Factors influencing the bacteriological quality of raw milk produced on dairy farms in Central South Africa

By

Celmarie Louw

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Magister Technologiae: Environmental Health,

School of Health Technology,

Faculty of Health and Environmental Sciences,

Central University of Technology, Free State

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Declaration

I, Celmarie Lynette Louw (student number 9108858), do hereby declare that this research project submitted to the Central University of Technology, Free State, for the degree Magister Technologiae: Environmental Health is my own independent work. This research project was conducted at the Central University of Technology, Free State, under the supervision of Prof. Annabel Fossey and co-supervised by Dr. Elsa Potgieter.

_____ _____

Signature of student Date
Celmarie Louw

I certify that this statement is correct.

_____ _____

Signature of supervisor Date
Prof. Annabel Fossey

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Abstract

Introduction

Dairy farms in central South Africa produce a substantial amount of milk, which is sold in Bloemfontein, Free State. Large volumes of unpasteurized (raw) milk is collected on the dairy farms, which undergoes further processing before it reaches the consumer at the end of the production line. There is a large proportion of the population that, in most cases unknowingly, consumes raw milk that has bacterial counts substantially higher than legal standards. Poor quality unpasteurized milk is either sold as fresh milk in the informal market, or as dairy products, such as cheese, manufactured from unpasteurized milk. Consumers are therefore, in most cases, unaware of the poor quality dairy products they consume. Milk quality is usually assessed in terms of bacterial content, which include *Escherichia coli,* coliforms and total bacterial count. The bacterial quality of milk is influenced by a number of factors, including farming practices, structural design of the milking shed, herd health and quality of water used in the dairy. If the highest level of hygiene practices is maintained, contamination of the milk by pathogenic microorganisms will be controlled, however, any drop in the vigilance of hygiene practices could result in unacceptable high levels of pathogenic microorganisms resulting in poor quality raw milk. Poor quality raw milk will inevitably result in poor quality pasteurized milk, containing unacceptably high levels of pathogenic organisms, which will eventually reach the consumer.

Objectives

The objectives of this study were to assess the quality of milk and influencing factors of milk produced on 83 dairy farms that supply milk intended for further processing to the greater Mangaung region, Central South Africa. Influencing factors investigated included, water quality and hygiene of milk contact surfaces, namely pulsator surfaces and milk pipeline surfaces.

Methods

Standard sampling procedures were followed when milk was sampled from bulk milk tanks, water at the point of use in the dairy, as well as collection of surface swabs. *Escherichia coli*, coliforms, total bacterial counts and somatic cell counts in milk were determined in terms of the regulations relating to milk and dairy

products, and for water in terms of drinking water standards. These data were analysed and the factors that directly influence bacterial quality of milk were identified.

Results

93% of the dairy farms displayed *E. coli* in their bulk milk containers, which did not comply with the legal standard. For coliforms, 86% of the milk samples did not comply with the legal standard. The total bacterial count of 85% of the milk samples did comply with the legal standard. The somatic cell count of 42% of the milk samples did not comply with the legal standard. The pulsator surfaces as well as the milk pipeline surfaces of 13% of the dairy farms displayed the presence of *E. coli.* 80% of the pulsator surfaces and 78% of the milk pipeline surfaces did comply with the legal standard pertaining to coliforms. The total bacterial count of pulsator surfaces revealed that 19% complied, whereas 29% of the milk pipeline surfaces complied with the legal standard. The water data further revealed that 31% of the dairy farms contained *E. coli* in the water used in the dairies. 63% of the dairy farms contained more than the allowable number of coliforms in their water. Chi-square tests revealed significant differences ($p > 0.05$) between the presence or absence of *E. coli* in milk and water; the presence or absence of *E. coli* in milk and milk pipeline surfaces; the presence or absence of *E. coli* in milk and pulsator surfaces and the presence or absence of *E. coli* in milk and the positioning of the cows in the milking shed. When milk quality indexes were calculated for all the farms, only four farms were classified with excellent milk, the remainder were all classified as producing poor quality milk. The hygiene quality indexes revealed that the hygiene practices on all the farms were not up to standard.

Discussion and conclusion

The study revealed that the milk produced for commercial processing and distribution in the greater Mangaung region of central South Africa was of poor quality. It is often mistakenly believed that the pasteurization process will remove all microorganisms from milk. As this is not the case, it is of major concern that milk delivered commercially is not of acceptable quality. Furthermore, it could be concluded that the quality of milk products from raw milk were also probably not of acceptable quality. The results further revealed that the possible contributing factors to the poor quality milk produced by the 83 commercial dairy farms were; poor quality water used in dairy sheds and contaminated milk contact surfaces. From this study it could be concluded that the overall status of milk production on the 83

commercial dairy farms studied, did not meet the standards required for milk quality, water quality and hygiene practices.

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Introduction

1.1 Introduction

Dairy farming is the fourth largest agricultural industry in South Africa with a representation of 6% of the gross value of overall agricultural production (Mkhabela *et al*. 2010). Compared to the red meat industry, which showed a decline in gross income of 9%, the dairy industry was one of South Africa's fastest growing agricultural sectors during the 2000/2001 season, with a growth of 17% (Mkhabela *et al*., 2010). The dairy industry is also a major contributor to the South African economy through employment, with about 60 000 farm workers employed by more than 4 000 milk producers.

The total number of milk producers, as recorded in January 2008 was 3 665, of which 919 milk producers were situated in the Free State Province (Mkhabela *et al*., 2010). Over and above the 2.37 million litres of milk produced per annum, South Africa also imports milk. In 2007 South Africa imported nearly 5 million litres of milk and 10 million kilograms of concentrated milk and milk powder (Mkhabela *et al*., 2010).

In South Africa over 300 processors and manufacturers buy and process milk, while approximately 500 producer-distributors supply liquid milk and fresh dairy products to the consumer. Even though large dairy companies represent a small fraction of all dairy processors, they process over 80% of the total volume of milk delivered to dairies, producing a wide range of dairy products including pasteurised milk, UHT milk and cheese (Mkhabela & Mndeme, 2010).

1.2 Aims and objectives

Dairy farms in central South Africa produce a significant amount of milk which is sold in Bloemfontein, Free State Province, South Africa. Large volumes of unpasteurized (raw) milk is collected on dairy farms in central South Africa, which undergoes further processing before it reaches the consumer in the form of pasteurized milk and other dairy products at the end of the production line.

There is however a large proportion of the population that, in some cases unknowingly, consumes raw milk that has bacterial counts significantly higher than legal standards as set out in the Regulations Relating to Milk and Dairy Products (Regulation R. 1555 of 1997) promulgated under The Foodstuffs Cosmetics and Disinfectants Act No 54 of 1972 (Lues *et al.,* 2003). This ignorant consumption of poor quality unpasteurised milk is either due to the selling of fresh milk in the informal market, or because of dairy products, such as cheese, manufactured from unpasteurized milk (Altalhi & Hassan, 2009). Consumers are therefore sometimes unaware of the poor quality dairy products they consume. The consumption of raw milk is unfortunately not well documented, consequently information is not available on the volumes of raw milk consumed (Claeys *et al*., 2013).

Despite numerous epidemiological studies showing the health risks involved in the consumption of unpasteurized milk, there is a current trend towards the consumption of products in their natural form (Oliver *et al*., 2009; Claeys *et al*., 2013). Consumption of unpasteurized milk continues even though people are aware that milk can be contaminated with various pathogens associated with disease in humans. Some of the reasons advocating this trend is the enhanced nutritional properties, taste and health benefits some consumers believe unpasteurized milk has (Oliver *et al*., 2009; Claeys *et al*., 2013).

Pasteurized milk is readily available to the consumer through different outlets, which can include retail supermarkets, general dealers, convenience stores, but also milk depots where the consumer collect milk in their own containers. Because the milk industry is diversified to a great extent, with participants operating at different levels of sophistication, it is creating a challenge for law enforcers to manage effective control measures to ensure milk safety. Large milk processing companies constantly implement measures to ensure safe milk with an extended shelf life. There are some of the smaller milk producers and milk processors that are causing concern regarding the quality of milk that reaches the consumer. The image of the dairy industry is thus, seriously damaged by irresponsible selling of insufficiently pasteurized milk, as well as raw milk sold illegally (The South African Milk Quality Forum).

Milk quality is usually assessed in terms of bacterial content, which is influenced by a number of factors. These factors include farming practices, structural design of the milking shed, herd health and quality of water used in the dairy. If the highest level of hygiene practices is maintained, contamination of the milk by pathogenic microorganisms will be controlled, however, any drop in the vigilance of hygiene practices could result in unacceptable high levels of pathogenic microorganisms resulting in poor quality raw milk. Poor quality raw milk will inevitably result in poor quality pasteurized milk, containing

unacceptably high levels of pathogenic organisms, which will eventually reach the consumer. Poor quality pasteurized milk, therefore, will not only have a limited shelf life, but poses a health risk to the consumer because of the possible presence of pathogenic organisms. One example is *Listeria monocytogenes*, which has been involved in several outbreaks and sporadic cases of disease associated with the consumption of pasteurized milk and dairy products in Switzerland, United States of America, Denmark and Finland (De Buyser *et al*., 2001). Unpasteurized milk was associated with 52 %, and pasteurized milk with 37% of milk-borne disease outbreaks in England and Wales between 1992 and 2000. *Salmonella* and *Campylobacter* was some of the most common pathogens detected in the England and Wales outbreaks (Baylis, 2009).

Through routine sampling of milk sold in Bloemfontein, the Environmental Health Division of Mangaung Local Municipality realized a rapid deterioration in the quality of milk that reaches the consumer (personal experience). This deterioration in the quality of the milk was significant with reference to milk sold from bulk milk tanks. A number of factors may contribute to this deterioration, but the Environmental Health Division decided to follow a Hazard Analysis Critical Control Point (HACCP) approach to this problem. Therefore the Division decided to monitor the production of milk on farm level, as well as the environment in which the milk is produced.

The aim of this study was, therefore, to investigate factors that influence bacteriological quality of raw milk produced on dairy farms in central South Africa. To address this aim, the following objectives were devised:

- To assess the bacterial quality of raw milk produced on selected farms in central South Africa (83 dairy farms were included in the study);
- To assess the bacterial quality of water used in the dairies;
- To evaluate the influence of dairy farm practices on bacterial quality of raw milk;
- To evaluate the influence of milking shed infrastructure on bacterial quality of raw milk;
- To evaluate the influence of hygiene maintenance on bacterial quality of raw milk; and
- To evaluate the influence of cow health on bacterial quality of raw milk.

