

5. Ayers, S.H., Cook, L.B. and Clemmer, P.W. (1918). The four essential factors in the production of milk of low bacterial content. U.S. Dept. Agric. Bull. 642, 63.

6. Baltimore Biological Laboratory (BBL) Manual (1968). Products and Laboratory Procedures. Cockeysville, Maryland. 21030. U.S.A.

7. Barthel, C. (1906). K. Landbr. Akad. Handl. Och Tidskr. 45, 48-56 (Abstract in Expt. Sta. Rec, 1907) 18, 75.

8. Bamm, D.A. (1954). A herd outbreak of mastitis caused by Pasteurella multocida. Can. J. Comp. Med. Vet. Sc. 18, 113-119.

9. Bergey, D.H. (1904). Source and nature of bacteria in milk. Penn. Dept. Agr. Bull. 125, 1-40.

10. Breed, S.R., Murray, E.G.D. and Smith, N.R. (1959). Bergey's Manual of Determinative Bacteriology 7th Edition. Williams and Wilkins Co., Baltimore.

11. Brew, J.B. (1949). Sanitary milk control in retrospect. J. Milk Food Technol. 12, 193-195.

12. Briggs, M. and Briggs, C.A.E. (1954). The Lactobacilli: a review of the literature with special reference to taxonomy. Dairy Sci. Abstract. 16, 252-268.

13. Bryan, C.S. Bryan, H.S. and Mason, K. (1946). Heat-resistant bacteria from an unclean milking machine invade the udder of cow. Milk Plant Monthly 35, 30-32.

14. Collins, C.H. and Lyne, P.M. (1970). Microbiological Methods Butterworth Sc. Publ. London.

15. Covington, J.L., Egdell, J.W. and Thomas, S.B. (1953). Recent investigations on thermoduric organisms in milk. 13th Int. Dairy Congress (The Hague) Proc. 2, 139-147.

16. Crowdy, S.H. (1939). Notes on the contamination of milk by bacteria in the tropics. Trop. Agr. Trin. 16, 124-129.

17. Crowley, N., Bradley, J.M. and Darrell, J.H. (1969). Practical Bacteriology. Butterworths, London.

18. Cruickshank, R. (1970). Medical Microbiology. McGraw-Hill, London.

19. Cullen, G.A. and Hebert, C.N. (1967). Some ecological observations on micro-organisms inhabiting bovine, skin, teat canals and milk. Br. Vet. J. 123, 14.

20. De Filippis, V. (1941). The bacterial count of raw milk. (Some irreducible factors in the bacterial count and their seasonal incidence). Dairy Sci. Abstr. 3, 193-194.

21. Difco Manual (1965). Dehydrated culture media for Microbiological and Clinical Laboratory Procedures. 9th Edition. Difco Laboratories Inc., Detroit, Michigan.

22. Dorner, W. (1930). The bacterial flora of aseptically drawn milk. N.Y. (Geneva) Agri. Expt. Sta. Tech. Bull. Vol.165.

23. Economic Survey of Ghana (1969). Domestic Livestock Population. Central Bureau of Statistics, Accra.

24. Erdman, I.E. and Thornton, R.H. (1951). Psychrophilic Bacteria in Edmonton milk and cream. II. Kinds. Ca. Jour. of Technol. 29, 238-242.

25. Foster, E.M., Nelson, F.E., Speck, M.L. Doetsch, R.N. and Olson, C.J. (1958). Dairy Microbiology. MacMillan and Co. Ltd., London.

26. Gibson, T. and Abdel-Malek, Y. (1957). The development of bacterial populations in milk. Can. J. Microbiol. 3, 203-213.

27. Harrigan, W.F. and McCane, E.M. (1966). Laboratory Methods in Microbiology. Academic Press, London.

28. Heeres, H. (1950). The connection between the quality of milk and the judging of farm conditions (English Summary). Neth. Milk Dairy J. 4, 10-21.

29. Hunter, O.W. (1919). The colon-aerogenes group of milk. J. Dairy Sci. 2, 108-129.