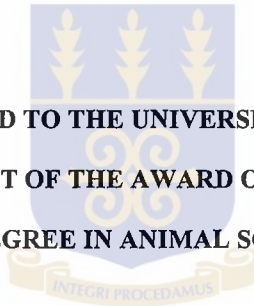


**A STUDY OF MILK QUALITY AND PUBLIC HEALTH HAZARDS
IN THE SMALLHOLDER PERI-URBAN DAIRY MARKETING
SYSTEM IN GHANA**

**BY
ERIC SAMPANE-DONKOR**

**A THESIS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN
PARTIAL FULFILLMENT OF THE AWARD OF MASTER OF PHILOSOPHY
DEGREE IN ANIMAL SCIENCE**



UNIVERSITY OF GHANA

AUGUST 2002

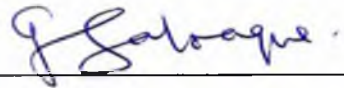
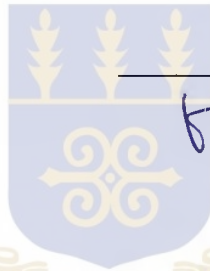
I, Eric Sampane-Donkor, author of this thesis entitled *A Study of Milk Quality and Public Health Hazards in the Smallholder Peri-Urban Dairy Marketing System in Ghana* do hereby declare that the research work presented in this thesis was done by me in the Department of Animal Science, Faculty of Agriculture, University of Ghana from August, 2000 to August, 2002.

This work has never been presented either in whole or in part for any other degree in this University or elsewhere.

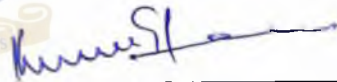


Eric Sampane-Donkor
(STUDENT)

This thesis has been presented for examination with our approval as supervisors.



for Prof. R.K.G Assoku
(MAJOR SUPERVISOR)



Dr. K.G. Aning
(CO-SUPERVISOR)

Dr. A. Omore
(CO-SUPERVISOR)

DEDICATION

This work is affectionately dedicated to my Lord and Saviour Jesus Christ, in whom is hidden all the treasures of wisdom and knowledge (Colossians 2:3).



To God whose grace and goodness made this work possible, I give thanks, praise, honour and glory.

I am greatly indebted to my principal supervisor Prof. R.K.G. Assoku for taking some time off his busy schedule to assist with the supervision of this work. His guidance, corrections, and advice have been of immense contribution to this work. My heart felt thanks also go to my co-supervisor Dr. K. G. Aning of the Animal Research Institute for the useful suggestions he offered me. I am also very grateful to my other co-supervisor, Dr. A. Omere of the International Livestock Research Institute (ILRI), Kenya for his guidance especially with the data analyses aspect of this work.

I acknowledge the technical advice from the following people. Mr. J. Quaye of the Animal Science Department, University of Ghana; Mr. F. K Kwarteng of the Animal Science Department, Kwame Nkrumah University of Science and Technology, and the entire staff of the Pathology Department of Animal Research Institute. I am also grateful to the entire staff of the Dairy Section of ILRI for the assistance they offered me during my period of stay there in connection with this work.

This thesis is an output of a research and development project funded by the UK Department for International Development (DFID) for the benefit of developing countries. I express my sincere gratitude to DFID for the financial assistance offered me, that enabled me carry out this work. Finally, I thank all the many other people who contributed in one way or the other to the success of this work.

	<u>Page</u>
DECLARATION.....	I
DEDICATION.....	II
ACKNOWLEDGEMENT.....	III
TABLE OF CONTENTS.....	IV
LIST OF TABLES.....	VI
LIST OF FIGURES.....	VII
ABSTRACT	VIII
CHAPTER ONE: INTRODUCTION.....	1
1.1 General introduction.....	1
1.2 Justification of the study.....	3
1.3 Objectives of the study.....	4
CHAPTER TWO: LITERATURE REVIEW.....	6
2.1 Microbial contamination of milk.....	6
2.2 Milk-borne zoonoses.....	12
2.2.1 Bovine tuberculosis.....	12
2.2.2 Bovine brucellosis.....	15
2.2.3 <i>Escherichia coli</i> 0157:H7.....	18
2.3 Major public health risks to consumers of milk.....	19
2.3.1 Pathogenic bacteria.....	19
2.3.2 Drug residues.....	20
2.3.2.1 Detection methods for drug residues.....	22
2.3.3 Aflatoxins.....	23
2.3.4 Adulteration of milk.....	25

CHAPTER THREE: MATERIALS AND METHODS space.ug.edu.gh	27
3.1 The study area.....	27
3.2 Data and sample collection.....	28
3.3 Preparation of laboratory media/reagents.....	29
3.4 Laboratory analyses.....	32
3.4.1 Detection of antimicrobial agents in milk by Charm Aim Test.....	33
3.4.2 Evaluation of bacteriological quality of milk by total and coliform bacteria plate counts.....	33
3.4.3 Examination for bacterial types in milk — cultural, microscopic and biochemical examinations.....	34
3.4.4 Detection of <i>Brucella abortus</i> antibodies in milk by the Milk Ring Test.	36
3.4.5 Determination of specific gravity and butterfat content of milk samples.....	36
3.5 Data analyses.....	37
CHAPTER FOUR: RESULTS (WITH DISCUSSIONS).....	39
4.1 Study 1: An evaluative study of the public health hazard of antimicrobial agents in marketed milk.....	39
4.2 Study 2: A study of the bacteriological quality and bacterial flora of milk samples.....	46
4.3 Study 3: A study of the compositional quality and adulteration of milk samples.....	61
4.4 Study 4: Evaluative study of the milk handling and hygienic practices of milk market agents.....	67
CHAPTER FIVE: GENERAL DISCUSSION AND CONCLUSIONS.....	74
5.1 Hygiene and handling of milk.....	74
5.2 Estimation of risk to consumers of milk.....	75
CHAPTER SIX: REFERENCES.....	79
CHAPTER SEVEN: APPENDICES.....	98

Table 1: Bacterial diseases transmissible to man through milk.....	2
Table 2: Comparison of plate counts on hand draw milk and machine drawn milk.....	8
Table 3: Pathogens found in milk: Their pathogenicity and diseases caused.....	10
Table 4: Prevalence of bovine tuberculosis in Ghana and some West African countries.....	14.
Table 5: Serological test results of bovine brucellosis in Ghana and some West African countries by Serum Agglutination Test, Rose Bengal Test, and Complement Fixation Test.....	17
Table 6: Market agents surveyed and sampled in Accra and Kumasi.....	28
Table 7: Milk samples collected by product type.....	29
Table 8: Effect of season on contamination of milk with antimicrobial agents for Accra and Kumasi.....	44
Table 9: Effect of season on total bacteria plate counts of raw milk for Accra and Kumasi.....	52.
Table 10: Means of coliform bacteria plate counts and total bacteria plate counts of raw and processed milk.....	52
Table 11: Prevalence of bacteria types in milk from Accra and Kumasi.....	54
Table 12: Overall means of butterfat, specific gravity, total solids, and solids-not-fat. of milk samples.....	62
Table 13: Effect of season on adulteration of milk with water for Accra and Kumasi....	65
Table 14: Milk marketing channels in Accra and Kumasi.....	69
Table 15: Refrigeration and heat-treatment of milk practised by market agents.....	70
Table 16: Container types used by market agents for marketing milk in Accra and Kumasi.....	70

Figure 1: Proportion of milk samples contaminated with antimicrobial agents in the dry and wet seasons.....	42
Figure 2: Proportion of milk samples contaminated with antimicrobial agents for Accra and Kumasi.....	43
Figure 3: Proportion of raw milk samples with unacceptable total bacteria plate counts in the dry and wet seasons.....	50
Figure 4: Proportion of raw milk samples with unacceptable total bacteria plate counts for Accra and Kumasi.....	51
Figure 5: Proportion of milk samples adulterated with water in the dry and wet seasons.....	63
Figure 6: Proportion of milk samples adulterated with water for Accra and Kumasi.....	64

Dairying is a young and developing industry in Ghana, and one of the major problems it faces is the low demand of the milk produced. This is partly due to public concerns over the safety and quality of the milk produced, and also the hygienic practices of informal agents. However, the extent of risks posed to human health by consumption of milk and dairy products in the country is not well documented. This project was therefore carried out to study the quality and the public health hazards inherent in marketed milk in Ghana. The study was conducted at two sites, namely Accra (Peri-urban Accra) and Kumasi (Peri-urban Kumasi) in Ghana. The areas or districts were chosen within these sites to represent a variation in consumer concentration, market access and dairy production intensity within each site. Data related to milk handling, and other market factors that affect milk quality, or pose a risk to public health were collected during the dry and wet seasons (1999-2000) from respondents selected by a stratified random sampling within each area. Milk samples were also obtained from the respondents for laboratory analysis for their quality and health risks. These involved the assessment of their bacteriological quality, determination of the bacterial flora, the evaluation of contamination with antimicrobial agents, and assessment of the compositional quality and adulteration. Laboratory data and the market level data were analysed to assess the milk quality and milk-borne health hazards, and their relationship with season, site, and marketing factors, including the handling and hygienic practices of market agents.

Overall, 35% of milk samples were found to be contaminated with antimicrobial agents, and three factors were found to significantly influence the contamination of milk with antimicrobial agents. These were site, type of milk trade practiced, and source of milk.

The most potent of these factors was milk source: milk sourced from the farm was found to be strongly associated ($p < 0.01$) with high levels of antimicrobial agents compared with other sources. The milk traded in Accra was associated with high levels of antimicrobial agents ($p = 0.04$), and likewise milk handled by farmers or producers ($p = 0.06$).

The mean coliform bacteria plate counts for raw and processed milk were $3.7 \log_{10}$ cfu/ml and $3.5 \log_{10}$ cfu/ml respectively, while both types of milk had the same mean total bacteria plate count ($5.9 \log_{10}$ cfu/ml). Bacteriological quality of milk was also significantly associated with milk pathways, type of milk trade, type of milk container, and mode of cleaning containers. High total bacterial counts were associated with use of gravels ($p = 0.04$) compared with sponge for cleaning milk containers. High coliform counts were strongly associated with milk associated with wholesalers ($p < 0.01$), and two milk pathways namely, Herdsman_Processor (milk sourced from herdsman to processor) ($p = 0.03$) and Trader/hawker_Retailer (milk sourced from trader/hawker to retailer) ($p = 0.04$). Plastic containers ($p = 0.06$), and the use of soap ($p = 0.06$) compared to detergent for cleaning containers, were also associated with high coliform counts.

In terms of compositional quality, the means of butterfat, specific gravity, solids-not-fat, and total solids of milk samples determined were 3.25%, 1.030kg/litre, 8.833%, and 12.078% respectively. All the means determined fall within the expected ranges of the

respective components or properties of normal bovine milk. On the whole, 18% of milk samples were found to be adulterated with water. Generally, adulteration was significantly higher in the wet season ($p < 0.10$) than the dry season.

Overall, the bacterial flora of milk sampled consisted of eight different potentially pathogenic bacteria, which were identified by culture or serology. The organisms so identified were *Escherichia coli*, *Brucella abortus*, *Klebsiella* spp., *Yersinia* spp., *Mycobacterium* spp., *Proteus* spp., *Bacillus* spp. and *Staphylococcus* spp. The bacterial flora of milk from Kumasi consisted of only the first four organisms enumerated above, while *Klebsiella* spp. was the only organism absent in milk from Accra. It was also observed that generally, a higher prevalence of bacteria was associated with milk samples from Accra than those from Kumasi, clearly indicating that milk from Kumasi was of better quality than those from Accra.

The high level of contamination of milk with various antimicrobial agents has obviously very serious public health implications, since antimicrobial agents have been implicated in various health problems like drug resistance and hypersensitivity. Similarly, the presence of pathogenic microorganisms in milk is a cause for great concern as several of the organisms identified have been implicated in many human disease outbreaks. Adulteration of milk with water observed also requires attention as this practice could introduce several health risks into milk produced and marketed in Ghana.

INTRODUCTION

1.1 General introduction

Milk and milk products have been used by man since prehistoric times, and because nearly all the constituents of milk are known to be essential for life, milk is referred to as nature's single most complete food (Frei, 1993). Milk also has an extremely high nutritional quality, serving several functions, including growth, supply of energy, reproduction, maintenance and repairs, and appetite satisfaction (O'Connor, 1994). However, there are also several health hazards that may affect its quality and pose a threat to public health. Pathogenic microorganisms constitute an important public health threat in food, and are usually introduced into milk through poor hygienic handling (Foster, 1990). Several of them have been identified in milk, some of which have been associated with disease outbreaks worldwide (Table 1) (WHO, 1962). As efforts are made to control milk-borne pathogens, new and emerging ones continue to evolve (Ryser, 1998). Additionally, drugs used in the treatment of animal diseases and various synthetic organic chemicals used in farming situations may occur in milk, resulting in many public health problems such as drug resistance and allergies (Aibara, 1983; Prescott and Baggot, 1993). Under situations where milk is adulterated, several microbial and chemical hazards could be introduced into it (O'Connor, 1994). Such interference also reduces the compositional, nutritional, and processing quality of milk as well as the economic value (Omore *et al.*, 2001). To protect public health against milk-borne health hazards, there are regulations that require proper hygienic handling of milk, its pasteurisation, and adherence to standard veterinary recommendations concerning drugs and chemicals.

Table 1: Bacterial diseases transmissible to man through milk

DISEASE	PRINCIPAL SOURCES OF INFECTION		
	Man	Animal	Environment
Cholera	X		
Diphtheria	X		
Shigellosis	X		
Typhoid fever	X		
Pathogenic <i>E. coli</i> Infection	X	X	
Paratyphoid Fever	X	X	
Salmonellosis	X	X	
Staph. Entero. Gastroenteritis	X	X	
Streptococcal Infections	X	X	
Tuberculosis	X	X	
Anthrax		X	
Brucellosis		X	
Leptospirosis		X	
Listeriosis		X	
Botulism (toxin)			X
<i>Clostridium perfringens</i> Infections			X
Enteritis (non-specific)			X
Rat-bite fever			X

(Source: WHO, 1962)

However, such regulations are not usually adhered to, especially in developing countries (Ombui, 1994; FAO/WHO, 1970; Ryser, 1998). Consequently, milk-borne health risks tend to be higher in developing countries than in the developed world (FAO/WHO, 1970; Ryser, 1998).