Milk safety and quality starts on the farm where the production of milk should be conducted in such a manner that milk quality is not compromised. There is however factors which have an impact on quality and safety of milk, from production to consumption, and strategies should be implemented to improve the overall quality of milk and other dairy products.

Chapter 2
Literature review

2.1 Introduction

Control of bacterial content in raw milk is very important for public health (Barbano *et al*., 2006), as a high bacterial count in raw milk contributes to the limited shelf-life of milk and dairy products produced from raw milk. Raw milk hygiene is one of the main contributors to safe milk in the dairy industry (Millogo *et al.*, 2010). It is important to note that many large milk processing companies calculate the milk price, to be paid to the farmer, according to the total bacterial count as well as the somatic cell count, both which must be as low as possible (Verdier-Metz *et al*., 2009). Good quality raw milk is therefore important for a profitable dairy industry (Van Schaik *et al*., 2005).

Numerous dairy programmes have been implemented in developing countries to increase milk production (Bonfoh *et al.*, 2003). These programmes do not necessarily include milk hygiene. Therefore, the objective of most dairy development programmes has been to increase the volume of milk intended for human consumption, but does not focus on the improvement of the quality of milk (Millogo *et al*., 2010). Consequently, milk hygiene does not necessarily feature in these programmes. This is evident, especially in developed countries, because management practices rather focus on aspects such as the implementation of new veterinary and biological technologies, which include artificial insemination and embryo transfers, machine milking and improved levels of disease control, all to increase the milk yield per cow. Dairy programmes have resulted in an approximate increase of 22% in milk production in the Organisation for Economic Cooperation and Development (OECD) countries between 1992 and 1996. This increase was because of a 49% increase in productivity, even though there was a steady decline in the number of cows milked during this period (Mkhabela *et al*., 2010).

2.2 Dairy farming in Africa

In African countries, including South Africa, dairy farming is commonly practiced as part of mixed farming. Farmers do not always consider dairy farming as a high priority, and therefore, dairy farming is often only a sideline business that is mainly conducted to subsidize main farming activities. These

farmers generally focus upon other activities, such as grain- and sheep-farming, which generates long-term income (Stewart, 2002). Because of the more subordinate role of dairy farming, the income generated is not always utilized to maintain and improve milking equipment and structural conditions, causing deterioration in the conditions under which milk is produced and stored (Personal experience).

In South Africa, milk processors have implemented a premium and penalty scheme relating to the somatic cell count, total bacterial count as well as fat and protein content of milk collected from dairy farms. This scheme entails that lower prices are paid for milk with high somatic cell and total bacterial counts, and low fat and protein content. Despite the implementation of this scheme, milk is seldom refused by milk processors, even if it is of poor quality. However, in cases where poor quality milk is refused by processors, the milk is sold in the informal sector. Dairy farmers will, therefore, inevitably receive some money for their milk, which means that even poorly managed dairy farms remain operational even though they do not always meet legal requirements for infrastructure and production of milk (Stewart, 2002).

2.3 Milk quality

2.3.1 Introduction

Generally the term "quality of milk" refers to the bacterial content of the milk, which is dependant on the entire milk production process, and thus should not only be associated with the product itself (Noordhuizen & Metz, 2005). Milk can be contaminated during different stages throughout the milking process. Because milk is such a nutritious medium, it is attractive for a variety of bacteria, including spoilage and pathogenic bacteria, to grow and multiply in. Factors contributing to the contamination of milk include contact with animals and personnel engaged in milk processing, unhygienic milking equipment, poor quality water used in the dairy and poor herd health (Altalhi & Hassan, 2009).

Towards the end of the 19th century pasteurization of raw milk was introduced to decrease bacterial content in order to improve the safety of milk (Lund *et al*., 2002). Pasteurization is defined as the process of heating milk for a predetermined time period at a predetermined temperature (Table 1.1) to destroy pathogens (LeJeune & Rajala-Schultz, 2009).

Table 1.1 Time and temperatures for pasteurization of fluid milk approved by the United States Food and Drug Administration (taken from LeJeune & Rajala-Schultz, 2009).

Temperature	Time in seconds
63ºC (145ºF)	1800
72ºC (161ºF)	15.0
89ºC (191ºF)	1.0
90ºC (194ºF)	0.5
94ºC (201ºF)	0.1
96ºC (204ºF)	0.05
100ºC (212ºF)	0.01

The process of pasteurization is responsible for the improvement of the safety and the lengthening of the shelf life of dairy products. This is accomplished by the reduction of the number of bacteria in milk before the end-product reaches the consumer. It is, however, important to note that pasteurization of raw milk is not effective in eliminating all microorganisms and their enzymes, spores and toxins. The thermal destruction process is logarithmic and eliminates bacteria at a rate that is proportional to the number of bacteria present in raw milk (LeJeune & Rajala-Schultz, 2009).

In most countries, restrictions and legislation on the marketing of unpasteurized milk have been introduced with the intention to minimize milk-associated health hazards. Even though pasteurization improves the bacterial quality of milk, it does not necessarily guarantee the safety of milk products, because in instances where the bacterial count is high in raw milk, pasteurization will not be able to kill all bacteria within the short period of time of its application (Lund *et al.,* 2002).

Psychrotolerant bacteria are considered to be a major cause of food spoilage, because of their ability to grow at low temperatures (Raats *et al.*, 2011). These organisms are able to grow at temperatures as low as 0ºC but have an optimal growth temperature of 20ºC (Maänistö & Puhakka, 2002). Even though psychrotolerant bacteria are destroyed by high temperature treatments, they are able to produce heat-resistant extracellular enzymes which can survive the pasteurization process, causing undesirable flavours, as well as a reduced shelf life of the milk and milk products (Elmoslemany *et al*., 2009a).

Thermoduric microorganisms are a group of bacteria that are able to survive the pasteurization process. This group includes the genera *Micrococcus*, *Microbacterium*, *Streptococcus*, *Lactobacillus*, *Bacillus*, *Clostridium*, the coryneforms, and occasionally some Gram-negative rods (Aaku *et al*., 2004).

The spore forming bacteria, *Bacillus* and *Clostridium* are of major public health concern. The spores that these bacteria form are able to survive for long periods of time in a variety of food products, including dairy products. These spores are also among the most resistant forms of living organisms, which are able to survive the pasteurization process, making them difficult to remove once they are present in the milk (Lin *et al*., 1998; Ranieri *et al*., 2009; Garde *et al*., 2011). Furthermore, vegetative cells, which are formed after spore germination, are able to multiply at a range of different temperatures (Carlin, 2011).

Despite pasteurization and legislation, numerous outbreaks of milk-borne diseases have occurred worldwide, mostly caused by either improper pasteurization or by post pasteurization contamination. Even though these outbreaks have occurred, the global incidence of foodborne diseases is difficult to estimate (Gran *et al*., 2003). It was estimated in 2005 - that 1.8 million people died from diarrhoeal diseases worldwide, caused by the consumption of contaminated food and drinking water (Velusamy *et al.,* 2010).

The presence of pathogenic bacteria in milk can be considered as a major public health risk, especially for people who consume raw milk and dairy products manufactured from raw milk (Chye *et al.*, 2004). A large number of people, especially in rural areas, consume raw milk directly. However, raw milk is indirectly consumed by a much larger part of the population, through the consumption of several types of cheeses manufactured from raw milk (Altalhi & Hassan, 2009).

The Environmental Health Sector of South Africa recently raised concerns about milk produced by some formal, and an increasing number of informal farmers, who often fail to meet the legal bacteriological standards. Many of these informal farmers are amongst the traditionally disadvantaged communities that are ignorant of practices that can contribute to production of good quality milk. For this reason, it is important to assess the state of milk hygiene and to investigate solutions in order to rectify problem areas that influence milk quality and safety (Lues *et al.*, 2003).

Some of the reasons why the dairy industry should focus more on the microbial quality of raw milk, include (Oliver *et al.*, 2005):

(1) outbreaks of diseases in humans traced back to the consumption of poor quality unpasteurized and pasteurized milk,
(2) milk producers, farm employees and their families, and neighbors often consume unpasteurized milk,
(3) unpasteurized milk is also consumed directly by a large number of people through the consumption of several types of cheeses manufactured from unpasteurized milk,
(4) pasteurization may not destroy all foodborne pathogens in milk, and
(5) inadequate pasteurization will not destroy all foodborne pathogens (Oliver *et al*., 2005).

There is also a wide range of pathogens that are able to survive and thrive in post-pasteurization processing environments which could lead to persistent contamination of dairy products. These pathways pose a risk to the consumer not only because of the direct exposure to foodborne pathogens present in unpasteurized dairy products, but also due to the consumption of dairy products that became re-contaminated after pasteurization.

2.3.2 South African milk standards

South African milk standards are set out in the Regulations Relating to Milk and Dairy Products (Regulation R. 1555 of 1997), promulgated under The Foodstuffs Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972). South African legislation provides clear prescriptions concerning bacterial content of milk, which include total bacterial count, presence of pathogens, including the presence of *Escherichia coli* and coliform bacteria.

Various bacterial indicators are used to describe the quality of milk and the general status of hygiene practices in a dairy. Total bacterial count is used as an indicator of the general quality of milk. The Regulations Relating to Milk and Dairy Products (Regulation R. 1555 of 1997), promulgated under The Foodstuffs Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972), stipulate that raw milk intended for further processing may not contain more than 200 000 colony forming units (CFUs) per 1.0 ml of bovine milk. The same legislation also stipulates that pasteurized milk may not contain more than 50 000 CFUs per 1.0 ml of milk. Because *E. coli* is naturally found in the intestinal tract of animals and

humans, its presence in milk is widely used as an indicator for faecal contamination of milk. This legislation thus specifies that raw milk intended for further processing may not contain any *E. coli* per 0.01 ml of bovine milk. Pasteurized milk may not contain any *E. coli* per 1.0 ml of bovine milk. Coliform bacteria on the other hand, are used as an indicator of the general hygiene throughout the milk handling process. The legislation stipulates that raw milk intended for further processing, may not contain more than 20 CFUs of coliform bacteria per 1.0 ml of bovine milk, while pasteurized milk may not contain more than 10 coliform bacteria per 1.0 ml.

2.3.3 Milk pathogens

Not all bacteria found in milk are harmful. Milk pathogens are considered to be those bacteria that have the potential to cause disease in humans. According to the Regulations Relating to Milk and Dairy Products (Regulation R. 1555 of 1997), promulgated under The Foodstuffs Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972), raw milk intended for further processing may not contain "pathogenic organisms, extraneous matter or any inflammatory product or other substance which for any reason whatsoever may render the milk unfit for human consumption".

Some of the pathogens that have been involved in food-borne outbreaks associated with the consumption of milk include *Listeria monocytogenes*, *Salmonella*, *Campylobacter*, *Yersinia enterocolitica*, *E. coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Clostridium botulinum* (Leclerc *et al*., 2002). It is for this reason that The Department of Health and Human Services in the United States of America has launched a "Healthy People 2010" initiative, with the aim of promoting health and disease prevention with the objective to improve the health of all people during the first ten years of the 21st century. Primarily the focus is upon improved food safety and the reduction in the incidence of foodborne diseases caused by *Campylobacter, E. coli* O157:H7*, Listeria* and *Salmonella* (Gandhi & Chikindas, 2007).

Listeria monocytogenes

Listeria spp. is a psychrotrophic pathogen that has the ability to grow and multiply in food products or other matter over a wide temperature range, namely 0ºC - 45ºC, at pH levels between 4.4 and 9.4 (Aygun & Pehlivanlar, 2006; Kalorey *et al.*, 2008).

Listeria spp. is widely found in soil, plants, silage, animal manure and contaminated water (Kalač, 2011). Silage contaminated with *L. monocytogenes* has been documented to be the cause of the initial infection of farm animals (Kalač, 2011). *Listeria monocytogenes* is able to pass through the intestinal tract of cows, without being affected. Consequently, *L. monocytogenes* can cause contamination of the dairy environment through the shedding of faeces by infected cows (Kalač, 2011). *L. monocytogenes* attaches to different surfaces and can later detach itself causing contamination of food, including milk, and often contaminates milk when milking and processing equipment has not been properly cleaned (Aarnisalo *et al*., 2006).

A study reported that 2 – 16% of healthy cows carry *L. monocytogenes* in their gastro-intestinal tract and excretes the organism into the farm environment, with potential contamination of milk during the milking process (Hassan *et al*., 2001). It is therefore understandable that, because of the ubiquitous nature of *Listeria* spp., frequent contamination of dairy products and other foodstuffs may occur (Meyer-Broseta *et al*., 2003; Aygun & Pehlivanlar, 2006).

Listeria monocytogenes may cause the disease listeriosis in humans when contaminated food, including milk, cheese and other dairy products are consumed. Listeriosis is a potentially lethal disease with a mortality rate of approximately 30% (O'Brien *et al.*, 2009). It is estimated that up to 99% of all human listeriosis cases appear to be acquired by means of the food-borne route (Ho *et al*., 2007). People that are mainly at risk of listeriosis are pregnant women, infants, immune-compromised patients, including cancer patients and HIV positive patients, as well as the elderly (Amagliani *et al*., 2004; O'Brien *et al*., 2009; Todd & Notermans, 2010). Symptoms of human listeriosis in otherwise healthy people are similar to flu, but in the high risk group of people, clinical symptoms are more severe, including septicaemia, meningitis and abortion (Hassan *et al.,* 2001).

Many outbreaks of listeriosis have been reported demonstrating why exposure should be prevented (Ho *et al*., 2007). An estimated 2 500 *L. monocytogenes* infections occurred in the United States of America during 1999, with approximately 500 deaths, with a mortality rate of 20% (Makino *et al*., 2005; Todd & Notermans, 2010). In 2006 in the Czech Republic, 78 cases with 13 fatalities in three outbreaks were reported after consumption of contaminated soft cheese. During 2007, 21 cases of which five fatalities were reported in Norway after the consumption of soft cheese manufactured from raw milk. During 2008 in Canada (Quebec), 21 cases of listeriosis were reported with one fatality, also after the consumption of soft cheese manufactured from raw milk (Todd & Notermans, 2010). Dairy

products contaminated with *L. monocytogenes* have been implicated in almost 50% of all the listeriosis outbreaks annually reported in Europe (Kousta *et al*., 2010).

***Salmonella* spp.**

Salmonella spp. are common contaminants of water and food. *Salmonella* spp. are non-fastidious pathogens which are able to grow at temperatures ranging from 5.2ºC to 46.2ºC, and at pH levels between 4.1 and 9.0 (Abushelaibi *et al.,* 2003). Even though effective pasteurization is able to kill *Salmonella* spp., freezing and refrigeration fails to destroy the pathogens (Abushelaibi *et al.*, 2003). This pathogen colonizes the intestinal tracts of humans, animals, birds and insects. Contamination of water and milk is therefore usually through faecal matter. Two most common *Salmonella* serotypes that cause salmonellosis are Salmonella Typhimurium and Salmonella Enteritidis *(*Abushelaibi *et al., 2003)*.

Salmonella was reported to be the causative agent of an estimated 1.4 million cases of foodborne illness and more than 500 deaths per year in the United States of America (Cetinkaya *et al.*, 2008). For example, in 1981 in the United States of America there were 321 cases of food poisoning after consumption of Mozarella cheese made of pasteurized milk contaminated with *Salmonella* (Kousta *et al*., 2010). More than 1 700 cases of food poisoning were reported after consumption of cheddar cheese made from unpasteurized milk (Kousta *et al*., 2010). *Salmonella* was also identified as the causative agent of a food poisoning outbreak in Illinois in 2006, after consumption of Latin-style aged cheese, affecting 85 people (Oliver *et al*., 2009). The majority of human *Salmonella* infections are thus derived from the consumption of contaminated food of animal origin (Cetinkaya *et al*., 2008). This contamination could occur through cross-contamination resulting from improper or insufficient cleaning and disinfection of equipment and food contact surfaces (Møretrø *et al*., 2012). Typical symptoms of *Salmonella* infections include nausea, vomiting and diarrhea, while complications associated with the infection include septicemia or reactive arthritis (Kousta *et al*., 2010).

***Campylobacter* spp.**

Campylobacter spp. are microaerophilic and thermotrophic bacteria (Kärenlampi & Hänninen, 2004; Whyte *et al*., 2011). They are different from other milk pathogens, as they are essentially

microaerophilic, and grow optimally in an atmosphere containing 10% CO_2 and 5% O_2 (Humphrey *et al.,* 2007). The optimal growth temperature of *Campylobacter* spp. ranges between 37ºC and 42ºC and the optimal pH value for growth is from 6.5 to 7.5, but are not able to grow in a pH below 4.9 (Whyte *et al.,* 2011). Of the 17 species within the genus *Campylobacter, Campylobacter jejuni* is considered to be one of the most important species from a food safety point of view (Horrocks *et al*., 2009).

Cattle can harbour *C. jejuni* in their gastrointestinal tract and therefore, the presence of *C. jejuni* in raw milk is most commonly derived from secondary faecal contamination during the milking process. It has also been reported that *C. jejuni* can be excreted directly into milk through soil or faeces from the udder of the cows (Heuvelink *et al.*, 2009). Because of heat sensitivity of *C. jejuni,* pasteurization is found to be effective in eliminating the organism in milk (Whyte *et al.,* 2011). It is therefore understandable that infections are usually caused by the consumption of unpasteurized milk, products manufactured from unpasteurized milk or inadequately pasteurized cows' milk (Lévesque *et al.,* 2008).

Campylobacter jejuni is a major cause of acute diarrhoeal illness in humans in developed countries (Lévesque *et al*., 2008). *Campylobacter jejuni* is associated with acute cases of bacterial diarrhoea that could also contribute to the risk of acquiring immune-mediated neuropathies such as Guillain-Barrè syndrome (Yan *et al*., 2005; Horrocks *et al*., 2009; Claeys *et al*., 2013). Furthermore, recent studies claim that *C. jejuni* infections can also lead to inflammatory bowel diseases such as Crohn's disease (Horrocks *et al*., 2009). It has also been documented that the number of reported *C. jejuni* cases exceeds 80 per 100 000 people in several developed countries. Of the estimated 5.2 million annual bacterial foodborne diseases in the United States of America, approximately 2.4 million may be because of *C. jejuni* infections (Horrocks *et al*., 2009). During 2002, 11 543 cases of *C. jejuni* enteritis were reported for Canada (Lévesque *et al.*, 2008). For the Canadian province of Quebec, approximately 3 000 cases are reported annually (Lévesque *et al.*, 2008).

In England and Wales, 44 342 cases were reported during 2005 (Schildt *et al*., 2006). In the United Kingdom, milk-borne transmission of *Campylobacter* occurred as a result of wild birds pecking milk-bottle tops. In another *C. jejuni* outbreak, a farming family of six members was infected through the consumption of unpasteurized milk from their own dairy (Schildt *et al*., 2006). Small family outbreaks of *C. jejuni* infection, caused by continual contamination of bulk milk may be more common than reported because patients with diarrhoea do not always seek medical care (Yan *et al*., 2005).

Yersinia enterocolitica

Yersinia enterocolitica is a facultative anaerobic, non-spore forming, psychrotrophic microbe from the family *Enterobacteriaceae*. This bacterium can survive and grow at temperatures between 0ºC and 45ºC, but are able to grow between 22ºC and 29ºC and optimally at 28ºC (Erkmen, 1996; Soltan-Dallal *et al.*, 2004; Barton, 2011). Therefore, *Y. enterocolitica* may pose a health risk to consumers because of their ability to survive and multiply in a wide range of temperatures, including refrigeration temperatures (Soltan-Dallal *et al*., 2004).

Yersinia enterocolitica infections in humans are associated with raw milk and products thereof, as well as with inadequately pasteurized milk. *Yersinia enterocolitica* can cause yersiniosis, gastroenteritis, enterocolitis and mesenteric lymphadenitis. Symptoms may be severe in children and people with underlying disease (Soltan-Dallal *et al*., 2004; Yucel & Ulusoy, 2006). The first documented, major foodborne outbreak of Yersiniosis occurred in 1976 in New York, when 222 children and employees of five schools were diagnosed with acute intestinal illness after consumption of chocolate milk contaminated with *Y. enterocolitica* (Bottone, 1999; Yucel & Ulusoy, 2006).

Escherichia coli

Coliform bacteria, both faecal and non-faecal are classical indicator organisms used in food and water testing (Forsythe & Hayes, 1998). The presence of coliform bacteria in milk is used as an indicator of possible contamination of bacteria either from the udder, milk utensils or water supply used (Chye *et al.*, 2004). Because coliforms are able to incubate on residual films of improperly cleaned milking equipment, it is of utmost importance that all contact areas should be cleaned and sanitized properly (Elmoslemany *et al.*, 2010).