Informal milk markets have been found to account for over 80% of milk sales in most sub-Saharan African countries (Omore *et al.*, 2001), and earlier studies have shown that consumers enjoy convenient delivery and lower prices from these informal milk markets (Omore *et al.*, 1999). However, there are regulations to discourage such markets for public health reasons, though these regulations are not generally implemented in many developing countries (Kang'ethe *et al.*, 2000). In Ghana, at present there is a growing urban and peri-urban population, which represents a potentially expanding market for milk and milk products. Consumers prefer imported processed milk to locally produced milk though the latter is cheaper (Dei, 1996). This may be partly due to public concerns over the quality and safety of locally produced milk (Chamberlain, 1989). Despite these preferences however, there is also evidence of demand for locally produced milk (Osafu *et al.*, 1999).

It is submitted that if efforts are made to produce safe and good quality local milk, it would not only protect public health, but also attract high patronage and stimulate growth of the dairy industry in Ghana.

1.2 Justification of the study:

Dairying is a young and a developing industry in Ghana, and much of the milk and milk products consumed are imported (Karikari *et al.*, 1998). Imported milk and milk products are now very expensive, with most people unable to afford them. Quite clearly, increasing local

milk production would be a good step to increasing consumption of milk, especially among the poor on a sustainable basis. In Ghana, local milk production is low, which is estimated at about 26,650 metric tonnes per year (Agyeman, 1998). At present, the bulk of the milk produced in the country is produced by the indigenous "Fulani" herdsman, using a technology untouched by time and science (Abraham and Laryea, 1968). Demand for their milk is low, partly because of public concerns over its safety and quality, including the hygienic practices of informal milk marketing agents (Karikari *et al.*, 1998). The extent of risks posed by consumption of milk and dairy products produced in the country to human health is also not well documented (Aning, 1999), and a study into the probable milk-borne health hazards encountered by consumers, and appropriate recommendations to address this problem is therefore necessary and long overdue.

This study is being carried out as part of an inter-country, interdisciplinary dairy systems research by The International Livestock Research Institute (ILRI) (Nairobi, Kenya) in several tropical and subtropical countries of Africa. The research takes a producer to consumer approach, including public health, processing, and marketing components, and their interactions.

1.3 Objectives of the study:

Overall objective of this research project was to study milk quality and public health risks inherent in the smallholder peri-urban dairy marketing system of Ghana.

Specific objectives however, were to:

- (i) determine the extent of, and evaluate the public health hazards of antimicrobial agents or drug residues in marketed milk.
- (ii) identify bacterial types, and evaluate their public health importance in milk.

- (iii) evaluate the milk handling and hygienic practices of milk market agents, and to demonstrate, if any, their effects both on the milk quality and public health.
- (iv) assess the bacteriological quality of milk samples collected.
- (v) assess the compositional quality, and the extent of adulteration of milk samples.

LITERATURE REVIEW

2.1 Microbial contamination of milk

Microorganisms are widely distributed in nature, and it is conceivable that they may be introduced into milk from a variety of sources (Adams and Moss, 1995). At one time it was generally accepted that milk as it was removed from the udder contained no bacteria (O'Connor, 1994). However, since the work of Ward (1900), it has been established that, in many instances the udder of the cow is a natural habitat for some bacteria. Other sources of contamination include milking utensils, dairy workers, the milking environment, soil, and cow dung (Aibara, 1983). Unless given adequate care, milking utensils and equipment may be the most important contaminating source (Hammer and Babel, 1957; Minja, 1998). Bacteriological quality of milk reflects the extent of milk contamination and also the keeping quality (Ombui *et al.*, 1995). Tests used to evaluate bacteriological quality include total bacteria counts and dye reduction tests using resazurin and methylene blue, and bacteria count of more than $5 \log_{10}$ cells per ml is evidence of poor hygiene (Adams and Moss, 1995; Ombui *et al.*, 1995).

Bacteriological quality of milk has been well documented over the years, and the work of several researchers indicate that bacteria counts in milk can be highly variable, ranging from zero to very high counts. Bergey (1904) had reported that 32% of 272 samples of milk drawn into sterile tubes contained no bacteria per ml., whilst 48.8% contained less than $2.7 \log_{10}$ bacteria per ml., and only 10.3% contained more than $3.7 \log_{10}$ bacteria per ml. Later, Dorner (1930) examined 933 samples drawn aseptically from 132 cows in 6 herds, and the herd counts ranged from $2.7 \log_{10}$ to $3.6 \log_{10}$ per ml. averaging $3.4 \log_{10}$ per ml. An average

bacterial count of $2.7 \log_{10}$ to $3 \log_{10}$ per ml. was reported by Foster and others (1958), while Omore *et al.* (2001) found that milk sampled from market agents had a mean count of $6.6 \log_{10}$ per ml.

Verma *et al.* (1944) found that milk produced under different conditions showed differences in bacterial counts, though they did not say how significant these differences were. Thus the farms with better milking conditions showed low counts. Similar observations have been reported in Ghana by Abraham and Laryea (1968) and Ayebo *et al.* (1976). The former found that the University of Ghana Agricultural Research Station situated on the Accra Plains had a mean bacterial count of $4.3 \log_{10}$ per ml., while the Fulani kraals also on the Accra Plains, had approximately a mean of $6.5 \log_{10}$ bacteria per ml. The latter found that the University of Ghana Agricultural Research Station had a mean count of $4.8 \log_{10}$ per ml., whilst the Ministry of Agriculture Dairy Farm also situated on the same plains had a mean count of $4.4 \log_{10}$ per ml.; the Fulani Kraals on the Accra Plains had a mean count of $5.5 \log_{10}$ per ml. McKenzie and Bowie (1946) had however observed that milk from certain farms, with seemingly unsatisfactory production conditions, were consistently graded as satisfactory, whereas milk from other farms with visually observable good conditions, 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. Preliminary statistics from the work of Covington *et al.*, (1953) 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 building and equipment are clean. Two methods of milking are in use, namely, hand milking and machine milking and they present somewhat different bacteriological problems. With hand milking, the external sources from which micro-organisms are added to milk include

stable air, coat of the animal, milk equipment, milker and other miscellaneous sources but with machines contamination from these sources is less important (Hammer and Babel, 1957). In general however, machines have refined the methods of producing milk but unless properly cleaned and sanitised, machines are an important source of microorganisms (Foster *et al.*, 1958). Table 2 shows comparisons of the bacteria counts on milk obtained by hand and by machine under defined conditions. The data shows that the counts commonly were higher with machine milking (Hammer and Babel, 1957).

Table 2: Comparison of plate counts on hand drawn milk and machine drawn milk

Number of Trials	Method of Milking	Bacteria per Millilitre of Milk
94	Hand	2.8 log ₁₀ to 4.8 log ₁₀
235	Machine	4.9 log ₁₀ to 4.3 log ₁₀
136	Hand	< 3 log ₁₀ (19.8%) 3 log ₁₀ to log ₁₀ 3.7 (44.1%) > 3.7 log ₁₀ (36.1%)
150	Machine	< 3 log ₁₀ (22.0%) 3 log ₁₀ to 3.7 log ₁₀ (55.3%) > 3.7 log ₁₀ (22.7%)
6	Hand	3.3 log ₁₀ to log ₁₀ 4.8
7	Machine	5.3 log ₁₀ to 6.1 log ₁₀
46	Hand	4.3 log ₁₀ (average)
45	Machine	4.7 log ₁₀ (average)
20	Hand	4.2 log ₁₀ (average)
20	Machine	5.8 log ₁₀ (average)
38	Hand	4 log ₁₀ (average)
38	Machine	5.7 log ₁₀ (average)

(Source: adapted from Hammer and Babel, 1957)

The important microorganisms of milk and milk products are the bacteria of the sub-order *Eubacteriaceae*, viruses of the order *Virales*, rickettsiae of the order *Rickettsiales*, yeasts and moulds (Foster *et al.*, 1958). Bacteria are the most encountered group of microorganisms in milk. The species of bacteria found in milk as it comes from the udder are limited to a few genera, the micrococci are generally present in the greatest proportion, followed by streptococci and rods primarily of the diphtheria type (O'Connor, 1994). However, many other microorganisms may get into the milk as a result of contamination from external sources. Thus a broad spectrum of microorganisms can be present in milk. These organisms play a role either in the spoilage of milk (Walter, 1967), in disease outbreaks, or in milk processing (O'Connor, 1994). Pathogenic microorganisms constitute an important group of microorganisms in milk due to their association with dairy-related disease outbreaks. Seaman (1963) had reported that the typical primary pathogens of milk are *Corynebacterium pyogenes*, *Streptococcus agalactiae* and *Staphylococcus aureus*, while *Mycobacterium tuberculosis* and *Brucella abortus* might be excreted in milk when the body had been extensively invaded. Salle (1967) reported that tuberculosis, food poisoning, infantile diarrhoea, poliomyelitis, septic tonsillitis, and typhoid fever, are among the numerous human diseases whose organisms survive in and are transmitted by milk. Vasavanda and Cousin (1992) had also reported on the pathogens found milk and dairy products, their pathogenicity, and diseases caused (Table 3). The pathogenicity of these pathogens is mediated mainly by toxins and enzymes. While enzymes are easily destroyed by heat, some of the toxins are heat stable and can still pose health risk even after milk is pasteurised (Ryser, 1998).

Several microorganisms have been associated with milk contamination in Ghana. Abraham and Laryea (1968) had reported the presence of several microorganisms in milk from the

Table 3: Pathogens found in milk and dairy products: Their pathogenicity and diseases caused.

Pathogens	Gram Stain	Pathogenicity	Diseases
<i>Listeria monocytogenes</i>	Positive	β . Listeriolysis lipase	Meningitis, Infectious abortion, Perinatal septicemia and encephalitis
<i>Bacillus cereus</i>	Positive	Heat-labile Diarrhoeal toxin, Enterotoxin and Heat stable emetic Enterotoxin	Emetic and Diarrhoegenic toxins
<i>Campylobacter jejunum</i>	Negative	Heat-labile Enterotoxin, Cytotoxin, Colonization, Invasiveness	Mastitis in cows, Mild enteritis/ Severe enterocolitis
<i>Escherichia coli</i>	Negative	Invasiveness, Heat-labile and Heat-stable Enterotoxins, Verotoxins	Haemorrhagic Colitis/blood Diarrhoea
<i>Salmonella spp</i>	Negative	Invasiveness, Heat-labile, Enterotoxin, Heat-table cytotoxin	Diarrhoea, abdominal cramps, vomiting and fever within 24 hours after consuming the contaminated food
<i>Staphylococcus aureus</i>	Positive	Seven enterotoxins (A, B, C ₁ , C ₂ , C ₃ , D and E), somewhat resistant to heat and proteolytic Enzymes	Vomiting, cramps and diarrhoea
<i>Yersinia enterocolitica</i>	Negative	Plasmid-mediated, (UHT) enterotoxin virulence	Gastroenteritis, mesenteric lymphadenitis, terminal ilectis and mimic acute appendicitis

(Source: Vasavanda and Cousin, 1992)

University of Ghana Agricultural Station and a Fulani kraal. These included *Escherichia coli*, *Staphylococcus spp.*, *Pseudomonas pyocyaneus*, and other groups of organisms including diphtheroids, Micrococcus, and yeast like cells. Generally, higher prevalence of the organisms were associated with milk from the Fulani kraal, while *E. coli* and *Pseudomonas pyocyaneus* were absent in milk from the University farm. They attributed this to poor hygiene of milking practiced at Fulani Kraals. Later, Ayebo *et al.* (1976) encountered a more extensive spectrum of microorganisms in milk samples obtained from the University of Ghana Agricultural Research station, the Ministry of Agriculture Dairy Farm, and a Fulani kraal. Generally, all the organisms reported by Abraham and Laryea (1968) had also been identified in this study. However, several additional microorganisms in milk had also been identified by these workers, which included *Brucella abortus*, *Corynebacterium spp.*, *Aerobacter spp.*, *Pasteurella spp.*, *Lactobacillus spp.*, and *Bacillus spp.* Milk from the Fulani kraal was associated with the highest prevalence of the various organisms, an observation they also attributed to poor hygiene of milking practised at Fulani kraals. In a recent study, Akyeh (2000) encountered several microorganisms in milk from a Fulani Kraal and the Ministry of Agriculture Dairy farm that had not been observed by the previous workers. These organisms include *Proteus spp.*, *Yersinia spp.*, *Enterobacter spp.*, *Citrobacter spp.*, *Salmonella spp.*, and *Erwinia spp.* Aning *et al.* (2002) examined milk from Accra and Kumasi for the following pathogens: *E. coli* O157:H7, *Brucella abortus*, and *Mycobacterium bovis*. With the exception of *Mycobacterium bovis*, all the other organisms were present in milk from the two sites. It is interesting to note that *Mycobacterium spp.* had also not been encountered in any of the work in Ghana mentioned above. However, high prevalence of *Mycobacterium bovis* has been reported in cattle in certain areas in the country (Aning, 1999).

2.2 Milk-borne zoonoses

Zoonoses are defined by World Health Organisation (WHO) as infections that are naturally transmitted between vertebrate animals and man. It encompasses a broad range of agents, from viruses to macroparasites. There are several zoonotic pathogens transmissible through milk to man (Table 1). Of these, the most widely reported in Africa are *Brucella* spp and *Mycobacterium bovis* (Chukwu, 1985; WHO, 1994). *Escherichia coli* O157:H7, a newly recognised zoonosis is gaining in importance as a milk-borne public health hazard (Ombui *et al.*, 1994; Ryser, 1998).

2.2.1 Bovine tuberculosis:

Mycobacterium tuberculosis (*M. tuberculosis*) is the most common cause of human tuberculosis, whereas *Mycobacterium bovis* (*M. bovis*) causes bovine tuberculosis, and is the agent of milk-borne tuberculosis (Ryser, 1998). Although it is less virulent than *M. tuberculosis*, *M. bovis* is an important cause of human tuberculosis. Cattle rarely become infected with *M. tuberculosis*, but they are susceptible to *M. avium* (McInerney *et al.*, 1995).

(a) Epidemiology of bovine tuberculosis:

The infected animal is the main source of infection. Organisms are excreted in the exhaled air, the sputum, faeces, milk, and urine (Minja, 1998). Infection of calves with *M. bovis* commonly occur through ingestion of contaminated milk of dams (Evangelista and Anda, 1996) although foetuses may be infected in utero (Blood *et al.*, 1983). Older animals usually acquire infection by ingestion usually through pasture, feed, and water contaminated with the organism shed in the faeces of infected cattle (Fraser and Mays, 1986). Inhalation is almost invariably the portal entry in housed cattle and even in those at pasture (Minja, 1998).

Housing predisposes animals to the disease (Blod/engapad@u.ug.edu.gh, 1983) as does congregation at watering-points (Bonsu, 1998).