Escherichia coli is a non-spore forming, faecal coliform bacterium that forms part of the *Enterobacteriaceae* family (Baylis, 2009). *Escherichia coli* is able to grow at temperatures between 7ºC and 46 ºC, but optimally between 35ºC and 40ºC, and at pH values between 4.4 and 10, but optimally at pH 6-7 (Desmarchelier & Fegan, 2011). The natural habitat of *E. coli* is the enteric tract of humans and warm-blooded animals, but it is also found in water, soil and food, as a result of faecal contamination (Yucel & Ulusoy, 2006; Baylis, 2009). *E. coli* is, therefore, considered to be a reliable indicator of faecal contamination. It may also implicate the possible presence of enteropathogenic and

toxigenic microorganisms, which constitute a health risk to consumers of contaminated milk. High counts of *E. coli* and total coliform in milk signify improper hygiene practices (Altalhi & Hassan, 2009). Thus, *E. coli* in dairy products is regarded as an indicator when assessing post-pasteurization contamination. Its presence may imply inadequate pasteurization, poor hygienic conditions during processing or even post-processing contamination (O'Brien *et al*., 2009).

Six recognized groups of diarrhoeagenic *E. coli* that are responsible for clinical disease in humans have been identified. These groups include the entero-pathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAggEC), diffusely adherent *E. coli* (DAEC), and Vero cytotoxin producing *E. coli* (VTEC) or Shiga toxin-producing *E. coli* (STEC) (Newell *et al.*, 2010). *E. coli* (EPEC) represents *E. coli* associated with urinary tract infections and newborn meningitis (Baylis, 2009), while particularly the VTEC strains of *E. coli* have been implicated in food associated outbreaks (Newell *et al*., 2010). *E. coli* O157:H7, which forms part of the VTEC strain of *E. coli*, is widely considered as an important foodborne pathogen (Newell *et al*., 2010).

Escherichia coli contaminated foodstuffs are known to cause diarrhea when consumed. A number of food poisoning outbreaks with *E. coli* as the causative agent were reported in the United States of America, France and Scotland during the period 1983-2004. These outbreaks resulted from the consumption of cheese such as Brie, Camembert and Gouda (Kousta *et al.*, 2010).

The use of unpasteurized milk for the production of cheese and other dairy products increase the risk of the final product to be contaminated by pathogenic bacteria. It is documented that *E. coli* O157:H7 can survive all stages of raw milk cheese production for up to 70 days after manufacturing. Therefore, it is evident that curing alone may not be sufficient in the elimination of *E. coli* O157:H7 from cheese (Marek *et al*., 2004). Most of the foodborne outbreaks of *E. coli* O157:H7 was associated with the consumption of contaminated food products originating from cattle, especially food contaminated with cattle faeces, as these animals are known to be asymptomatic carriers of the organism (Öksüz *et al.,* 2004).

The presence of *E. coli* in milk implicates the possible presence of pathogenic coliforms as well as bacteria from the *Enterobacteriaceae* family, for example *Salmonella* (Altalhi & Hassan, 2009). Therefore, the higher the *E. coli* and coliform counts are in milk, the greater the risk of enteric pathogens being present in milk (Yucel & Ulusoy, 2006).

Staphylococcus aureus

Staphylococcus aureus do not produce endospores. This organism is facultatively anaerobic, but grows best under aerobic conditions. *S. aureus* is able to grow at temperatures between 7ºC and 48ºC, but optimally at 37º, and at pH values between 4 and 10, but optimally at pH 6-7 (Asperger & Zangerl, 2011).

Staphylococcus aureus is considered to be the third most important cause of foodborne diseases around the world, because of its ability to produce a wide range of heat-stable enterotoxins (Kousta *et al.,* 2010). These heat-stable enterotoxins are able to retain their biological activity even after the milk has been subjected to pasteurization (Rall *et al.*, 2008; Mhone *et al.,* 2011). The load of heat-resistant enzymes in milk is determined by the microbial count and somatic cell count (SCC) (Barbano *et al.*, 2006). Consequently, milk contaminated with *S. aureus* and accompanying heat-stable enterotoxins in raw milk, pose a health risk to consumers of various dairy products, including pasteurized milk (Smit, 2003).

The presence of this organism in milk can either be because of direct excretion from udders with clinical or subclinical staphylococcal mastitis, or because of contamination from the environment during handling and processing of raw milk. Furthermore a positive correlation has been found between the presence of *S. aureus* and *E. coli* (Peles *et al.*, 2007).

Staphylococcus aureus is frequently implicated as the causative agent of mastitis in cattle, therefore it is considered to be a common contaminant of raw milk (Mhone *et al.,* 2011). In Hungary, *S. aureus* has been shown to be responsible for 30% - 40% of all recorded mastitis cases annually (Peles *et al.*, 2007).

Bacillus cereus

Bacillus cereus is a ubiquitous, resilient, spore-forming bacterium that is commonly found in the environment (Svensson *et al*., 2004; Bartoszewics *et al*., 2008). *Bacillus cereus* is widely detected in the environment, and is thus considered to be a common contaminant of raw milk through a variety of mediums, these include soil, manure and milking equipment (Bartoszewicks *et al*., 2008; Coorevits *et al*., 2008). *Bacillus cereus* spores ingested by the cow, through contaminated silage, are not affected

during passage through the intestinal tract of the cow. Consequently, *B. cereus* spores are excreted in the cow's faeces, which can then be transferred to milk through faecal contamination of the udder (Kalač, 2011). *Bacillus cereus* is of special importance as a contaminant of milk, because of its ability to form spores that can survive the pasteurization process. Thus, raw milk is the major source of *B. cereus* in pasteurized milk (Salo *et al*., 2006).

The *B. cereus* bacterium is also a potential food poisoning organism, because of its ability to produce several enterotoxins and an emetic toxin, which cause diarrhoea and vomiting, respectively (Peng *et al*., 2001; Svensson *et al*., 2004; Bartoszewics *et al*., 2008). Psychrotrophic strains of *B. cereus* are known to limit the shelf life of milk stored at temperatures above 6ºC, because of their ability to proliferate at low temperatures especially in the presence of nutrients (Svensson *et al*., 2004; Bartoszewics *et al*., 2008). It causes sweet curdling and bitterness defects in milk, and is thus also regarded as being an important organism that influences the shelf life and quality of pasteurized milk and other dairy products (Peng *et al*., 2001).

Bacillus cereus spores are able to survive the pasteurization process, which explains the presence of *B. cereus* in pasteurized milk and dairy products (Coorevits *et al*., 2008). Because of the heat resistant nature of *Bacillus* spp. spores, it is important to prevent initial contamination of milk (Coorevits *et al*., 2008).

***Clostridium* spp.**

The *Clostridium* genus comprises of a heterogeneous group of microorganisms. They are anaerobic and endospore-forming bacteria. Because of the spore's resistance to extreme chemical and physical conditions, they are widely spread in the environment, and germinate when conditions are favourable. The growth temperature of this organism is between 3.3ºC and 80ºC, with an optimum growth temperature range of 25ºC to 40ºC (Aurelli *et al*., 2011).

It is generally accepted that the presence of *Clostridium* spp. spores in cheese is because of the contamination of milk during the milking process. Poor quality silage has been identified as the main source of raw milk contamination (Garde *et al*., 2011). When cows consume silage contaminated with *Clostridium* spores, the spores pass through the intestinal tract of the cows without the spores being affected. The spores are finally excreted in the faeces of the cows (Kalač, 2011).

Clostridium spp. in dairy products mainly originates from contaminated raw milk (Aurelli *et al*., 2011). Because the spores are able to survive the pasteurization process they are found in many dairy products, such as cheeses, cheese sauces, cream, pasteurized milk, powdered milk, sweetened condensed milk, yogurt and ice cream (Aurelli *et al*., 2011).

2.3.4 Somatic cells

The somatic cell count (SCC) is the number of white blood cells, known as leucocytes, present in milk (Auldist, 2011). The Regulations Relating to Milk and Dairy Products (Regulation R. 1555 of 1997), promulgated under the Foodstuffs Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972), stipulates that raw milk intended for further processing may not contain more than 500 000 somatic cells per 1.0 ml of bovine milk.

SCC in bovine milk is an important indicator of udder health. It provides an indication of the level of sub-clinical mastitis present in a particular dairy herd (Philips, 1996; Van Schaik *et al.*, 2005; Elmoslemany *et al.*, 2009a). Mastitis is defined as the inflammation of the mammary gland, affecting lactating animals (Karimuribo *et al*., 2005). An elevated SCC is usually an indication of mastitis (Le Maréchal *et al*., 2011), which is caused by pathogenic bacteria that enter the mammary gland through the teat canal. *Staphylococcus* spp*., Streptococcus* spp., *Listeria* spp. and *E. coli* are the main causative bacterial agents of mastitis in cows (Ruegg, 2003; Gröhn *et al*., 2005; Le Maréchal *et al*., 2011). A high SCC is because of an influx of leucocytes to the site of infection as part of the cow's immune response to this inflammation caused by the intruding bacteria (Le Maréchal *et al.,* 2011).

The type of mastitis is categorized according to the severity of the symptoms. Mastitis can be categorized into four groups, namely: subclinical mastitis, mild clinical mastitis, moderate clinical mastitis and severe clinical mastitis. With subclinical mastitis the inflammation of the mammary gland is not visible, and is detected through a diagnostic test, of which SCC is most commonly used (Le Maréchal *et al*., 2011). Subclinical mastitis is the most prevalent form of mastitis, being 15 to 40 times more prevalent than the clinical form (Sharif & Muhammad, 2008). Another form of mastitis is mild clinical mastitis, which is detectable through abnormalities in the milk. Although the mammary gland displays little or even no signs of swelling, the milk produces clots or presents with flakes. Moderate clinical mastitis is characterized by a swollen mammary gland, but the absence of systemic illness. The milk produced by these udders also present with abnormalities such as clotting and flakes. Severe

clinical mastitis is characterized by an inflamed udder accompanied by a sudden onset of systemic and local symptoms (Le Maréchal *et al.*, 2011).

Elevated SCC in milk can cause changes in milk composition and thus has an influence on milk quality (Lindmark-Månsson *et al*., 2006; Sharif & Muhammad, 2008). The change in milk composition occurs because of the inflammatory reaction, which results in the reduction in synthesis activity of the main components of milk, including fat, lactose and casein, and also because of the increased presence of blood elements (Hortet & Seegers, 1998). Furthermore, milk with an elevated SCC has higher levels of proteolytic and lipolytic enzymes, which not only affect the shelf life of dairy products, but also the flavour, causing mainly rancidity and bitterness (Elmoslemany *et al.*, 2009c).

2.4 Factors that influence milk quality

2.4.1 Introduction

High quality raw milk is important for the production of high quality pasteurized milk and dairy products. High quality milk starts at the farm and is influenced by many hygiene practices related to the milking shed. Poor hygiene practices are considered as one of the major causes of spoilage of products, resulting in a loss of income for the dairy farmer (Bonfoh *et al*., 2003).

Microbial contamination of bulk tank milk (BTM) occurs through three main sources: bacterial contamination from the external surface of the udder and teats, from the surface of the milking equipment, and from mastitis organisms from within the udder (Elmoslemany *et al*., 2010). Compromised hygiene practices could thus result in the contamination of raw milk by means of these routes, which in turn could produce unacceptable high bacterial levels that will result in processed milk with a limited shelf life, even when refrigerated (Smit, 2003). It is therefore important that a clean environment free from any source of contamination (pests, rodents, pathogenic and spoilage microorganisms), is maintained for the assurance of quality and safety of milk and dairy products (Dioguardi & Franzetti, 2010). An important strategy to follow is to identify pathogen sources and farm management practices that could lead to possible contamination of milk and to put preventative measures in place (Doyle & Erickson, 2012).

Other factors that have the potential to contribute to spoilage and contamination of milk include factors such as non-functional dairy design and layout of the milking shed and equipment, quality of water used in the dairy and herd health. When designing a dairy, attention should be paid to a functional layout, location of the milking shed, and the use of non-absorbing and non-corrosive finishing materials that comes into contact with milk (Dioguardi & Franzetti, 2010). Accumulation of dirt, along with possible contamination of milk, can be minimized through proper design of the milking shed, effective cleaning programmes, as well as the utilisation of non-absorbing and non-corrosive finishing materials that can be effectively cleaned. Figure 1.1 shows the different routes of milk contamination and the different organisms involved (Hassan & Frank, 2011).

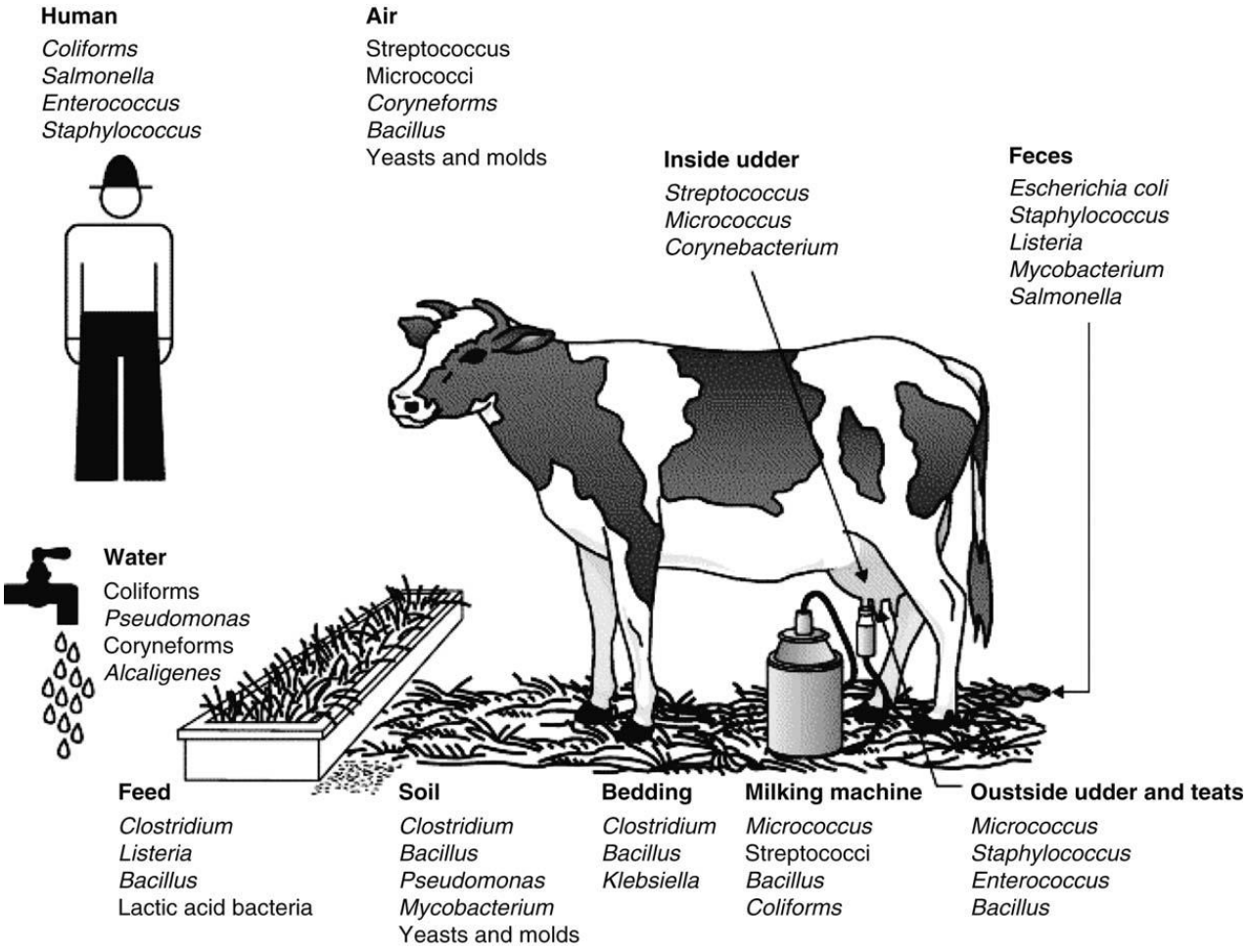


Figure 1.1 Examples of milk contamination sources at the dairy farm (Hassan & Frank, 2011).

2.4.2 Farming practices

Storage practices, including storage temperatures and the time that elapses between milk production and collection may have an impact on the final bacterial quality of milk and dairy products (Soler *et al*., 1995; Niza-Ribeiro *et al*., 2000). The Regulations relating to hygiene requirements for milking sheds, the transport of milk and related matters (Regulation R. 961 of 2012), promulgated under the Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972), stipulates that the bulk farm milk tank should be equipped to cool the milk in such a tank to 5ºC or a lower temperature within three hours. Thereafter, the milk should be kept at a temperature between 1ºC and 5ºC. Milk stored at temperatures greater than 5ºC, along with improper sanitation, may result in milk with high bacterial counts, which culminates in inferior quality milk with a limited shelf life (Van Schaik *et al.*, 2005).

Another important factor that could influence the quality of milk and derived products is pre-milking preparation of the cows (Heuvelink *et al*., 2009). Both thermoduric bacteria and psychrotrophic bacteria are commonly found on exterior teat and udder surfaces. The teat and udder surfaces are often contaminated with organisms generally associated with bedding materials. These organisms include streptococci, staphylococci, coliforms and other Gram-negative bacteria, such as *Campylobacter* (Heuvelink *et al.*, 2009; Reinemann & Rasmussen, 2011). *Bacillus cereus* is an important milk pathogen that has the ability to form spores that has been found to multiply in milk cow bedding (Carlin, 2011). Therefore to minimize the risk of transferring bacteria commonly found in bedding to milk, including *Bacillus cereus* cells or spores, proper cleaning and disinfection of the cow's udder and teats should be performed before milking (Andersson *et al*., 1995).

Improper cleaning and disinfecting of a cow's udder prior to milking could inevitably result in high bacterial loads in the bulk tank milk. These bacteria do not only contaminate the milk before entering the milk pipeline, but also result in the incubation of the bacteria in the milk handling equipment, thereby also causing continual contamination of milk (Reinemann, 2011). The balance between the microbial counts in milk, the spoilage flora and useful bacteria in the production of cheese are highly influenced by a combination of milking practices. These practices include the hygienic condition of the milking equipment, pre-milking and post-milking udder preparation (Verdier-Metz *et al.*, 2009)

2.4.3 Dairy design and infrastructure

The Regulations relating to hygiene requirements for milking sheds, the transport of milk and related matters (Regulation R. 961 of 2012), promulgated under the Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972), has clear prescriptions pertaining to the structural requirements of milking sheds, including the milking parlour, milk room, change room and scullery.

Regulation 6.(2) prescribes the following pertaining to the milking parlour :

a. "there shall be no direct connection with a latrine or with a room where gases, smoke, vapours, dust or a root deposit are present or may originate owing to the nature of the activities in such room;
b. which provides standing-room for more than one row of dairy stock parallel with one another, there shall be a dividing corridor of at least one meter wide between the rows;
c. the partitions, if any, that separate dairy stock form each other when they are being milked, shall be of a smoothly finished non-absorbing and corrosion resistant material free of any open seams and cracks;
d. mangers shall be arranged so that fodder which accumulates behind the mangers can be removed and be disposed of appropriately;
e. the exterior walls –
    i. shall be at least 2.4 meters high in the inside;
    ii. shall, at places where dairy stock are milked, extend to at least 2.1 meters above the level on which the dairy stock stand;
f. the interior surfaces of the walls, if provided shall be made of impervious materials with no toxic effect in intended use;
g. the ceilings, if provided or overhead structures and fixtures shall be constructed and finished to minimize the build-up of dirt and condensation, and the shedding of particles;
h. the floors shall be constructed to allow adequate drainage and cleaning;
i. such parlour shall be adequately ventilated and illuminated;
j. such parlour shall be provided with at least one water tap with running water to which a flexible pipe may be connected for washing purposes; and
k. the entrances and exits for dairy stock shall have a floor covering with an impenetrable surface connected to a disposal system, and such floor covering shall be installed in such a way that

any milk animal entering or leaving the milking parlour shall walk on it for a distance of at least 4 meters."