M. bovis infection in humans is mainly a result of consumption of unpasteurised milk contaminated with the organism (O’Rielly and Daborn, 1995), but does also occur through inhalation (WHO, 1993). Transmission through consumption of infected meat does occur, but it is considered to be a rare event as cooking will easily destroy the causative agent (Kleeberg, 1984). Housing cattle in poor ventilated houses together with people is another mode of transmission from cattle to man (Minja, 1998)

The prevalence rates of bovine tuberculosis in Ghana and some West African countries are reported in Table 4 (Aning, 1999). Very high prevalence rates were reported for Cote d’Ivoire (50%) and Nsawam (60%) in Ghana. In Ghana, bovine tuberculosis seemed to be rather high at the Transitional zone (19% and 60%) compared to the Coastal Savanna (1–13.8%) and Accra Plains (7.0%). Though the overall prevalence in Nigeria was relatively low (0.2%), Northern Nigeria had a prevalence rate of 2.5%.

(b) Control and prevention of bovine tuberculosis:

The control of tuberculosis in cattle must be part of the overall strategy for controlling the disease in man (Edelston, 1995). In developed countries, tuberculosis was virtually eliminated in cattle by the end of the second world (Vasavanda and Cousin, 1992). For example in 1979, only 0.18% of the herds in Great Britain were found positive for tuberculosis (Collins and Grange, 1983). Eradication of bovine tuberculosis in developed countries was achieved through systematic slaughter of tuberculosis-positive animals in the herds (Ryser, 1998) and vaccination using Bacillus Calmette Guerin (BCG) *M. bovis* vaccine

strains (Edelston, 1995). However, vaccination of cattle using the BCG vaccine has been considered in some countries such as Malawi (Edelston, 1995) but with little successful results (WHO, 1993). Treatment of infected cattle with *M. bovis* using Isomazid is considered inappropriate, mainly because of the danger of emergence of drug-resistance strain (WHO, 1994). However, it was used in combination with the test and slaughter policy

Table 4: Prevalence of bovine tuberculosis in Ghana and some West African countries.

Country	Location	Prevalence (%)	Source
Ghana	Accra Plains <i>Coastal Savanna</i>	7.0	VSD, 1997
	Katamanso	2.6	ARI, 1997
	Nungua	1.0	Bonsu, 1998
	Everytime	4.0	Bonsu, 1998
	Dangbe West	13.8	Bonsu, 1998
	Volta Region <i>Transitional Zone</i>	1.0	VSD, 1997
	Pokoase	19.0	ARI, 1997
	Nsawam	60.0	Bonsu, 1997
	Nigeria	Northern Nigeria	2.5
National		0.2	Alonge & Ayanwale, 1984
Cote d'Ivoire		50.0	WHO, 1993

(Source: adapted from Aning, 1999)

to successfully control bovine tuberculosis in a herd of cattle in Brazil (Sorensen *et al.*, 1993). The test and slaughter policy is the most successful method of controlling bovine tuberculosis (Blood *et al.*, 1983; WHO, 1993; WHO, 1994; Ryser, 1998). However, owing to its high cost, developing countries are unable to implement it fully (WHO, 1993). Its application is obstructed by other factors such as limited resources, poorly equipped laboratories, inadequate infrastructure and other pressing disease priorities (WHO, 1994).

Pasteurisation is effective in the control of *M. bovis* infection in humans. Compulsory pasteurisation by the high, short time or Vat pasteurisation is highly effective in killing *M. bovis* (Ryser, 1998).

2.2.2 Bovine brucellosis

Brucellosis is caused by organisms of the genus *Brucella*, and *Brucella abortus* is usually the species involved in bovine brucellosis (Berman, 1981). *Brucella* organisms are small, non-motile, non spore forming rods or coccobacilli growing singly or in small groups (Berman, 1981). Nine biotypes of *B. abortus* are recognised (Blood *et al.*, 1983). Chukwu (1985) reported that biotypes 1,2,3,4,5,6,7,8, and 9 are present in Africa. However only biotypes 1,2,3 and 4 have been identified in Nigeria (Eze, 1978; Bale and Kumi-Diaka, 1981) and biotypes 1,6 and 8 in Cote d'Ivoire (Pile-Moron *et al.*, 1979). There appears to be no information on the biotypes in Ghana (Aning, 1999).

(a) Epidemiology of bovine brucellosis

The major mode of transmission from cattle to cattle is by ingestion of contaminated fodder or feeding stuff and water, penetration of the intact skin or conjunctiva and contamination of the udder during milking (Blood *et al.*, 1983). The disease is also transmitted through

infected placentae and aborted foetuses, which contaminate the environment (Berman, 1981).

Infected bulls used for artificial insemination can be the cause of introducing widespread infection into a clean herd (Bale and Kumi-Diaka, 1981; Fraser and Mays, 1986)

The major mode of transmission of brucellosis from cattle to humans is consumption of unpasteurised milk and dairy products, uncooked or undercooked contaminated meat (Blood *et al.*, 1983). The disease could also be transmitted through contact with infected animals (Buxton and Fraser, 1977). *Brucella* species have been isolated from raw milk, and local cheese from milk of cows and goats (Ayebo *et al.*, 1976; Eze, 1978). Regular consumption of these products has made human brucellosis endemic in many parts of the world (Stiles, 1989 cited by Aning, 1999). This may be true in Africa, too (Eze, 1978).

The prevalence rates of bovine brucellosis in Ghana and some West African countries are given in Table 5 (Aning, 1999). In Ghana, it appears studies on brucellosis have focused in the south (Accra Plains) where prevalence rates as high 55.3% have been reported. In Nigeria, the prevalence of bovine brucellosis appears to be higher in Southern Nigeria (68.7% and 1.5-60%) than the Northern part (3.6-7.1%). In the other West African countries, prevalence rates ranging from 6.5% (Guinea) to 41.0% (Togo) have been reported.

(b) Control and prevention of brucellosis in cattle and humans.

Identification and slaughter or segregation of carriers removes them as a source of infection, and vaccination both protects the susceptible and reduces spread by lowering the abortion rate (Berman, 1981). The brucellosis control method of combining vaccination with test and elimination has been very successfully applied in many countries in Europe, the USA and New Zealand (Blood *et al.*, 1983; O'Neil, 1996). Efforts are being made towards eradication

Table 5: Serological test results of bovine brucellosis in Ghana and some West African countries by Serum Agglutination Test (SAT) Rose-Bengal Plate Test (RBPT) and Complement Fixation Test (CFT).

Country	Prevalence (%)	Reference
Togo	41.0	Akakpo <i>et al</i> , 1981
Benin	10.4	Akakpo <i>et al</i> , 1984
Cote d' Ivoire	10.8	Pile-Moron <i>et al</i> , 1979
Guinea	6.5	Diallo, 1994
Mali	19.7(5.3-35.9)	Maiga <i>et al</i> , 1996
Ghana, Accra Plains		
South	23.47	Oppong, 1966
South	55.3	VSD, 1997
Nigeria, Northern	3.6-7.1	Nuru & Dennis, 1975
Southern	68.7	Falade <i>et al</i> , 1981
National	3.0-50.0	NVRI, 1983
Southern	1.5-60.0	Esuoroso, 1974

(Source: adapted from Aning, 1999)

in some African countries. Control of brucellosis in West Africa does not seem to have received much attention beyond the prevalence studies reviewed above (Aning, 1999). Prevention of brucellosis in man will depend upon successful control in animals, mandatory pasteurisation of milk and ageing of cheese (Ryser, 1998). These practices have drastically reduced the number of brucellosis cases in certain developed countries such as the United States (Ryser, 1998).

2.2.3 *Escherichia coli* O157:H7:

E. coli O157:H7 infection is a newly recognised bacterial zoonosis that poses serious threat to human health. The organism causes haemorrhagic colitis leading to bloody diarrhoea, haemolytic ureamic syndrome, and kidney damage (Jay, 1992; Besser *et al.*, 1993).

(a) *Epidemiology of Escherichia coli* O157:H7

Cattle appears to be the main reservoir of *E. coli* O157: H7 although this pathogen has been isolated from other meat sources including pork and poultry (Riley *et al.*, 1983). *E. coli* O157:H7 resides in the intestinal tracts of live animals, and are shed in the faeces that may contaminate food, water, and the environment. Transmission to human is associated with consumption of a number of contaminated foods: meat, especially undercooked ground beef, raw milk, yoghurt, salamis, cheese, and unpasteurised apple cider (Doyle and Shoen, 1984; Doyle, 1991; Riley *et al.*, 1983). Transmission of the organism from person to person can occur via faecal-oral route in close personal setting such as families, nursing homes, and day care centres (Doyle, 1991). Cross contamination of contaminated food products such as raw meat to ready-to-eat products may also occur (Doyle, 1991). The first publicised outbreak of *E. coli* O157:H7 occurred in 1982, when many people developed bloody diarrhoea after eating hamburger from a fast food restaurant (Riley *et al.*, 1983). Since then several outbreaks have occurred in several countries. In the United States, since 1982, more than 25 outbreaks incriminating *E. coli* O157:H7 have occurred (Doyle, 1991). In June 1996, the largest outbreak occurred in Japan in which 9000 people, became sick and 10 died (Doyle, 1991). Contaminated radish sprouts were incriminated as one of the sources of infection.

The risk of acquiring *E. coli* O157:H7 through ingestion of raw milk is well documented. Bleem (1994) cited by Ryser (1998) reported on two separate raw milk-related outbreaks,

which occurred in Oregon during 1992 and 1993 <http://the-first-of-its-kind-outbreaks>, *E. coli* O157:H7 infections developed in 9 individuals aged 9 months to 73 years after consuming raw milk. Testing the entire herd of 132 animals revealed 4 cattle as positive for *E. coli* O157:H7. Furthermore, strain-specific typing demonstrated that 6 of the 9 human isolates were identical to the 4 bovine strains. In the second raw milk-related outbreak, 5 cases of *E. coli* O157:H7 were identified, including 2 cases of HUS. Subsequent faecal sampling of the entire herd of 60 animals revealed *E. coli* in 4 postweaned heifers. Subtyping again demonstrated that the cattle and human isolates were identical.

(b) Prevention and Control of Escherichia coli O157:H7

Given the probability for contamination of milk during milking, consumption of raw milk should be avoided (Ryser, 1998). However, if milk is properly pasteurised, it poses little risk because *E. coli* O157:H7 is readily inactivated during high-temperature, short time pasteurisation (D'Aoust *et al.*, 1988). Raw milk cheeses and soft-ripened cheeses should be prepared or aged properly so that they do not pose public health concern, as the organism is reasonably acid tolerant (Ryser, 1998).

2.3 Major public health risks to consumers of milk:

2.3.1 Pathogenic bacteria:

The presence of pathogenic bacteria in milk has been a matter of public health concern since the early days of the dairy industry (Vasavanda and Cousin, 1992). Bacteria are responsible for more than 90% of all reported cases of dairy-related illness, while other groups of microorganisms including rickettsiae, parasites, and viruses are each generally responsible for less than 1% (Ryser, 1998). The first important evidence of spread of pathogenic microorganism through milk was obtained from Michael Taylor of Penrith, England in 1857

(Hammer and Babel, 1938). He reported that 13 cases of typhoid fever among 7 rural families were due to contaminated milk. Since then milk has been implicated in a number of disease outbreaks worldwide. For example between 1919 and 1948, consumption of raw milk was epidemiologically linked to 11 diphtheria outbreaks in the United States (Bryan, 1979) with 3 similar outbreaks also reported in Australia (Bryan, 1979) during this period. In most of these outbreaks, dairy workers who either exhibited active infections or carried *Corynebacterium diphtheria* (the causative agent) asymptotically were assumed to have contaminated the milk during milking or subsequent handling.

Public health concerns impacting the dairy industry continue to change in response to advances in sanitation, milk handling and animal husbandry practices. Ryser (1998) noted that common Pre-World War II milk-borne illnesses such as diphtheria, scarlet fever, tuberculosis, and typhoid fever in industrialised countries no longer pose a threat to consumers, but have been replaced by more immediate concerns such as staphylococcal poisoning, salmonellosis and campylobacteriosis based on their continued high incidence since the early 1980s. Although responsible for comparatively few out-breaks of illness, several recently identified milk-borne pathogens including *E. coli* O157:H7, *Listeria monocytogenes*, *Yersinia enterocolitica* and *Clostridium botulinum* have received widespread attention because of the particularly severe or fatal complications produced by these organisms (Foster, 1990; Ryser, 1998).

2.3.2 Drug Residues

Several countries have introduced financial penalties for farms that produce milk with antimicrobial residues (Prescott and Baggot, 1993). These steps are in response to human

health concerns and also to the problems produced by such residues in milk used for manufacturing.

Antimicrobial agents in food can produce life-threatening allergic reactions, including anaphylactic shock, in susceptible individuals. About 10% of the human population is sensitive to penicillin, the most allergenic drug (Oslo and Sanders, 1975), while about 3.4% is sensitive to sulfonamides (Bigby *et al.*, 1986). Given the current long life expectancy of humans, increased suppression of the human immune system through long term exposure to low levels of antibiotics in the milk supply is a cause of concern.

Another public health issue relates to development of new antibiotic resistant bacterial pathogens as a result of long term exposure to low levels of antibiotics in milk. Resistance in this case probably results from mutation in the bacterial population, followed by continual selection to establish the resistant species (Bryan, 1984).

The advent of multiple drug resistance was first reported in 1955 in Japan and has been observed in a large number of bacterial genera (Bryan, 1984). In Ghana, Akyeh (2000) observed that several of the bacteria he isolated from milk were resistant to several antibiotics including ampicillin, nitrofurantoin, co-trimoxazole, and tetracycline. Several of the bacteria were also found to exhibit multiple drug resistance to these drugs. In 1985, the largest known food borne outbreak involving more than 16,000 cases of salmonellosis in the Chicago area was traced to pasteurised milk that contained a very rare multi-antibiotic-resistant strain of *Salmonella typhimurium* (Ryan *et al.*, 1987; Schuman *et al.*, 1989). The fact that this organism also contained several plasmids encoding resistance to 14 different antibiotics highlights the potential danger of antibiotic misuse on the farm.

Certain antibiotics such as nitroimidazole are potential human carcinogens (Prescott and Baggot, 1993). Aplastic anaemia (an irreversible and potentially fatal bone marrow disease) and various neurological disorders caused by chloramphenicol and ivermectin (an anti-worming agent) are other potential hazards of antibiotic contamination (Prescott and Baggot, 1993; Ryser, 1998). This has led to the banning of chloramphenicol for use in meat animals in several countries (Prescott and Baggot, 1993).