Regulation 6.(3) prescribes the following pertaining to the milk room –

a. "such milk room shall comply *mutatis mutandis* with the provisions of sub-regulation (2) (e)(i), (f), (g), (h) and (i);
b. where the scullery forms and integral part of the milk room, there shall be sufficient space to allow for the cleansing and disinfection of all milk containers, and the storage of milk;
c. such milk room shall be provided with at least one sink, with hot and cold water (or temperature controlled water), and running water with the run-off connected to a disposal system;
d. such milk room shall be erected so that a milk pipe form a milk tanker can be connected to a bulk farm tank through a door, and the distance between the two connection points shall not exceed 6 meters;
e. such milk room shall be rodent-proof;
f. the doors should have smooth, non-absorbent surfaces, and be easy to clean and, where necessary disinfect;
g. windows should be easy to clean, be constructed to minimize the build-up of dirt and where necessary, be fitted with removable and cleanable insect-proof screens. Where necessary windows should be fixed;
h. such milk room may be equipped with a farm tank referred to in regulation 7(3) for the storage of milk."

Regulation 6.(4) prescribes the following pertaining to a change room –

a. "comply *mutatis mutandis* with sub regulation (2)(e)(i), (f), (g), (h) and (i);
b. have at least one hand wash-basin and shower provided with hot and cold running water, soap, disinfectant and disposable towels, and the used water from such hand wash-basin and shower shall adequately drain into a disposal system;
c. be within easy reach of the milking parlour and milk room."

Other important design and infrastructure aspects to consider are the distance between the cattle shed and the entrance to the milking shed, as well as the type of flooring around the milking shed. A soil floor around the milking shed can contribute to contamination of milk and dairy products, because of

soil and cow manure brought into the milking shed by the cows (Dioguardi & Franzetti, 2011). The contamination of cows with manure, soil and dirty water may be reduced by paving and proper drainage of the area adjacent to the milking shed and also through the prevention of stagnant water pools in the vicinity of the milking shed (Chatterjee *et al*., 2006). Layout and location of the dairy should, therefore, be executed in such a way that the production flow and finishing materials will limit cross-contamination (Dioguardi & Franzetti, 2010).

2.4.4 Hygiene maintenance

Milking equipment is regarded as a major source of milk contamination. Effective cleaning and sanitation programmes that combine thermal, chemical and physical processes should be implemented (Carlin, 2011). Failure in any one of these processes could result in the build-up of dirt, which in turn provides nutrients for the growth and multiplication of bacteria between milking sessions (Elmoslemany *et al*., 2009b).

A major contributor to poor quality milk is contamination by the cow. Soiled udders and teats allow bacteria normally found in manure, soil and water to find their way into the milking operation (Chatterjee *et al*., 2006). Ineffective cleaning and sanitizing of cow udders and milking equipment result in the contamination of milk with thermoduric bacteria, including coliforms (Elmoslemany *et al.*, 2009c). Such contamination can be minimized by clipping the cow, as well as washing of the udder with pure water and a germicidal solution before milking (Chatterjee *et al.*, 2006). These practices are supported by The Regulations relating to hygiene requirements for milking sheds, the transport of milk and related matters (Regulation R. 961 of 2012), promulgated under the Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972), Regulation 9 (7), which stipulates that "all flanks, udders, bellies and tails of visibly dirty milk animals shall before the milking process be cleaned, and if necessary dried with a disposable or clean towel."

Coliforms are inhabitants of the intestinal tract of cows, and are therefore generally found in manure, bedding material, soil and contaminated water. Thus, the presence of coliforms is often used as an indication of inadequate pre-milking cow preparation, which can include improper cleaning of the udder and teats before milking (Pantoja *et al*., 2009). Soiled udders and teats are known sources of coliforms in milk (Hassan & Frank, 2011). It is therefore important to wash teats with high concentrations of

germicide and by using paper towels to clean udders before milking to minimize the contamination risk (Verdier-Metz *et al.*, 2009).

Another important aspect to consider in the cleaning and disinfecting of milking equipment, is the ability of bacteria to form biofilms. Under suitable conditions bacteria have the tendency to form microbial cell clusters on surfaces, known as biofilms. Biofilms may comprise of microorganisms from a single species or could represent a consortium of different species (Oulahal *et al.*, 2008). Dairy biofilms not only contain microorganisms, but also contain milk residues with protein and minerals, which not only pose a potential source of milk contamination, but also increase the corrosion rate of milking equipment (Bremer *et al.*, 2006). Disease outbreaks associated with the presence of biofilms have been related to pathogens, such as *L. monocytogenes, Y. enterocolitica, C. jejuni, Salmonella* spp., *Staphylococcus* spp. and *E. coli* O157:H7 (Simões *et al.*, 2010).

Some bacteria naturally have a higher tendency to produce a biofilm than others (Salo *et al.*, 2006). *Escherichia coli, L. monocytogenes, S. typhimurium, C. jejuni, B. cereus, Klebsiella pneumoniae* and *Y. enterocolitica,* to mention only a few, have the ability to produce biofilms on food contact surfaces, causing a challange to the dairy farmer in effective cleaning and disinfection of food processing facilities including milking equipment (Salo *et al.*, 2006). *Listeria monocytogenes* is commonly found on the surface of packaging machines, coolers, freezers, floors and drainage systems (Salo *et al.*, 2006). The presence of biofilms on milk contact surfaces have the potential to act as a chronic source of microbial contamination of milk, which inevitably compromise milk safety and quality (Oulahal *et al*., 2008). In addition to this, it is also important to note that the formation of biofilms on dairy surfaces, including floors, walls, drains and dairy equipment, pose a significant risk to the safety and quality of dairy products because they form a protective environment for bacteria and may also serve as a reservoir from which bacteria can spread to other dairy products (Knight & Craven, 2010).

Effective use of chlorine or iodine sanitizers has been associated with reduction in psychrotrophic bacterial levels (Reinemann, 2011). In addition, studies have shown that biofilm cells of *Listeria monocytogenes* are found to be more resistant to disinfectants containing chlorine, iodine, quaternary ammonium and anionic acid compounds, than planktonic cells of the organism (Salo *et al.*, 2006).

Escherichia coli on their own adheres poorly to surfaces. However, *E. coli* has the ability to embed itself in the organic matrix of the biofilm and cause hygiene problems if the biofilm formed is not removed completely during the cleaning process. Studies have shown that acid-adapted *E. coli*

O157:H7 showed enhanced survival and prevalence in biofilms on stainless steel surfaces (Salo *et al*., 2006). Even though stainless steel is frequently used for the production of dairy equipment, including mass cooling tanks, pipelines and utensils, polypropylene is becoming more popular in the production of this equipment. In dairies, these surfaces are continuously in contact with milk, therefore, if not cleaned and sanitized effectively, increasing the risk of milk being contaminated with microorganisms contained in biofilms (Oulahal *et al.*, 2008).

Areas in the food handling environment more likely to favour the development of biofilms include airhandling- and cooling systems, milk transfer lines, on conveyors, in packaging machines, in heat exchangers, on ultra-filtration surfaces, in mixers, tanks, on floors and in drains (Salo *et al*., 2006). Studies on the bacterial adherence to milk contact surfaces indicated that biofilm development may especially occur on gaskets in cases where the cleaning-in-place procedures are insufficient (Salo *et al*., 2006).

The increase in automation of dairy plants and the use of more complex milking equipment may contribute to the contamination of milk and other dairy products through the presence of bacterial biofilms (Bremer *et al*., 2006). Old, worn-out rubber parts are especially associated with elevated levels of thermoduric bacteria, such as coliforms (Reinemann, 2011). Control of biofilms in the dairy environment should involve a process called Clean-In-Place (CIP). CIP is defined as the "cleaning of complete items of plant or pipeline circuits without dismantling or the opening of the equipment and with little or no manual involvement on the part of the operator" (Bremer *et al.*, 2006). This CIP process includes the spraying of surfaces or circulation of cleaning chemicals through the dairy equipment with increased turbulence and flow velocity in order to obtain a biofilm-free environment (Bremer *et al*., 2006; Shi & Zhu, 2009). Sometimes residual microflora persists on milk contact surfaces, even after CIP treatment (Shi & Zhu, 2009). Significant accumulation of thermoduric microorganisms in milk residue may take several days or even weeks to reach a point where the total bacterial count is influenced. Presence of thermoduric organisms in milk is therefore an indication of persistent cleaning failure (Reinemann, 2011).

A study showed that a three day old biofilm containing *Bacillus* spp. treated with 125ºC wash water for 30 minutes, failed to completely inactivate it, even though this treatment was found to be an effective cleaning method for planktonic cells of *Bacillus* spp. (Shi & Zhu, 2009). Furthermore, elevated coliform counts in bulk tank milk can also occur when coliforms grow on residual milk left on milk contact surfaces or in inadequately disinfected milking equipment (Pantoja *et al.*, 2009; Hassan & Frank, 2011).

Psychrotrophic bacteria are often associated with improper cleaning and disinfecting of bulk milk tanks. Contamination of milk with psychrotrophic bacteria furthermore has the potential to become dominant if milk is stored at temperatures as low as 4.4ºC (Reinemann, 2011).

In cases where bulk tank milk bacterial counts indicate that a cleaning failure is the likely cause of elevated bacterial counts, the following aspects should be evaluated (Reinemann, 2011):

1. Is the cleaning programme implemented properly and with the appropriate frequency?
2. Proper implementation of the cleaning programme could include prescribed concentration of cleaning chemicals, as well as the temperature of wash water.
3. Instructions on product labels should be followed, to ensure that cleaning chemicals are used correctly.

Another important factor contributing to milk contamination is the employees involved in milk processing, because of poor personal hygiene (Aarnisalo *et al.*, 2006; Altalhi & Hassan, 2009). Even if personnel do not physically touch the milk, they could touch a multitude of other surfaces that comes into contact with the milk, for example the milk clusters and bulk cooling tanks (Aarnisalo *et al*., 2006). *Listeria monocytogenes* and *S. aureus* are two organisms that were found to be present on hands of employees engaged in food handling (Aarnisalo *et al.*, 2006; Kousta *et al.*, 2010).

A milk pathogen commonly transmitted through milkers' hands is *S. aureus*, an important causative agent of mastitis in dairy cows (Kousta *et al.,* 2010). The human nose is considered to be the principal site for multiplication of *S. aureus*. Milk can be contaminated with *S. aureus* when milk handlers have infected wounds or skin lesions or even by coughing and sneezing. Human contamination is therefore considered to be one of the most important factors in staphylococcal food poisoning, as skin lesions are common and often ignored by food handlers (Asperger & Zangerl, 2011). It is furthermore important to note that milk handlers can even be asymptomatic carriers of *S. aureus* (Kousta *et al.,* 2010).