Contamination of milk with even minute levels of antibiotics also has created several potential safety-related problems for manufacturers of fermented dairy products. These include inadequate milk clotting and improper cheese ripening, inadequate acid and flavour development in buttermilk, and more importantly, diminished starter culture growth and acid production during cheese making which allow pathogens such as *Salmonella* and *Staphylococcus* to grow (Marth and Ellickson, 1972). Starter culture failure remains a major cause of disease outbreaks involving cheese and other fermented dairy products (Ryser, 1998).

2.3.2.1 Detection methods for drug residues

The methods used for the detection of antibiotics residues are mainly microbiologic, with confirmation by electrophoresis and chemical methods mainly high performance liquid chromatography (Prescott and Baggot, 1993). Microbiologic procedures involve the use of sensitive bacterial strains (eg. *Bacillus stearothermophilus*) that are incubated overnight on plates in the presence of tissue or fluid suspected to contain antibiotics. The earliest method were based on the inability of test bacteria to produce acid, reduce dyes, or grow on solid media in the presence of antibiotics (Bishop and White, 1984). These time consuming assays, which required overnight incubation, were eventually replaced by the qualitative and

Certain antibiotics such as nitroimidazole are potential human carcinogens (Prescott and Baggot, 1993). Aplastic anaemia (an irreversible and potentially fatal bone marrow disease) and various neurological disorders caused by chloramphenicol and ivermectin (an anti-worming agent) are other potential hazards of antibiotic contamination (Prescott and Baggot, 1993; Ryser, 1998). This has led to the banning of chloramphenicol for use in meat animals in several countries (Prescott and Baggot, 1993).

Contamination of milk with even minute levels of antibiotics also has created several potential safety-related problems for manufacturers of fermented dairy products. These include inadequate milk clotting and improper cheese ripening, inadequate acid and flavour development in buttermilk, and more importantly, diminished starter culture growth and acid production during cheese making which allow pathogens such as *Salmonella* and *Staphylococcus* to grow (Marth and Ellickson, 1972). Starter culture failure remains a major cause of disease outbreaks involving cheese and other fermented dairy products (Ryser, 1998).

2.3.2.1 *Detection methods for drug residues*

The methods used for the detection of antibiotics residues are mainly microbiologic, with confirmation by electrophoresis and chemical methods mainly high performance liquid chromatography (Prescott and Baggot, 1993). Microbiologic procedures involve the use of sensitive bacterial strains (eg. *Bacillus stearothermophilus*) that are incubated overnight on plates in the presence of tissue or fluid suspected to contain antibiotics. The earliest methods were based on the inability of test bacteria to produce acid, reduce dyes, or grow on solid media in the presence of antibiotics (Bishop and White, 1984). These time consuming assays, which required overnight incubation, were eventually replaced by the qualitative and

is a close correlation between the level of aflatoxin concentration in the diet and occurrence of hepatocarcinoma in communities surveyed (WHO, 1979). Four major forms of aflatoxins designated aflatoxin B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁), and G₂ (AFG₂) are currently recognised (Ryser, 1998). From the milk hygiene point of view, AFB₁ and AFM₁ (formed from AFB₁) are the most important (Aibara, 1983). AFM₁ has the strongest physiological activity and its oral carcinogenicity is known to be unparalleled among all the known chemical substances (Aibara, 1983). It is most often found in moldy peanuts and animal feeds containing corn or other grains. When dairy cattle ingest contaminated feed, AFB₁ is metabolised to AFM₁, some of which is shed in the milk. Studies by Applebaum *et al.* (1982) showed that cows given pure and impure aflatoxin B₁ alongside toxin administration produced milk containing 1.05 ppb to 10.58 ppb of aflatoxin M₁. The Food and Drug Administration (USA) research group calculated the aflatoxin feed/tissue ratio from published data in the world and estimated that the most likely ratio of feed level of AFB₁ to milk level of AFM₁ was 300 (Aibara, 1983). There are no international guidelines for AFM₁ in liquid milk. However, as of 1987, at least 34 countries had active or proposed legislation regarding aflatoxin limits in animal feed, with the United States and many European countries also having legislated maximum acceptable levels of AFM₁ in fluid milk and dairy products (Van Egmond, 1989 cited by Ryser, 1998).

Present evidence indicates that levels of AFM₁ in milk and dairy products are relatively unaffected by pasteurisation, sterilisation, fermentation, cold storage, freezing, concentrating, or drying (Yousef and Marth, 1989 cited by Ryser, 1998). However, treating milk with agents such as hydrogen peroxide has proven effective in experimentally reducing levels of AFM₁ in contaminated milk (Yousef and Marth, 1986).

is a close correlation between the level of aflatoxin concentration in the diet and occurrence of hepatocarcinoma in communities surveyed (WHO, 1979). Four major forms of aflatoxins designated aflatoxin B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁), and G₂ (AFG₂) are currently recognised (Ryser, 1998). From the milk hygiene point of view, AFB₁ and AFM₁ (formed from AFB₁) are the most important (Aibara, 1983). AFM₁ has the strongest physiological activity and its oral carcinogenicity is known to be unparalleled among all the known chemical substances (Aibara, 1983). It is most often found in moldy peanuts and animal feeds containing corn or other grains. When dairy cattle ingest contaminated feed, AFB₁ is metabolised to AFM₁, some of which is shed in the milk. Studies by Applebaum *et al.* (1982) showed that cows given pure and impure aflatoxin B₁ alongside toxin administration produced milk containing 1.05 ppb to 10.58 ppb of aflatoxin M₁. The Food and Drug Administration (USA) research group calculated the aflatoxin feed/tissue ratio from published data in the world and estimated that the most likely ratio of feed level of AFB₁ to milk level of AFM₁ was 300 (Aibara, 1983). There are no international guidelines for AFM₁ in liquid milk. However, as of 1987, at least 34 countries had active or proposed legislation regarding aflatoxin limits in animal feed, with the United States and many European countries also having legislated maximum acceptable levels of AFM₁ in fluid milk and dairy products (Van Egmond, 1989 cited by Ryser, 1998).

Present evidence indicates that levels of AFM₁ in milk and dairy products are relatively unaffected by pasteurisation, sterilisation, fermentation, cold storage, freezing, concentrating, or drying (Yousef and Marth, 1989 cited by Ryser, 1998). However, treating milk with agents such as hydrogen peroxide has proven effective in experimentally reducing levels of AFM₁ in contaminated milk (Yousef and Marth, 1986).

2.3.4 Adulteration of milk.

Adulteration of milk implies addition or subtraction of any of its components (Omore *et al.*, 2001). Milk may be adulterated in several ways (O'Connor, 1994), but the common form is the addition of water. Adulteration of milk with water is influenced by amount of milk supply, which also depends on season (Gyiele *et al.*, 1999). During the dry season, milk supply is low as a result of scarcity of feed (grass) for cattle. Thus there is a tendency to add water to milk to increase the quantity. However, during the wet season, when grass is abundant and milk supply is normal, addition of water is not a common practice. Adulteration of milk with water could pose a public health threat to consumers. This could be very serious under situations where untreated water such as streams is used to adulterate milk. Untreated water could contain several pathogenic microorganisms mainly from faecal pollution, and such organisms include *Clostridium perfringes*, *Streptococcus faecalis*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhi*, and *Vibrio cholerae* (Round *et al.*, 1971; Skinner and Schewan, 1977; Ryser, 1998). Apart from pathogenic microorganisms, untreated water could also contain several chemical hazards such as cyanide, mercury, antibiotics, and other synthetic organic chemicals used in farming (Aibara, 1983; O'Connor, 1994). Such chemicals, which enter water through pollution, could also end up in milk through adulteration and cause problems to human health. Chemical hazards could also be introduced directly into milk through addition of antimicrobial agents and alkali by traders to preserve milk and reduce its acidity respectively (O'Connor, 1994; Omore *et al.*, 2001).

Adulteration of milk has been observed by several workers, and is more likely to be a problem in the developing world, where milk marketing is mostly informal and there is little or no monitoring of the handling practices of market agents (Ombui *et al.*, 1995;

Omore *et al.*, 2001). In Ghana, there appears to be little information on milk adulteration though Karikari *et al.* (1998) had reported that 122 milk samples screened were not adulterated.

3.2. Data and sample collection

Data collection focused on milk handling practices and other market factors that affect milk quality or pose public health risk to consumers (appendix 4). Respondents were selected by a stratified random sampling procedure from each area within the two sites. A total of 242 and 177 respondents were interviewed from Accra and Kumasi respectively for both the dry and wet seasons (Table 6). Attempts were made to interview the same respondents during the two seasons. Where it was not possible to interview the same respondent during the second season, a replacement was selected within the same locality. A total of 516 milk samples were collected aseptically into tubes and transported to the laboratory on ice for analyses. Four hundred and seventy eight of the samples collected were obtained from respondents interviewed, while 38 were obtained from other milk traders in the study sites. Most of the samples collected were fresh milk (Table 7).

Table 6: Market agents surveyed and sampled in each site and season

Market agents	Kumasi		Accra	
	Dry	Wet	Dry	Wet
Producers	86	67	48	50
Processors	15	14	6	4
Wholesalers	6	6	29	20
Retailers	26	22	14	6
Total	133	109	97	80

3.2. Data and sample collection

Data collection focused on milk handling practices and other market factors that affect milk quality or pose public health risk to consumers (appendix 4). Respondents were selected by a stratified random sampling procedure from each area within the two sites. A total of 242 and 177 respondents were interviewed from Accra and Kumasi respectively for both the dry and wet seasons (Table 6). Attempts were made to interview the same respondents during the two seasons. Where it was not possible to interview the same respondent during the second season, a replacement was selected within the same locality. A total of 516 milk samples were collected aseptically into tubes and transported to the laboratory on ice for analyses. Four hundred and seventy eight of the samples collected were obtained from respondents interviewed, while 38 were obtained from other milk traders in the study sites. Most of the samples collected were fresh milk (Table 7).

Table 6: Market agents surveyed and sampled in each site and season

Market agents	Kumasi		Accra	
	Dry	Wet	Dry	Wet
Producers	86	67	48	50
Processors	15	14	6	4
Wholesalers	6	6	29	20
Retailers	26	22	14	6
Total	133	109	97	80

Table 7: Milk Samples collected by product type

Type of product	n	%
Fresh milk	430	83.3
Processed milk (Wagashie, Fermented, Ghee)	86	16.7
Total	516	100

3.3 Preparation of laboratory media/reagents

(a) Phosphate buffered water (BDH)

This reagent was employed as a diluent for milk samples. It was prepared by dissolving 0.0425g of potassium dihydrogen phosphate per litre of distilled water and adjusting the pH to 7.2.

(b) Standard plate count agar (Oxoid)

This is a general purpose medium usually employed in enumeration of bacteria. It was prepared by dissolving 23.5g of dehydrated powder of the medium in a litre of distilled water and sterilising the mixture at 121⁰ C for 15 minutes.

(c) Violet red bile agar (Oxoid)

Violet red bile agar is a differential medium usually used for enumeration of coliform bacteria. It was prepared by dissolving 52g of dehydrated powder of the medium in a litre of distilled water and sterilising the mixture at 121⁰C for 15 minutes.

(d) Triple sugar iron agar (Merck)

This is a semi-solid medium used to differentiate enterobacteria based on their production of acid, hydrogen sulphide, and gas as a result of fermentation of sugars. In preparing this medium, 65g of dehydrated powder of the medium was dissolved in a litre of distilled water and boiled. It was then dispensed in test tubes and sterilised by autoclaving at 121⁰C for 15 minutes at a pressure of 1.1 kgm⁻².

(e) Simmons citrate agar (Difco)

This is a semi-solid medium used to differentiate enterobacteria based on their ability to utilise citrate. In preparing the medium, dehydrated powder (23g) of the medium was dissolved in a litre of distilled water with boiling. The mixture was distributed into tubes and sterilised at 121⁰C for 15 minutes, followed by cooling to 50⁰C.

(f) MacConkey agar (Oxoid)

This is a differential and low selective medium, used to distinguish lactose fermenting from non-lactose fermenting bacteria. It is usually employed in the isolation of enterobacteriaceae. The medium was prepared by dissolving 52g of macConkey agar powder in a litre of distilled water, and sterilised by autoclaving at 121⁰C for 15 minutes at a steam of 1.1 kg/cm². It was then cooled to between 45-50⁰C and poured aseptically into petri dishes.

(g) Blood Agar (Merck)

Blood agar is used to grow a wide range of bacteria particularly those that are more difficult to grow. It is required to detect and differentiate haemolytic bacteria such as *Streptococcus* and *Staphylococcus*. To prepare the medium, dehydrated blood agar base (40g) was added to a litre of water and dissolved by boiling gently. The dissolved medium was cooled to about 50⁰C, and sterile aerated sheep blood added aseptically in a proportion of 1 part of blood to

about 4 parts of the medium to give a 5% blood agar. It was then mixed by rotating gently and poured into sterile petri dishes.

(h) *Salmonella* and *Shigella* agar (Oxoid)

This is a highly selective medium for *Salmonella* spp and *Shigella* spp. The medium was prepared by dissolving 60g of dehydrated agar powder of the medium in a litre of distilled water. The mixture was sterilised by autoclaving at 121⁰C for 15 minutes at a steam of 1.1 kg/cm². It was then cooled to between 45-50⁰C and poured aseptically into petri dishes.

(i) Urea agar base (Difco)

Urea agar base is a semi-solid medium used to differentiate enterobacteria based on their ability to produce the enzyme urease, which hydrolyses urea. The medium was prepared by dissolving 2.4g of dehydrated powder of the medium in 95 ml of distilled water. The mixture was then sterilised by autoclaving at 121⁰C for 20 minutes and then cooled to about 50⁰C. Five millilitres of 40% urea supplement (SR 20) was aseptically added to the mixture, distributed into tubes, and allowed to set in a slanted position.

(j) Lowenstein Jensen medium agar (Beton Bickinson)

Lowstein Jensen medium is used to cultivate and isolate *Mycobacterium* spp. In preparing this medium, dehydrated powder of Lowstein Jensen base was dissolved in 600 ml. of distilled water. The mixture was sterilised at 121⁰C for 15 minutes and then cooled to 50⁰C. After this whole egg (1000 ml) was prepared and added to it with gentle mixing. The final mixture was distributed into tubes in a slanted position, allowed to coagulate and inspissated at 85⁰C for about 45 minutes.

(k) Nutrient agar (Oxoid)

Nutrient agar is a basic culture medium and it can be used to subculture pathogens prior to performing biochemical and serological tests. The medium was prepared by dissolving 28g of nutrient agar powder in a litre of distilled water, and sterilised by autoclaving at 121⁰C for 15 minutes at a steam of 1.1 kg/cm². It was then cooled to between 45-50⁰C and poured aseptically into petri dishes.

(l) Bacto peptone (Oxoid)

Bacto peptone is an enrichment medium and can be used to culture a wide range of microorganisms. The medium was prepared by dissolving 1g of dehydrated powder of the medium in a litre of distilled water. The mixture was distributed into tubes and then sterilised by autoclaving at 121⁰C for 15 minutes, followed by cooling to about 50⁰C

(m) Lysine indole motility medium (Difco)

This is a semi-solid medium used to differentiate enterobacteria species by their lysine utilisation, motility, and indole production. It was prepared by dissolving 38.5g of dehydrated powder of the medium in a litre of distilled water. The mixture was then dispensed in test tubes and sterilised by autoclaving at 121⁰C for 15 minutes at a pressure of 1.1 kgm⁻².

3.4 Laboratory analyses

The milk samples collected were analysed in the laboratory for the following:

- (i) antimicrobial agents
- (ii) total bacteria plate count
- (iii) coliform bacteria plate count
- (iv) bacteria species and types

(v) butterfat content

(vi) specific gravity

3.4.1 Detection of antimicrobial agents in milk by Charm Aim Test:

The Charm Aim-96 anti-microbial inhibition assay screening kit (Charm Sciences Inc., USA) was used according to the manufacturer's recommendations. This test detects beta-lactams, tetracyclines, aminoglycosides, macrolides and sulphonamides at levels above maximum residue limits recommended by the European Union.

Fifty microlitres of each sample was added to microtitre plate followed by 200 μ l of a mixture of *Bacillus stearothermophilus* spore tablet and lyophilised medium dissolved in 22mls of deionised water. The plate was then sealed tightly, secured by screws and incubated for 3-4 hours. Positive and negative controls were included in the assay. The positive milk control consisted of milk mixed with penicillin G standard or sulfamethazine standard. To 50 μ l of the positive control milk, 200 μ l of bacterial spore and lyophilised media were added. The negative control consisted of 50 μ l of negative control tablet dissolved in distilled water and 200 μ l of the test bacteria and media dissolved in deionised water.

The test results were read using colour contrasts and scored from 1-5 (negative =1-3 and positive = 4-5).

3.4.2 Evaluation of bacteriological quality of milk by total and coliform bacteria plate counts

Milk samples were assessed for total and coliform bacteria plate counts using direct culture methods described by Marshall (1992) and Speck (1984). For each sample, tenfold serial

dilutions (10^{-1} - 10^{-8}) were prepared in sterile phosphate buffered water diluent, pH 7.2. The wide range of dilutions was selected in anticipation of variations in bacterial counts.

Dilutions for culture for total bacteria plate count ranged from 10^{-4} - 10^{-8} . One millilitre of dilutions of each sample was pipetted into 90mm diameter petri dish and mixed well with about 20ml of sterile standard plate agar. After cooling and solidification of the medium, plates were incubated at 32°C or 37°C for 48 hours. Following this period, plates with colonies ranging from 25-250 colony forming units (cfu) were counted with a colony counter, and used to compute total bacteria plate count of milk samples following guidelines by Speck (1984).

For coliform bacteria plate count, sample dilutions ranging from 10^{-1} - 10^{-4} were cultured in molten violet red bile agar using the same volumes and procedures as above for total bacteria plate count. However, the plates were incubated at 37°C for 24 hours. Plates showing typical red coliform colonies in the countable range of 15-150 cfu per plate were counted with a colony counter, and used to compute coliform bacteria plate count of milk samples following guidelines by Speck (1984).

3.4.3 Examination for bacteria types in milk- Cultural, microscopic and biochemical examinations:

Milk samples were centrifuged at 3000rpm for 15 minutes and about 100µl of the sediments inoculated onto slopes of Lowstein Jensen medium. The inoculated slopes were incubated at 37°C for 2-6 weeks. One millilitre of milk samples were also inoculated into peptone broth and incubated at 37°C for 18-24 hours. After incubation, about 100µl of the inoculated peptone broth were subcultured onto plates of blood agar, macConkey agar, and *Salmonella* and *Shigella* agar, and further incubated at 37°C for 18-24 hours. Discrete colonies on the

agar plates were purified for biochemical tests by subculturing onto nutrient agar plates and incubating them at 37°C for 18-24 hours. Identification of bacteria was done by colony morphology on the various media, staining and microscopy, and the results of biochemical examination (Cheesebrough, 1994).

(a) Staining and microscopical examination

(i) Ziehl Nielsen stain:

This is a differential staining procedure used to identify acid-fast bacteria such as *Mycobacterium* spp. It was performed as follows. A small bit of bacterial culture suspected to be *Mycobacterium* spp. was aseptically transferred onto a drop of distilled water on a slide and emulsified. The smear was fixed with methanol for about 3 minutes and air-dried. Carbol-fuchsin stain was used to flood the stain and heat applied for about 8 minutes. The stain was washed off with water and decolourised with 3% acid alcohol for 5 minutes. Water was used to wash off the decolouriser and the smear counterstained with methylene blue for about 2 minutes. The counterstain was washed with water and the smear examined microscopically for acid-fast bacilli, which appear as red (Atlas, 1995).

(ii) Gram stain:

A small bit of bacterial culture was aseptically transferred onto a drop of distilled water on a slide and emulsified. The smear was fixed by passing the slide gently over a flame before staining. Crystal violet solution was used to flood the fixed smear, allowed to react for one minute and then washed off with water. Lugol's iodine solution was also used to flood the smear, allowed to react for one minute and washed off with water. The smear was then decolourised with acetone-ethanol for about 10 seconds and washed with water. A counter stain of Gram's safranin was added to the smear, and washed off with water after about 45

seconds. The smear was then examined microscopically. Bacterial cells which stained purple were recorded as Gram positive, while those stained pink were recorded as Gram negative (Atlas, 1995).

(c) Biochemical examination

Biochemical tests were also employed in the identification of enterobacteria. Pure cultures of Gram negative bacteria on nutrient agar plates were used to inoculate the following prepared biochemical media and incubated at 37⁰C for 18-24 hours:

-Triple sugar iron

-Lysine indole motility medium

-Simmons citrate agar

-Urea agar base

After incubation, Kovac's reagent (2-3 drops) was added to Lysine indole motility medium to check for production of indole. Changes in the various biochemical media were noted and used as a guide in the identification of enterobacteria.

3.4.4 Detection of *Brucella abortus* antibodies in milk by the Milk Ring Test:

The test was performed by adding and mixing one drop of stained *Brucella abortus* antigen with 1ml of milk sample in a miniature test tube, followed by incubation at 37⁰C for 1hour (Huber and Nicoletti, 1986).

3.4.5 Determination of Specific gravity and Butterfat content of milk samples:

In determining the specific gravity of a milk sample, the sample was mixed gently and poured into a measuring cylinder (300-500ml). A lactometer was allowed to sink slowly into the milk and the reading just below the meniscus of the milk recorded. Where the temperature of

milk was different from the calibration temperature of the lactometer, a temperature correction was calculated. For each unit temperature ($^{\circ}\text{C}$) above the calibration temperature, 0.2 units was added to the lactometer reading and for each temperature unit below 0.2 unit was subtracted.

Butterfat content of milk samples was determined by the Gerber method (O'Connor, 1994). The test was performed by adding 11 mls of milk sample to 10 mls of concentrated sulphuric acid in a butyrometer. Next, 1 ml of amyl alcohol was added to the mixture and shaken carefully until the curds dissolved and no white particles were seen. The butyrometer with its contents were centrifuged at 1100 rpm for 5 minutes and then put in a water bath maintained at 65°C for 3 minutes before taking the butterfat readings.

For each sample, the butterfat content and specific gravity, were used to calculate the solids-not-fat (SNF) and total solids (TS) content of the milk by the Richmond's formulae as follows: $\% \text{ SNF} = (0.2 \times \% \text{ fat}) + (0.25 \times \text{L}) + 0.48$ and $\text{TS} = \text{SNF} + \text{BF}$.

SNF values of milk samples computed were used to determine whether the samples were adulterated with water. Milk samples with $\text{SNF} < 8.5$ were considered to be adulterated while those with $\text{SNF} > 8.5$ were considered unadulterated (O'Connor, 1994).

3.5 Data analyses

Laboratory analyses results and data collected by questionnaire were entered into MS-ACCESS and MS-EXCEL and analysed in STATA (Nagda, 2000) to:

- Identify sources and pathways of marketed milk and milk products;
- Assess milk bacteriological quality;

- Quantify the prevalence of different bacteria types in milk.
- Assess presence of potentially harmful levels of antimicrobial residues in marketed milk;
- Assess milk handling practises by market agents;
- Assess the influence of market factors (e.g., pathways and handling factors) on milk quality;
- Assess the influence of season and site on milk quality and public health risks;
- Quantify the risks of milk-borne public health hazards.

The approaches taken to analyse the data were as follows: Firstly, descriptive analyses were carried out on the laboratory and questionnaire data. Variables were described by frequencies; geometric means; ranges; standard deviations; and proportions above given standards. Following the descriptive analyses, regressions were conducted using milk-borne hazards and quality indicators as response variables. Ordinary least squares (OLS) were conducted on total bacteria plate count, coliform bacteria plate count, and solids-not-fat (appendix 1) while maximum likelihood logistic regression models were conducted on bacteria types and drug residues above EU maximum residue limits (appendices 2 and 3). For each response variable, tests of significance (p-values) were used to identify significant associations ($p < 0.10$) with explanatory variables. Only factors considered to potentially explain variations in each response were included as explanatory variables. The explanatory variables included season, site, area within site, trader type, sources and pathways of marketed milk, time, distance, milk temperature, and milk handling practices.

CHAPTER FOUR

RESULTS (WITH DISCUSSIONS)

This study was organized into four investigative phases as follows:

- (1) Evaluation of antimicrobial agents as a public health hazard in marketed milk
- (2) Bacteriology of marketed milk and the public health implications of microorganisms so identified
- (3) Adulteration of milk and its compositional quality
- (4) Milk handling and hygienic practices of market agents of the commodity

4.1. Study 1: An evaluative study of the public health hazard of antimicrobial agents in marketed milk.

Summary:

Milk samples obtained in the dry and wet seasons from Accra and Kumasi were screened for five families of antimicrobial agents by the Charm Aim Test. The families of antimicrobial agents screened were: beta-lactams; tetracyclines; aminoglycosides; macrolides; and sulphonamides.

Overall, 35% of milk samples were found to be contaminated with one or more of the antimicrobial agents screened. There was no significant difference ($p>0.10$) in the levels of contamination between the dry season (33.3%) and wet season (37.0). However, the level of contamination observed for Accra (35.1%) was significantly higher ($p=0.04$) than that of Kumasi (34.9%). Seasonal changes in milk contamination for each of the sites was however not significant ($p>0.10$). Whereas for Accra, the extent of adulteration was higher in the dry season (37.3%) than in the wet season (33.8%), the reverse was observed for Kumasi (dry season=33.6%; wet season=39.4%). The high levels of contamination of milk with

antimicrobial agents observed may probably be due to non-observance of withdrawal requirements of veterinary drugs or lack of its awareness or both. These findings have serious public health implications as antimicrobial agents in food may cause drug resistance and hypersensitivity in susceptible individuals.

Introduction:

Antimicrobial agents (antibiotics and other anti-bacterials) in milk constitute an important public health risk. Consumption of milk contaminated with them may indeed cause hypersensitivity in sensitive people (Osion and Sanders, 1975). Drug resistance (Nijstien *et al.*, 1996) and specific tissue damage (Schultz *et al.*, 1963) may also occur. Occurrence of antimicrobial agents in milk usually results from drugs used in the treatment of animal diseases (Aibara, 1983) and contamination of animal feed (McEvoy *et al.*, 2000). Because milk from various sources is typically comingled, antimicrobials can contaminate large volumes of milk (Ryser, 1998). Antimicrobial agents in milk have been reported in both developed and developing countries (Chewulukei, 1978; Prescott and Baggot, 1993; Ryser, 1998). However, in the former, widespread testing, educational programmes and control have reduced the occurrence of antimicrobials agents in milk (FAO/WHO, 1970). In Ghana, there is limited information about the occurrence of antimicrobial agents in milk.

The objective of this study therefore, is to determine the extent of, and evaluate, the public health hazard inherent in antimicrobials in marketed milk in Ghana. The Charm Aim Test was used to screen antimicrobial agents in milk, as described in Chapter 3.4.1. The test detects antimicrobial agents in milk at levels above the maximum residue limits recommended by the European Union.

Results:

Overall, 35% (121) of 346 milk samples analysed were found to be contaminated with one or more of the five families of antimicrobial agents screened, namely:

- (i) beta-lactams,
- (ii) tetracyclines,
- (iii) aminoglycosides,
- (iv) macrolides and
- (v) sulphonamides.

Figure 1 shows the proportions of milk samples contaminated with antimicrobial agents in the wet and dry seasons. There was no significant difference ($p>0.10$) between levels of contamination between the two seasons. In the dry season, 33.3% (66) of 184 milk samples analysed were contaminated with antimicrobial agents, while in the wet season 37.0% (55) of 162 milk samples analysed were contaminated. Figure 2 illustrates the proportions of milk samples contaminated with antimicrobial agents from Accra and Kumasi. The level of contamination from Accra was significantly higher ($p=0.04$) than that of Kumasi. For Accra, 35.1% (53) of 151 milk samples analysed were contaminated with antimicrobial agents, whereas for Kumasi, 34.9% (68) of 195 milk samples analysed were found to be contaminated. The effect of season on contamination of milk with antimicrobial agents for Accra and Kumasi is also shown in Table 8. Whereas the extent of contamination was higher in the dry season than in the wet season for Accra, the reverse was observed for Kumasi: For Accra, the extent of contamination decreased from 37.3% in the dry season to 33.8% in the wet season while for Kumasi, it increased from 33.6% in the dry season to 39.4% in the wet season. However, the seasonal changes in milk contamination with antimicrobial agents observed for each site was not significant ($p>0.10$).

Figure 1: Proportions of milk samples contaminated with antimicrobial agents (% AG) in the dry and wet seasons.