The Regulations relating to hygiene requirements for milking sheds, the transport of milk and related matters (Regulation R.961 of 2012) has clear prescriptions regarding milkers and handlers of milk. Regulation 10 stipulates the following:

(1) "In addition to sub-regulation 6(4), personnel hygiene facilities shall be made available to ensure that an appropriate degree of personal hygiene can be maintained and to avoid contaminating milk, where appropriate facilities shall include-

  (a) Adequate means of hygienically washing and drying hands, including hand wash basins and a supply of hot and/or cold water and soap and disinfectant;

  (b) Toilets of appropriate hygienic design; and

  (c) Adequate changing facilities for personnel;

(2) Such facilities shall be suitably located and designed.

(3) The hands and fingernails of every milker or handler of milk shall be washed thoroughly with soap and water, and there shall be no accumulation of grime under the nails when milk is handled.

(4) Each person handling milk, shall daily before the commencement of his activities or work put on clean and undamaged over-clothes and gumboots and wear them continuously while he is handling milk in the interests of milk safety and suitability to use.

(5) Milk, shall not be handled by any person –

  (a) who has on his or her body a suppurating abscess or a sore or a cut or abrasion, unless such abscess, sore, cut or abrasion is covered with a moisture proof dressing which is firmly secured to prevent contamination of the milk;

  (b) who is or who is suspected of suffering from or being a carrier of a disease or condition in its contagious stage that can be transmitted by food or animals, unless any such person immediately reports the disease or condition to the person in charge and a certificate by a medical practitioner stating that such person is fit to handle food is submitted;

  (c) whose hands or clothing are not clean."

2.4.5 Water quality

Water, similarly to milk, is an excellent vehicle for the transmission of bacteria and pathogens. Because water is used in the cleaning and sanitizing process in a dairy, its quality is of the utmost importance (Elmoslemany *et al*., 2009c). A study performed in Ontario, Canada, showed that wash water contaminated with *E. coli* can be associated with higher bacterial counts in raw milk samples. Therefore poor quality water could have a detrimental effect on the effectiveness of the cleaning process and consequently may lower the quality of the raw milk produced (Bonfoh *et al.*, 2006; Perkins *et al*., 2009). To maintain high levels of hygiene in a dairy, good quality water should be used so as to avoid contamination of cleaned surfaces and milking equipment and the subsequent contamination of milk (Bonfoh *et al.*, 2006; Perkins *et al*., 2009).

Regulation 6.(2)(j) in The Regulations relating to hygiene requirements for milking sheds, the transport of milk and related matters (Regulation R. 961 of 2012), promulgated under the Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972) stipulate clearly that a milking parlour shall be provided with running water. The South African Bureau of Standards (SABS) has guidelines (SANS 241 of 2011) for drinking water with reference to bacterial and chemical quality. The SABS guideline stipulates that drinking water may not contain more than 10 coliforms per 100 ml and may not contain any *E. coli* per 100 ml. Even though this guideline determines that drinking water may also not contain more than 1 000 colony forming bacteria per 1.0 ml of water, the Mangaung Local Municipality in Central South Africa implemented a Water Action Plan in 2002 (Potgieter *et al*., 2007) with an alert value of 100 colony forming bacteria per 1.0 ml.

High coliform counts and the presence of other pathogens, such as *Pseudomonas* spp. and other Gram-negative bacteria in raw milk could be the result of contaminated wash water (Afif *et al.*, 2008; Elmoslemany *et al.*, 2009c). Therefore water quality could be considered as a basic determinant of milk quality (Perkins *et al*., 2009). The bacteriological quality of water used in a dairy could be improved by either boiling the water or by adding chlorine (Bonfoh *et al*., 2006).

South African dairies generally rely on boreholes for their water supply. Cows that are kept in close proximity to the water source, as well as the fertilization of cultivated fields with manure could increase the risk of contaminating the water supply, and subsequently could result in the contamination of raw milk (Pierce, 2009; Rosengren *et al*., 2010).

It is not only the bacteriological quality of water that could have an influence on the quality of milk. Chemical quality as well as wash water temperature must also be taken into account. Therefore it is important to consider the impact of water hardness, due to dissolved minerals, and temperature on the effectiveness of the washing process (Reinemann, 2011). Increased water hardness could not only lead to the formation of milk residues on the milking system, but may also require higher concentration of cleaning and disinfectant chemicals to have the same cleaning efficiency (Elmoslemany *et al*., 2009c; Reinemann, 2011). Dairies with medium or high water hardness are 2.5 to 4.7 times more likely to have high bacterial counts in bulk tank milk, than dairies with acceptable water hardness (Elmoslemany *et al*., 2009c).

The temperature of water used in cleaning of the milking system is another important aspect to bear in mind, as hot water is necessary for emulsifying fat and dispersing milk protein. Hot water supply should be checked regularly in order to ensure effective cleaning (Bonfoh *et al*., 2006). Studies showed that hot water used with alkaline detergent and wash solution contribute to low bacterial counts in bulk tank milk (Elmoslemany *et al.,* 2009c). It is therefore expected that correct concentrations of detergents along with good quality hot water should remove milk residues and inevitably reduce the number of microorganisms on dairy contact surfaces as well as in the milk itself (Bonfoh *et al.*, 2006).

It is important for dairy farmers to be aware of the bacteriological and chemical quality of the water used in the dairy and more specifically the water used in the cleaning and sanitizing process. This can be established through regular microbiological and chemical monitoring of the quality of the water used in a dairy. When deterioration of the water quality is identified necessary remedial actions can then be implemented (Elmoslemany *et al*., 2009c).

2.4.6 Herd Health

Animals, including cows play an essential role in the maintenance of zoonotic infection in nature (Hallaj, 2010). The World Health Organisation (WHO), the Food and Agriculture Organization (FAO) as well as the World Organization for Animal Health / “Office International des Epizooties” (OIE) define emerging zoonosis as: “one that is newly recognized or newly evolved or that has occurred previously but shows an increase in incidence or expansion in geographical, host or vector range.” According to the WHO, over three-quarters of the new, emerging or re-emerging human diseases in the past three decades were caused by pathogens originating from animals or products of animal origin (Hallaj, 2010).

The World Health Organization (WHO) (Hallaj, 2010) defines zoonotic diseases as follows:

> *"Zoonotic diseases are infectious diseases that are naturally transmissible from vertebrate animals to humans."*

Dairy cows serve as major reservoirs of these organisms even though they appear to be clinically healthy (LeJeune & Rajala-Schultz, 2009). Organisms commonly found in the milk produced by asymptomatic cows include *Coxiella burnettii, Listeria* spp., *Mycobacterium avium* subspecies *paratuberculosis* (MAP), *Campylobacter* spp., coliforms, which include *E. coli*, and *Salmonella enterica* (LeJeune & Rajala-Schultz, 2009).

Brucellosis is considered by the WHO and IOE as the most important and wide spread zoonotic disease worldwide, especially in developing countries (Schelling *et al.,* 2003). Bovine brucellosis is caused by bacteria of the genus *Brucella* presenting with lesions that include necrotic placentitis and interstitial mastitis in pregnant cows, fibrinous pleuritis with interstitial pneumonia in aborted fetuses and in newborn calves (Carvalho Neta *et al*., 2010). Brucellosis in humans is caused by *Brucella melitensis* and *Brucella abortus,* mainly as a result of the consumption of contaminated raw milk and cheese or as a result of occupational exposure to infected animals or carcasses, secretions from cow's uteruses or aborted foetuses (Carvalho Neta *et al*., 2010). Clinical symptoms of human brucellosis include fever, anorexia, polyarthritis, meningitis, and with serious complications the musculo-skeletal, cardiovascular and central nervous systems can also be affected (Carvalho Neta *et al*., 2010, Schelling *et al*., 2003). According to the World Health Organization data (Taleski *et al*., 2002) there are 500 000 cases of human brucellosis worldwide and 10 000 – 20 000 cases occur in Europe each year. High risk areas include Portugal, Spain, Southern France, Italy, Greece, Turkey, South and Central America, Eastern Europe, Asia, Africa, the Caribbean and the Middle East (Taleski *et al*., 2002).

The pathogen *Mycobacterium bovis* causes bovine tuberculosis. It is transmitted to humans through inhalation of infectious droplets from infected cattle as well as the consumption of contaminated, unpasteurized milk and dairy products (Rodwell *et al*., 2010). Rodwell *et al*. (2010) found that tuberculosis caused by *M. bovis* in Southern California was as a result of the consumption of unpasteurized dairy products, including unpasteurized cheese, commonly referred to as queso fresco, manufactured with milk from infected dairy cows. In the early 1900's an estimated 30% of human tuberculosis cases in Europe were caused by the cattle tuberculosis pathogen, *M. bovis* (Rodwell *et al.*, 2010).

Human tuberculosis is on the increase in populations with a high incidence of HIV infection, especially in Africa (Bernard *et al.*, 2005). Because HIV infection suppresses the immune system dairy employees or farmers infected with mycobacteria have an increased risk of contracting tuberculosis, which in turn can also lead to transmission of tuberculosis from humans to cattle (Regassa *et al.*, 2008).

The United States of America (USA) Department of Agriculture conducted a study amongst US dairy herds, which estimated that at least 68% of all US dairy herds are infected with *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Infected cows secrete MAP in their milk, which puts consumers of contaminated milk and products of the milk at risk, particularly because this organism is not completely killed during the pasteurization process. MAP can therefore be present in pasteurized milk, unpasteurized milk as well as cheese and other dairy products manufactured from contaminated milk (Pierce, 2009). MAP is the causative agent of Johne's diseas, which is a chronic, progressive gastroenteritis of ruminants. There is much debate about whether there is an association between Johne's and a similar condition in humans, namely Crohn's disease (Lund *et al*., 2002; LeJeune & Rajala-Schultz, 2009). Crohn's disease is linked to the consumption of unpasteurized milk and cheese as well as untreated drinking water from wells or springs (Pierce, 2009).

Another zoonotic disease transmitted through the consumption of contaminated unpasteurized milk, is the rickettsial disease Q-fever. The causative agent of Q-fever is *Coxiella burnetti*, which can be transmitted by ticks to farm and domestic animals. The modes of transmission from livestock to humans are similar to that of brucellosis, namely consumption of contaminated raw milk and dairy products, close contact with infected animals and carcasses or through contact with aborted foetuses (Schelling *et al*., 2003). *Coxiella burnetii* is a rickettsia, which is an obligate intracellular parasite. The organism is extremely resistant to chemical and physical disinfectants, and is one of the most heat resistant pathogens in milk (Hassan & Frank, 2011).