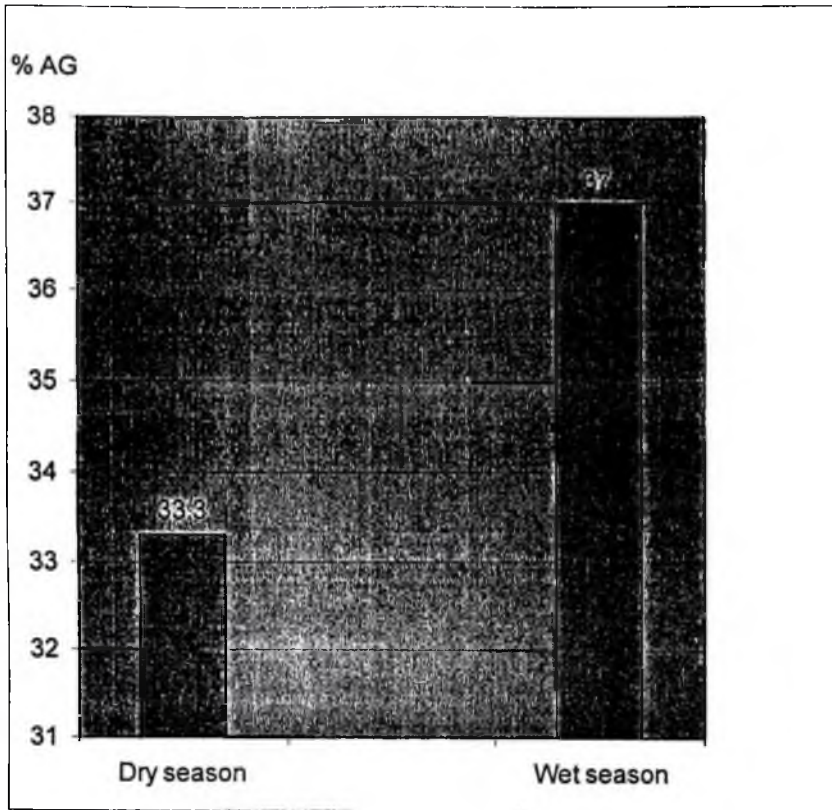


Figure 2: Proportions of milk samples contaminated with antimicrobial agents (%AG) for Accra and Kumasi.

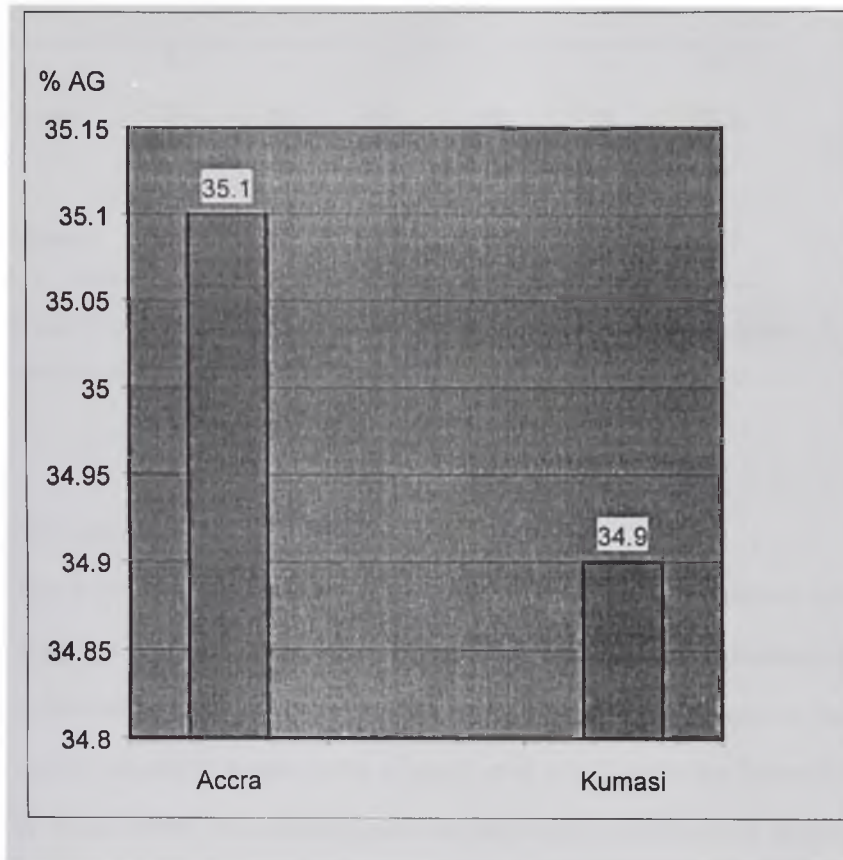


Table 8: Effect of season on contamination of milk with antimicrobial agents for Accra and Kumasi.

	Dry season			Wet season		
	N	n	%	N	n	%
Accra	83	31	37.3	68	23	33.8
Kumasi	101	34	33.6	94	37	39.4

n refers to number of samples contaminated with antimicrobial agents, % refers to its corresponding percentage, and N refers to number of milk samples analysed.

Discussion:

This is probably the first time contamination of milk with antimicrobial agents has been quantified in Ghana. The overall prevalence of 35% indicates that contamination of milk with antimicrobial agents may be a problem in the country. Lower prevalence rates have been reported recently in Kenya (9.4%) by Aboge *et al.* (2001) and in the United States (<0.15%) by Ryser (1998). Antimicrobial agents in milk usually originate from farm-level practices (Omoro *et al.*, 2001). The non-observance of withdrawal requirements of veterinary drugs or lack of its awareness or both may account for the high overall prevalence observed in this study. These practices are probably not affected by seasonal changes, and this probably account for the non-significant difference in levels of contamination between the two seasons. Withdrawal periods are probably flouted more in Accra where the level of contamination was significantly higher, than in Kumasi. The high levels of milk contamination with antimicrobial agents observed in this study has serious public health implications. Unlike bacterial health risk, antimicrobial agents in milk cannot be eliminated simply by heat treatment. Consumers are therefore undoubtedly faced with the hazards of

4.2. Study 2: A study of the bacteriological quality and bacterial flora of milk

Summary:

Milk samples obtained in the dry and wet seasons, and from Accra and Kumasi, were analysed to evaluate the bacteriological quality and catalogue the bacterial flora of same.

Total bacteria and coliform bacteria plate counts were employed to evaluate bacteriological quality, while bacterial floral profile was determined by culture, biochemical, and serological tests.

There were no significant differences ($p > 0.10$) between the two seasons and sites in both total bacteria plate count (TPC) and coliform bacteria plate count (CPC) of raw milk samples. The ranges of TPC and CPC of raw milk were found to be 0 to 9.4 log₁₀ cfu/ml and 1 log₁₀ to 6.2 log₁₀ cfu/ml respectively. Whereas 23.5% of the raw milk samples did not meet the standard of the Ghana Standards Board for TPC (6.3 log₁₀ cfu/ml.), none of them did meet the standard for CPC (0.7 log₁₀ cfu/ml.). The mean TPC of raw milk (5.9 log₁₀ cfu/ml) was the same as that of processed milk, while the mean CPC of raw milk (3.7 log₁₀ cfu/ml) was not significantly higher ($p > 0.10$) than that of processed milk (3.5 log₁₀ cfu/ml). Overall, a spectrum of eight bacteria genera and species was identified in the examined milk by culture or serology involving assay of antibodies. The bacteria so identified were *Escherichia coli*, *Brucella abortus*, *Klebsiella* spp., *Yersinia* spp., *Mycobacterium* spp., *Proteus* spp., *Bacillus* spp. and *Staphylococcus* spp. The most prevalent organism was *Brucella abortus* (30.5%), while the least prevalent was *Mycobacterium* spp. (0.008%). The bacterial flora of milk from Kumasi consisted of four of the eight organisms identified (*Escherichia coli*, *Brucella abortus*, *Klebsiella* spp., *Yersinia* spp.), while that of Accra consisted of seven with *Klebsiella* spp. being the only one absent. It was also observed that, generally, the prevalence rates of different bacteria in milk from Accra were generally higher than those of the

4.2. Study 2: A study of the bacteriological quality and bacterial flora of milk.

Summary:

Milk samples obtained in the dry and wet seasons, and from Accra and Kumasi, were analysed to evaluate the bacteriological quality and catalogue the bacterial flora of same.

Total bacteria and coliform bacteria plate counts were employed to evaluate bacteriological quality, while bacterial floral profile was determined by culture, biochemical, and serological tests.

There were no significant differences ($p > 0.10$) between the two seasons and sites in both total bacteria plate count (TPC) and coliform bacteria plate count (CPC) of raw milk samples. The ranges of TPC and CPC of raw milk were found to be 0 to 9.4 log₁₀ cfu/ml and 1 log₁₀ to 6.2 log₁₀ cfu/ml respectively. Whereas 23.5% of the raw milk samples did not meet the standard of the Ghana Standards Board for TPC (6.3 log₁₀ cfu/ml.), none of them did meet the standard for CPC (0.7 log₁₀ cfu/ml.). The mean TPC of raw milk (5.9 log₁₀ cfu/ml) was the same as that of processed milk, while the mean CPC of raw milk (3.7 log₁₀ cfu/ml) was not significantly higher ($p > 0.10$) than that of processed milk (3.5 log₁₀ cfu/ml). Overall, a spectrum of eight bacteria genera and species was identified in the examined milk by culture or serology involving assay of antibodies. The bacteria so identified were *Escherichia coli*, *Brucella abortus*, *Klebsiella* spp., *Yersinia* spp., *Mycobacterium* spp., *Proteus* spp., *Bacillus* spp. and *Staphylococcus* spp. The most prevalent organism was *Brucella abortus* (30.5%), while the least prevalent was *Mycobacterium* spp. (0.008%). The bacterial flora of milk from Kumasi consisted of four of the eight organisms identified (*Escherichia coli*, *Brucella abortus*, *Klebsiella* spp., *Yersinia* spp.), while that of Accra consisted of seven with *Klebsiella* spp. being the only one absent. It was also observed that, generally, the prevalence rates of different bacteria in milk from Accra were generally higher than those of the

corresponding bacteria in milk from Kumasi. The high CPC of raw milk samples, and the occurrence of several enteric bacteria, some of which had shown high prevalence, reflect the poor hygienic state in which milk is handled. However, the presence of bacteria such as *Mycobacterium* spp. in milk samples may also indicate that infected cattle may be an important source of such microbial contamination and/ health hazards. All the bacteria identified here are potentially pathogenic and their presence in milk therefore is of great public health concern.

Introduction:

On account of its nutritional composition, milk is an excellent medium for multiplication of bacterial contaminants (Seaman, 1963). Bacteriological quality of milk reflects the temperature of the milk, time elapsed since milking and level of hygiene (Omore *et al.*, 2001). Whereas total bacteria population in milk mainly reflects time elapsed since milking and ambient temperature (if milk is not chilled), coliform bacteria are especially associated with level of hygiene, since they are mainly of faecal origin. Poor hygiene often arises from poor handling at the farm, collection centres, during transportation and at retail points. A high bacterial count reduces the shelf life and enhances the risk of milk-borne bacterial infections and intoxication if milk is not properly heated (Andrew and Russel, 1984; Kayihura *et al.* 1987).

The bacterial flora of milk includes a wide range of bacteria and other microorganisms (WHO, 1962; O'Connor, 1994). While some of the bacterial agents cause milk spoilage only, others cause diseases, including food poisoning (Seaman, 1963). The importance of milk-borne infections is due to the morbidity and mortality they cause in humans. In developed countries, pasteurisation has contributed greatly to reduction in the incidence of milk-borne

infections in humans (Ryser, 1998). The incidence of human milk-borne infections is probably still higher in developing countries where non-heat treated milk and cheese from raw milk is consumed routinely (Castell-Monslave *et al.*, 1996). In Ghana, several pathogenic microorganisms have been reported in raw milk (Abraham and Laryea, 1968; Ayebo *et al.*, 1976). Some of these pathogens are zoonotic, and it is worthy of note that high prevalence of zoonotic diseases has been reported in certain farming areas in the country (Oppong, 1966; Assoku, 1988; Turkson and Boadu, 1993; Aning, 1999). The objective of this study is to assess the bacteriological quality and bacterial flora of milk samples collected, and evaluate the public health implications.

Bacteriological quality and bacterial flora of milk samples were assessed and/ or measured by total bacteria and coliform bacteria plate count methods as described in Chapter 3.4.2. Bacteria types in milk especially were identified by culture, biochemical tests, and serological tests which are also described in Chapter 3.4.3.

Results: bacteriological quality of milk

In Ghana, the Standards Board specification for raw unprocessed milk considers good quality milk in terms of TPC and CPC, as one containing not more than $6.3 \log_{10}$ cfu/ml and $0.7 \log_{10}$ cfu/ml respectively. These standards were applied in this study to determine raw milk samples with unacceptable bacterial counts (bacterial counts above these standards). The range of CPC of raw milk samples was $1 \log_{10}$ to $6.2 \log_{10}$ cfu/ml. Thus all the raw milk samples analysed in the dry season and in the wet season had CPC higher than the acceptable limit. Likewise all the milk samples analysed from Accra and Kumasi had CPC higher than the acceptable limits. There was no significant difference ($p > 0.10$) in CPC between the two seasons and the two study sites. The range of TPC of raw milk samples was 0 to $9.4 \log_{10}$

Figure 3: Proportions of raw milk samples with unacceptable total bacteria plate count (% ATPC) in the dry and wet seasons.

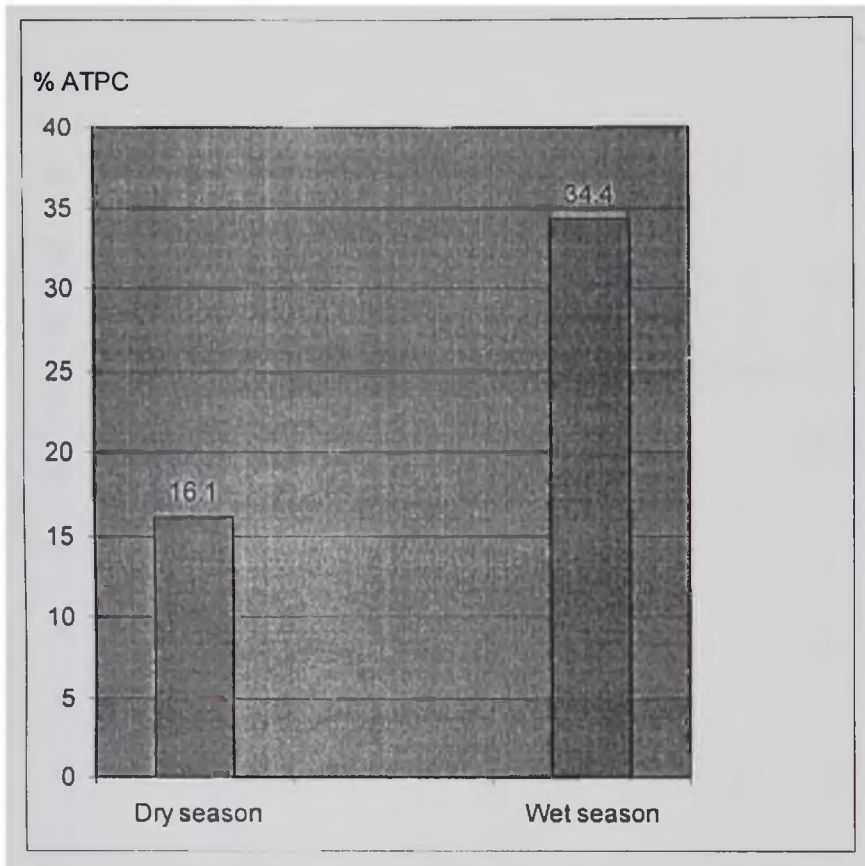


Figure 4: Proportions of raw milk samples with unacceptable total bacteria plate counts (% ATPC) for Accra and Kumasi.

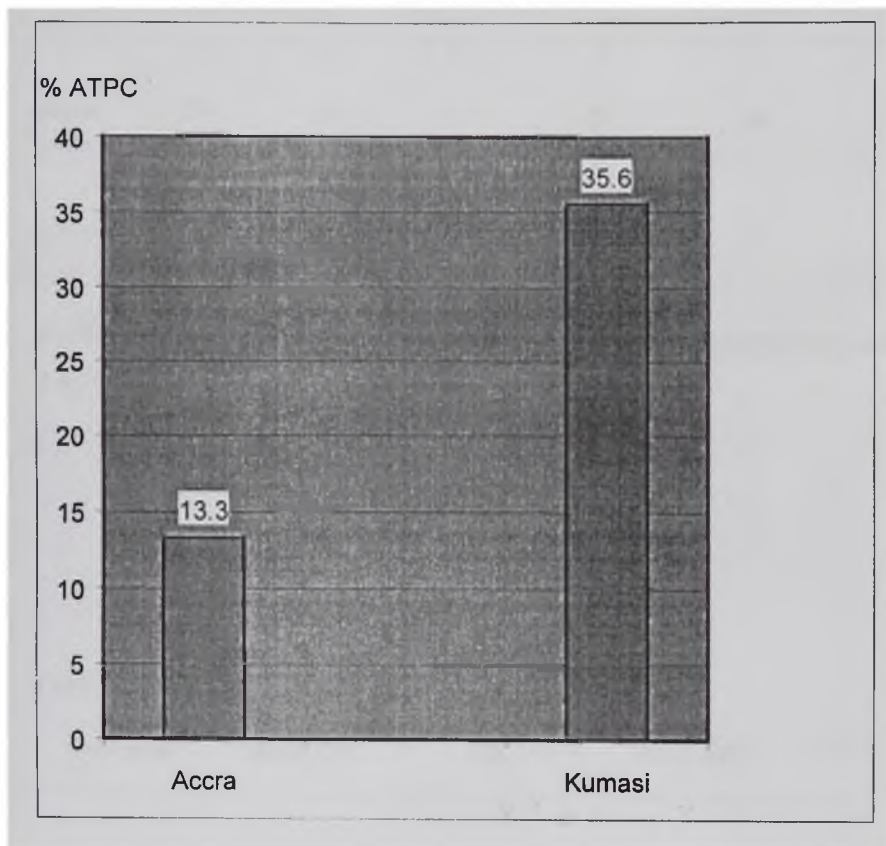


Table 9: Effect of season on total bacteria plate count of raw milk for Accra and Kumasi.

	Dry season			Wet season		
	N	n	%	N	n	%
Accra	89	60	67.4	57	38	67.7
Kumasi	110	36	32.7	71	23	32.4

n refers to number of samples with unacceptable TPC, % is its corresponding percentage and N refers to number of samples analysed

Table 10: Means of CPC and TPC of raw and processed milk.

Milk type	Mean CPC	SD	Mean TPC	SD
Raw milk	3.7 log ₁₀ cfu/ml	1.255	5.9 log ₁₀ cfu/ml	1.312
Processed milk	3.5 log ₁₀ cfu/ml	1.051	5.9 log ₁₀ cfu/ml	1.291

Kumasi. Out of the eight bacteria types identified in milk samples, only four were identified in milk from Kumasi namely *Klebsiella* spp., *Escherichia coli*, *Yersinia* spp. *Brucella abortus*. However, the only bacteria not identified in milk from Accra was *Klebsiella* spp. Three bacteria were present in milk from both Accra and Kumasi, which were *Escherichia coli*, *Yersinia* spp. and *Brucella abortus*. Table 11 also shows the prevalence of the various bacteria identified in milk. The overall prevalence rates were 0.008-30.5%; the most prevalent organism was *Brucella abortus* while the least prevalent was *Mycobacterium* spp. The prevalence rates of the various bacteria in milk for Accra and Kumasi were 0.008-37.6% and 2.6-25.1% respectively. The most prevalent bacteria in milk for both sites was also *Brucella abortus*. The least prevalent bacteria in milk for Accra was *Mycobacterium* spp while *Klebsiella* spp was the least prevalent for Kumasi. For the three bacteria present in milk from both Accra and Kumasi, a higher prevalence of *Brucella abortus* and *Yersinia* spp. were associated with milk of Accra, while a higher prevalence of *E. coli* was associated with milk of Kumasi. However, significant difference between the two sites was observed for only *Escherichia coli* ($p=0.06$).

Discussion: bacteriological quality of milk

The results indicate that whereas season and site were not very important factors in affecting bacteriological quality of raw milk, hygienic practices of milk handlers require serious consideration. Though minority of raw milk samples had TPC higher than the acceptable limit of the Ghana Standards Board, all the raw milk samples had CPC higher than the acceptable limit. Similar observations had been reported by Akyeh (2000) who had carried out bacteriological examination on milk samples obtained from a traditional dairy farm and the Ministry of Agriculture Dairy Farm, both situated on the Accra plains. He found that all the raw milk samples analysed had CPC higher than 0.7 log₁₀ cfu/ml and TPC less than

Table 11: Prevalence of bacterial types in milk from Accra and Kumasi

Bacteria type	Accra		Kumasi		Overall	
	n	%	n	%	n	%
<i>Escherichia coli</i>	52	17.3	51	23.7	103	20
<i>Brucella abortus</i> (antibodies)	56	37.6	49	25.1	105	30.5
<i>Mycobacterium</i> spp.	1	0.008	0	0	1	0.008
<i>Klebsiella</i> spp.	0	0	1	2.6	1	2.6
<i>Proteus</i> spp.	7	12.0	0	0	7	12.0
<i>Yersinia</i> spp.	15	25.9	4	10.5	19	19.3
<i>Bacillus</i> spp.	11	11.2	0	0	11	11.2
<i>Staphylococcus</i> spp.	14	24.1	0	0	14	14.6

6.3 log₁₀ cfu/ml. As CPC reflects the level of hygiene (Omore *et al.*, 2001), the results of this study indicate that milking or milk handling is done under very poor hygienic conditions. It was observed that milking of the cow was usually done in the kraals, which often were contaminated with mud, cow dung and urine. Several flies usually surrounded the milk during milking and it was not unusual to find particles of dirt, dung and some flies in the milk during and after collection.

Although, most of the processed milk samples had been subjected to boiling which destroys bacteria, the mean bacterial counts of processed milk were not significantly lower than those of raw unprocessed milk. Two factors may contribute to this. Firstly, bacterial cells can recover after thermal injury under the favourable tropical temperatures that prevail during transportation or at retail outlets that do not have chilling facilities (Andrew and Russel, 1984; Kayihura *et al.*, 1987). Secondly, there is the tendency of recontamination of processed

milk after heat treatment as a result of poor hygiene. This finding indicate that the shelf life of the raw milk and that of the processed milk probably were not different and as long as the problem of poor hygiene persists, milk processing to extend shelf life might not be worthwhile..

The poor hygiene of milking or milk handling as reflected in this study may also have serious public health consequences as several pathogenic microorganisms were introduced into milk under such situations. Improvement in hygienic handling of milk in many developed countries has eliminated several milk-borne pathogens, which no longer pose health threat in such countries (Ryser, 1998).

Discussion: bacterial flora of milk

Bacteria consist of a large number of genera that are widely distributed in nature and may be introduced into milk from a great variety of sources (Aibara, 1983). Consequently, a broad spectrum of bacteria can be present in milk as observed in this study. The poor hygiene of milking, or milk handling reflected in the first part of this study, may account largely for the presence of these organisms in milk. However, the presence of organisms like *Mycobacterium* spp. and *Brucella abortus* antibodies also indicate that infected cattle may be an important source of contamination. Generally, in terms of bacteria flora, milk from Kumasi was of better quality than that from Accra. This may be due to the training lessons on hygienic handling of milk offered milk traders in Kumasi by the Animal Science Department of the University of Science and Technology (Anonymous, 2002). All the bacteria identified in milk in this study are potentially pathogenic. Pathogenic bacteria in milk constitute an important public health concern, as several of them are responsible for dairy-related disease outbreaks. Though, some of them occurred in few samples, their presence in milk is still of

public health importance as the practice of pooling milk can lead to gross contamination of milk with such organisms. The public health implications of the various bacteria identified in milk are discussed below:

(i) *Bacillus* spp.

Bacillus spp are known to be common contaminants of raw milk (Ryser, 1998). Some species are however, pathogenic (for example *B. anthracis* and *B. cereus*). Therefore, its presence in milk is of public health concern. *B. cereus*, the main species associated with milk and milk products, enter milk from the soil, cattle feed, milking equipment or the udder during milking. However, *B. cereus* can also be shed into milk as a result of mastitis (Logan, 1988). The organism produces spores that are able to survive pasteurisation and germinate, producing toxins that cause food poisoning. Since its recognition as a food borne pathogen in the early 1950s, the organism has been involved in several outbreaks (Ryser, 1998). In Ghana, no outbreak has occurred yet. However, its high prevalence observed in this study is a cause for concern. It was surprising that *Bacillus* spp., a common contaminant of milk, was absent in milk from Kumasi. Considering the widespread occurrence of *B. cereus* in the natural environment, unless adequate care is taken, the organism could still pose milk-borne health risks despite its absence in milk from Kumasi in this study.

(ii) *Brucella abortus*

Brucella abortus causes brucellosis in both cattle and humans. In humans, the disease presents as a febrile flu-like illness and is common among pastoralists in Africa (Berman, 1981; Chukwu, 1987). Unlike other microorganisms investigated in this study, which were done by culture, *Brucella abortus* was identified in milk by detection of its antibodies in milk. Antibodies produced to *Br. abortus* infection in cattle usually appear in the serum and

milk (Nielsen *et al.*, 1996), and the antibodies in the two fluids correlate well (Smith *et al.*, 1989). In Ghana, the presence of the organism has been reported in milk (Ayebo *et al.*, 1976) and in cattle (Table 5) (Aning, 1999). In recent serological studies carried out on bovine serum in Southern Ghana, a prevalence rate of 38% of *Brucella abortus* antibodies was reported (VSD, 1997). This finding is in agreement with the prevalence of 37.6% observed in milk for Accra (Southern Ghana) in this study. This high prevalence of *Brucella abortus* antibodies observed in milk has several public health implications. Firstly, it indicates a high risk of contamination of milk with the *Brucella abortus* organism. Secondly, it shows that bovine brucellosis poses a great health threat to cattle herdsman since the disease can be transmitted to humans through contact with infected animals and consumption of raw milk (Buxton and Fraser, 1977). While the high incidence of bovine brucellosis may be due to the extensive cattle production system practised by farmers in this country, it also reflects the absence of effective brucellosis control measures in this country.

(iii) *Enterobacteria*

The four enteric bacteria identified (*Klebsiella* spp. *Proteus* spp. *Yersinia* spp. *E. coli*) indicate probable faecal contamination of the milk. Enteric bacteria are common inhabitants of the intestinal tract of various domestic animals including cow (Foster *et al.* 1958; Cruickshank, 1970), and are commonly found in cow dung, which was observed to be abundant at the milking environments and therefore easy contaminant of milk. All the enteric bacteria identified here are potentially pathogenic (Vasavanda and Cousin, 1992; Ryser, 1998). *E. coli*, which was the most abundant enteric bacteria found in milk in this study, has been accepted as a normal and probably beneficial inhabitant of the human bowel, but it has been implicated in some cases of appendicitis and urinary infections (Prescott *et al.*, 1993).

University of Ghana <http://ugspace.ug.edu.gh>
E. coli O157:H7, a serotype of enterohaemorrhagic *E. coli* has attracted attention in recent times due to its implication in several dairy related outbreaks (Riley *et al.*, 1983; Doyle, 1991; Ombui *et al.*, 1994). The organism is an important human health hazard causing haemorrhagic colitis and ureamic syndrome (Besser *et al.*, 1993).

The main species of *Yersinia* associated with food-borne illness is *Yersinia enterocolitica* (Vasavanda ad Cousin, 1992; Ryser, 1998). Although capable of growing at refrigeration temperatures, *Y. enterocolitica*, is generally regarded as an unusual cause of milk-borne diseases because of the low incidence of human pathogenic strains and the high susceptibility of the organism to pasteurisation (Ryser, 1998). However, under situations where milk is usually consumed unpasteurised as in Ghana and in other developing countries, the presence of the organism could be dangerous to public health causing problems such as gastroenteritis, and appendicitis (Abraham and Laryea, 1968; Vasavanda ad Cousin, 1992).

The other two enteric bacteria identified, *Klebsiella* spp. and *Proteus* spp. are not commonly associated with milk (WHO, 1962; O'Connor, 1994). However, apart from this study, their presence in milk in Ghana has also been reported by Akyeh (2000). These organisms are probably gaining importance as milk-borne pathogens in the country and need further investigation. *Klebsiella pneumoniae*, the main *Klebsiella* pathogen causes pneumonia, while *Proteus* organisms are associated with urinary tract and wound infections (Atlas, 1995)

(iv) *Staphylococcus* spp.

The presence of *Staphylococcus* in milk observed in this study is of public health concern, as some of its species are pathogenic. The main human staphylococcal pathogen is *Staphylococcus aureus* (Prescott *et al.*, 1993), which is usually present on the udder of the

University of Ghana <http://ugspace.ug.edu.gh>
cow and human skin. It may get access into milk during milking or handling of milk. The presence of this organism in milk in Ghana has also been reported by several workers (Abraham and Laryea, 1968; Ayebo *et al.* (1976); Akyeh (2000). When conditions favour growth of the organism in its environment, its presence is likely to be dangerous as it causes health problems such as osteomyelitis and abscesses (Wilson and Miles, 1957; Prescott *et al.*, 1993). It produces enterotoxins that cause food poisoning (Ryser, 1998). Although, *S. aureus* is readily destroyed in milk by pasteurisation, the toxins are heat stable and are not easily inactivated in food during cooking (Vasavanda and Cousin, 1992).

(v) *Mycobacterium* spp

Mycobacterium spp. was identified in one milk sample, which was obtained from Accra. However, Ayebo *et al.* (1976), and Aning *et al.* (2002) did not isolate *Mycobacterium* from milk taken from Accra or Kumasi. Two main pathogenic species of *Mycobacterium* are encountered in milk, namely *M. bovis* and *M. tuberculosis*, both of which are implicated in human tuberculosis (Grange *et al.*, 1994). Whereas *M. bovis* is usually introduced into milk from infected cattle, *M. tuberculosis* in milk results from poor handling practices (WHO, 1962; Ryser, 1998). Though *Mycobacterium* spp. was identified in only one milk sample (0.008%), its public implications should be taken seriously. The habit of mixing milk from different sources increases the chances of gross contamination as reported by Kleeberg (1984). He had indicated that one cow could excrete enough bacilli to contaminate the milk of up to 100 cows when milk is pooled. In recent times, there has been an increase in the number of human tuberculosis cases in Ghana. The findings of this study indicate that milk may play an important role in this situation.

4.3 Study 3: A study of the compositional quality and adulteration of milk samples

Summary:

Milk samples obtained in the dry and wet seasons, and from Accra and Kumasi were analysed for butterfat content (BF) and specific gravity (Sp.G). These were used to determine solids-not-fat (SNF) and total solids (TS) of the milk samples. Adulteration of the samples with water was also determined using the SNF values.

The mean BF, Sp.G, SNF, and TS determined were 3.25%, 1.030 kg/litre, 8.833% and 12.078% respectively. Overall, 18% of the milk samples were found to be adulterated with water. Adulteration observed in the wet season (51%) was significantly higher ($p=0.035$) than that of the dry season (25.8%). However, there was no significant difference ($p>0.10$) in adulteration between Accra (44.8%) and Kumasi (30.9%). Seasonal change in adulteration associated with Kumasi was highly significant ($p<0.001$) while that of Accra was not significant ($p>0.10$): Whereas for Accra, the extent of adulteration was higher in the dry season (59.6%) than in the wet season (46.5%), the reverse was observed for Kumasi (dry season= 41%; wet season= 53 %). The adulteration of milk with water observed could have serious public health consequences as the water used could introduce several health risks into milk.

Introduction:

On account of its composition, milk is regarded as nature's single most complete food (Frei, 1993). It consists of proteins, carbohydrates (lactose), lipids (fat), minerals, vitamins, and water. Good quality milk should have its physical and chemical constituents within certain ranges (Vanstone and Dougall, 1960). Usually when milk is adulterated changes occur in its

constituents. The specific gravity of milk measured at about 20°C is normally 1.026-1.032kg/litre (Ombui *et al.*, 1995). If the milk is mixed with air, for example by bumping during transport, the specific gravity would be 1.020 kg/litre or lower (FAO, 2000). The changes that occur in milk as a result of adulteration reduce its nutritional and processing quality, palatability, and market value (Omore *et al.*, 2001). Additionally, adulteration could also introduce chemical and microbial hazards into milk and pose problems to human health (O'Connor, 1994).

Indices of adulteration of milk include its specific gravity, butterfat content, total solids and, solids-not-fat. However, SNF, which combines specific gravity and butterfat, is a better measure of adulteration than specific gravity or butterfat (Omore *et al.*, 2001).

The objective of this study was to assess the compositional quality of milk samples and also the extent of adulteration with water. The specific gravity, butterfat content, total solids, solids-not-fat, and adulteration of milk samples were determined as described in Chapter 3.4.5.

Results:

The means of butterfat, specific gravity, total solids, and solids-not-fat of milk samples were 3.25%, 1.030 kg/litre, 12.1%, and 8.8% respectively. Overall, adulteration of milk with water was 18 % as determined by SNF values (SNF<8.5). Figure 5 shows the proportions of milk samples adulterated with water in the dry season and wet season. Adulteration observed in the wet season was significantly higher ($p=0.035$) than that of the dry season. In the wet season, 51% (103) of 202 milk samples analysed were found to be adulterated with water

University of Ghana <http://ugspace.ug.edu.gh>
while in the dry season, 25.9% (35) of 135 milk samples analysed were adulterated. Figure 2 illustrates proportions of milk samples adulterated with water for Accra and Kumasi.

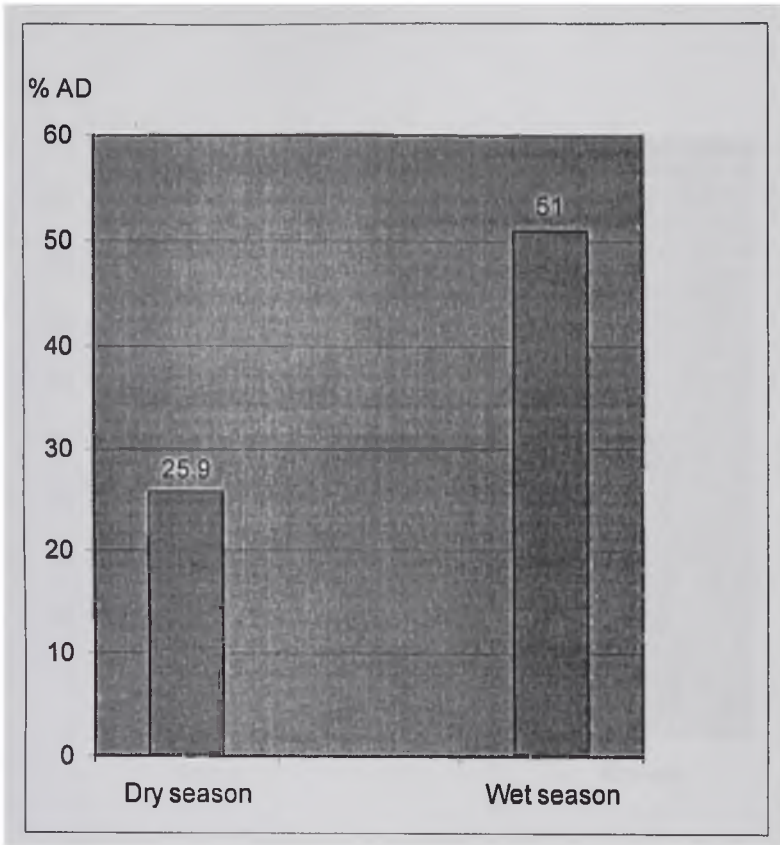
For Accra, 44.8% (64) of 143 milk samples analysed were adulterated with water, while for Kumasi, 30.9% (60) of 194 milk samples analysed were found to be adulterated. However, adulteration between the two sites was not significant ($p>0.10$). The effect of season on adulteration of milk with water for Accra and Kumasi is also shown in Table 8. Whereas the extent of adulteration was higher in the dry season than in the wet season for Accra, the reverse was observed for Kumasi. For Accra, the extent of adulteration decreased from 59.6% in the dry season to 46.5% in the wet season, while for Kumasi, it increased from 41% in the dry season to 53% in the wet season. However, the seasonal change in adulteration associated with Kumasi was highly significant ($p<0.001$) while that of Accra was not significant ($p>0.10$).

Table 12: Overall means of butterfat, specific gravity, total solids, and solids-not-fat of milk samples

Parameter	N	Mean	SD
Specific gravity	344	1.030	0.003
Butterfat	337	3.250	0.992
Solids-not-fat	337	8.833	1.151
Total solids	337	12.078	1.589

N refers to the number of milk samples analysed

Figure 5: Proportion of milk samples adulterated with water (%AD) in the dry season and wet season.



University of Ghana <http://ugspace.ug.edu.gh/>
Figure 6: Proportion of milk samples adulterated with water (%AD) for Accra and Kumasi.

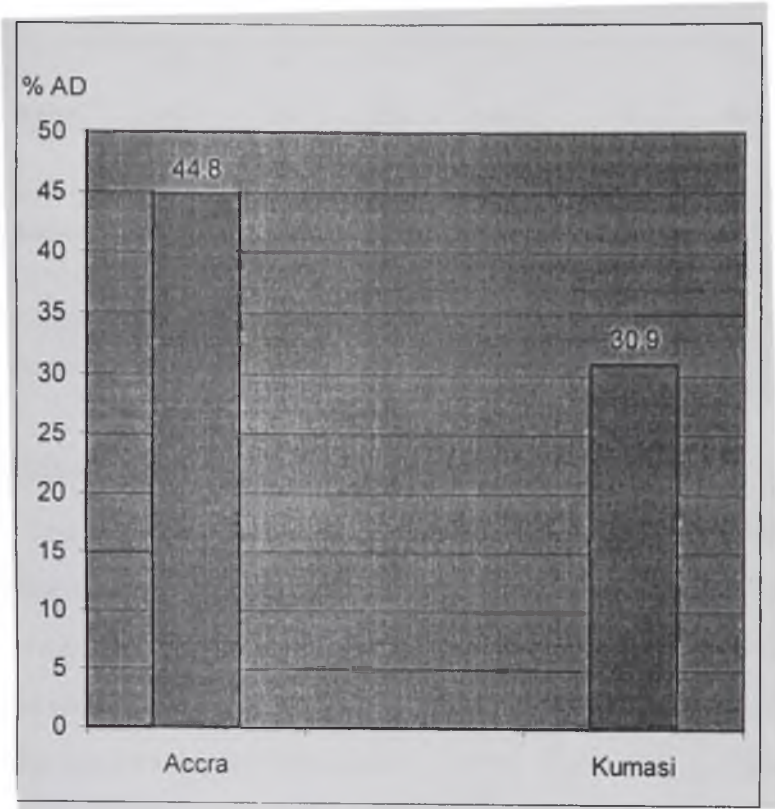


Table 13: Effect of season on adulteration of milk with water for Accra and Kumasi

	Dry season			Wet season		
	N	n	%	N	n	%
Accra	57	34	59.6	86	40	46.5
Kumasi	78	32	41	116	62	53

n refers to number of samples adulterated with water, % refers to its corresponding percentage, and N refers to number of milk samples analysed.

Discussion:

The overall means of Sp.G, BF, SNF, and TS of milk samples determined all fall within the expected ranges of the respective components of normal bovine milk (Vanstone and Dougall, 1960). This indicates that the overall compositional quality of the milk is good and the extent of adulteration with water may not be much, and was found to be 18%. A similar observation has also been reported by Karikari *et al.* (1998) about milk from Kumasi. However, lower rates of adulteration of milk with water (4.7-10.4%) have been reported in Kenya by Ombui *et al.* (1995) and Omore *et al.* (2001).

The practice of adding water to milk could have serious public health consequences, as the water used could be a source of health risks in the milk. It was observed that water sources used by most milk traders was not treated water, but rather other sources like streams, rivers, wells, rain etc. These latter forms of water are not usually treated in anyway, and these are likely to introduce microbial risks into the milk if used for adulteration. Untreated water could contain several pathogenic microorganisms mainly from faecal pollution, and such organisms include *Clostridium perfringes*, *Streptococcus faecalis*, *Escherichia coli*, *Proteus*

vulgaris, *Salmonella typhi*, and *Vibrio cholerae* (Round *et al.*, 1971; Skinner and Schewan, 1977; Ryser, 1998). Some of these organisms, such as *Vibrio cholerae*, produce toxins that could affect human health adversely even in the absence of the organisms (Atlas, 1995). Two of the organisms (*Escherichia coli* and *Proteus*) were also found in milk samples analysed (Study 2), and may have been introduced into it probably through adulteration. Apart from pathogenic microorganisms, untreated water could also contain several chemical hazards such as cyanide, mercury, antibiotics, and other other synthetic organic chemicals used in farming (Aibara, 1983; O'Connor, 1994). Such chemicals, which enter water through pollution, could also end up in milk through adulteration and cause problems to human health (Aibara, 1983). Though overall, a small proportion of milk samples were adulterated, with bulking, which was commonly observed among market agents, large volumes of milk could become contaminated with hazards introduced through adulteration. This is particularly dangerous where the milk serves a large number of people.

There was no significant difference in adulteration between Accra and Kumasi, indicating that site was not an important factor in adulteration. Adulteration of milk therefore seems to be a practice that generally occurs across the two sites. Season was found to be the important factor affecting adulteration, with the wet season associated with a higher adulteration than the dry season. This contrasts with the normal tendency to add water to milk during periods of low milk in the dry season, observed by Omore *et al.* (2001). This indicates that, in Ghana, adulteration of milk with water may not be primarily related to amount of milk supply. Further studies are required to establish the main factors underlying the practice of adulterating milk with water in the country. Such information would be necessary to protect the public against hazards associated with milk adulteration.

Study 4: An evaluative study of the milk handling and hygienic practices of milk market agents and their effect on milk quality and public health.

Summary:

Data were collected in the dry and wet seasons, and from Accra and Kumasi to assess milk handling and hygienic practices of market agents, and the effect on milk quality and public health risk.

Handling containers were found to be predominantly plastic, with over 99% of market agents using them. None of the market agents refrigerated milk, while only 8.5% boiled or pasteurised milk before sale. Milk from the farm was the major source of milk, and milk sourced from the farm to producers constituted the biggest pathway accounting for 61.4% of all the milk pathways.

Several milk handling and other marketing factors were found to influence milk quality and public health hazards significantly ($p < 0.10$). High bacterial counts were associated with use of plastic containers, and also the use of soap (compared with detergent) and gravels (compared with sponge) for cleaning milk containers. Milk handled by wholesalers was strongly associated with high bacterial counts. Milk of two milk pathways namely Herdsman_Processor (milk sourced from herdsman to processor) and Trader/hawker_Retailer (milk sourced from Trader/hawker to retailer) were also associated with high bacterial counts. High levels of contamination of milk with antimicrobial agents were associated with only milk sourced from the farm, and milk handled by farmers or producers. The association of the pathway: Trader/hawker_Retailer, and milk of wholesalers with poor hygiene (High bacteria counts) indicates that milk contamination from poor hygiene is not limited to only the milking environment at the farm, but includes the hygienic practices of milk traders outside