Another dairy disease of public health concern is that of mastitis, which inevitably influences the bacteriological quality of raw milk. A high somatic cell count is generally used as an indication of mastitis (Le Maréchal *et al*., 2011). Mastitis, which is inflammation of the mammary gland, is recognised as the single disease that has the most significant impact on milk quality (LeJeune & Rajala-Schultz, 2009). The disease is considered worldwide to be one of the most important causes of economic losses in the dairy industry (Karimuribo *et al.*, 2005). Thus, a diseased herd could not only impact the profitability of a dairy farm through the disease itself, but also because of the presence of antibiotic residues in milk used to the treatment of the disease (Gröhn *et al*., 2005). Antibiotic residues

should not be present in raw milk intended for further processing (Act 54 of 1972). The presence of antibiotic residues in milk is of human health concern because of their association with varying degrees of allergies, gastrointestinal conditions, liver damage, anaphylaxis and drug resistance (Kang'ethe *et al*., 2005).

Even though *S. aureus* is indicated to be one of the main causative agents of mastitis, the disease can be caused by a range of more than 150 different contagious or environmental microorganisms, including *Streptococcus agalactiae, Lactococcus lactis, Klebsiella pseudomoniae* and *L. monocytogenes* which can consequently be shed in the milk (Ruegg, 2003; Sommerhäuser *et al*., 2003; Kuang *et al*., 2009). Recent studies found that *E. coli* has emerged as an important mastitis pathogen resulting in high milk loss and death of infected cows, even on well-managed farms (Oliver *et al*., 2011).

The consequences of each type of mastitis on milk quality will differ. Animals with subclinical mastitis for instance can produce milk that is not really noticeably different from milk produced by healthy animals, and therefore it is frequently mixed with the rest of the herd's milk in the on-farm bulk storage tank (LeJeune & Rajala-Schultz, 2009; Le Maréchal *et al*., 2011). This practice of mixing mastitis milk with fresh high quality milk may not only result in high microbial load of bulk milk, but also increase the risk of pathogens, toxins and antibiotic residues in the milk (Chye *et al*., 2004; Sharif & Muhammad, 2008). It is therefore inevitable that the shelf life and processing quality of milk are reduced when high concentrations of somatic cells are present in bovine milk (Van Schaik *et al*., 2005; Oliver *et al*., 2009).

In the Regulations relating to hygiene requirements for milking sheds, the transport of milk and related matters (Regulation R. 961 of 2012), promulgated under the Foodstuffs, Cosmetics and Disinfectants Act (Act 54 of 1972), there are clear prescriptions pertaining to dairy herds, which include the following:

Regulation 9. (1) "Every milk animal shall be marked with a distinguishing and indelible mark by which such an animal can be identified.

(2) A register shall be kept of each separate milk animal's diseases, each withdrawal from the dairy herd, each return to the dairy herd for milking purposes and all veterinary examinations and treatment records with the name of the veterinarian, if involved in such examinations or treatments.

(3) Each individual milk animal shall be examined by a veterinarian at least once in every two-year cycle, provided that milk animals be further examined as required; and a report shall be obtained from the veterinarian after each examination.

(4) The milk of any milk animal that is or appears to be ill shall not be made available for human consumption until such time as the holder has made sure that that animal is not suffering from a disease mentioned in subregulation (5).

(5) The milk of dairy stock that suffer from mastitis, indurations of the udder, a secretion of bloody or ropy milk or milk otherwise abnormal, tuberculosis, salmonellosis, acute fever with the inclusion of anthrax, anaplasmosis, redwater, ephemeral fever and lumpy skin disease, septic metritis, septic multiple mange, serious tick infection or brucellosis, or that have any open or septic wounds which may contaminate milk, milk containers, or apparatus or equipment or people who work with the milk animals, shall not be made available or used for human consumption unless steps have been taken to the satisfaction of the local authority to eliminate such health hazard.

(6) Substances and materials used in the milking process or on dairy stock shall be kept in containers that are free of foreign or toxic matter and dirt, and such containers when not in use shall be covered with tight-fitting lids. Where applicable, such substances and materials shall be approved in terms of the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act, 1947 (Act 36 of 1947)."

Chapter 3
Materials and methods

3.1 Introduction

Milk, dairy surfaces and water from 83 dairy farms that supply milk to the Mangaung region where assessed in this study (Figure 3.1). These farms were assessed for the presence of *E. coli,* coliforms and total bacterial content. The milk was furthermore also assessed in terms of somatic cell quantification. A milk quality index (MQI) as well as a hygiene quality index (HQI) were developed for each of the dairy farms included in the study.

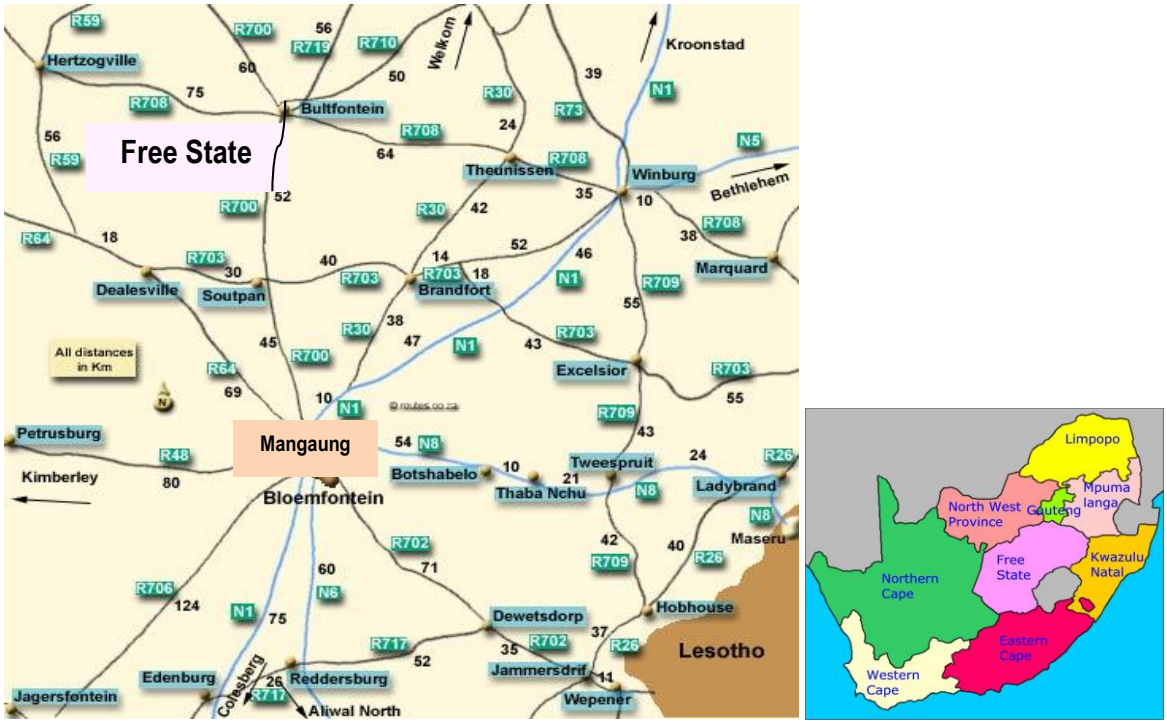


Figure 3.1 Map displaying study area in the Free State (obtained from Routes Travel Info, 1999; South African Travel Online, 2010).

The 83 dairy farms included in this study are located in the vicinity of Bainsvlei, Jagersfontein, Petrusburg, Dealesville, De Brug, Tierpoort, Reddersburg, Bultfontein, Tweespruit, Dewetsdorp, Brandfort, Verkeerdevlei, Glen, Roodewal, Vaalbank Zuid, Riverside and Theunissen. Table 3.1 includes information about the location of the farm, the town or suburb nearby which the farm is located

and the GPS co-ordinates. Information about dairy layout as well as the size of each of the enterprises in terms of the number of cows and number of staff is also provided.

Table 3.1 Farm locality, number of cows and staff.

Dairy Farm no.	Area	No. of Cows	GPS co-ordinates	No. of Staff	Dairy layout
1	Bainsvlei	95	S2900.847E02555.820	4	Stanchion
2	Bainsvlei	90	S2857.691E02554.143	4	Tandem
3	Bainsvlei	163	S2903.369E02557.223	7	Stanchion
4	Bainsvlei	105	S2900.338E02607.124	4	Stanchion
5	Bainsvlei	150	S2856.636E02606.352	6	Tandem
6	Bainsvlei	97	S29801.845E2556.673	5	Stanchion
7	Bainsvlei	200	S2902.948E02605.891	11	Stanchion
8	Bainsvlei	35	S2855.803E02601.613	3	Stanchion
9	Bainsvlei	18	S2859.816E02604.873	2	Stanchion
19	Bainsvlei	58	S2911.951E02600.478	8	Stanchion
10	Jagersfontein	80	S2914.995E02600.690	4	Stanchion
11	Jagersfontein	56	S2920.117E02554.682	3	Tandem
12	Jagersfontein	24	S2918.746E02556.848	4	Stanchion
13	Jagersfontein	44	S2915.435E02605.712	4	Stanchion
14	Jagersfontein	50	S2911.205E02608.270	4	Stanchion
15	Jagersfontein	65	S2915.116E02602.387	6	Stanchion
16	Jagersfontein	42	S2917.379E02559.220	2	Herringbone
17	Jagersfontein	120	S2912.995E02603.532	2	Herringbone
18	Jagersfontein	32	S2913.536E02605.752	4	Tandem
20	Petrusburg	40	S2919.057E02542.990	4	Stanchion
21	Petrusburg	35	S2915.243E02548.155	7	Stanchion
22	Petrusburg	80	S2915.417E02552.715	4	Herringbone
23	Petrusburg	80	S2917.378E02550.337	3	Stanchion
24	Dealesville	26	S2839.204E02545.556	4	Stanchion
25	Dealesville	92	S2837.943E02555.334	6	Herringbone
26	Dealesville	45	S28.20.281E 25.43.820	5	Herringbone
27	Dealesville	120	S28.23.343E25.40.297	6	Herringbone
28	Dealesville	200	S2837.650E02545.502	12	Stanchion
29	Dealesville	162	S2853.815E02549.431	9	Herringbone
30	Dealesville	39	S2833.023E02541.050	7	Stanchion
31	Dealesville	100	S2835.954E02547.562	8	Stanchion
32	Dealesville	60	S2837.156E02545.910	3	Stanchion
33	Dealesville	80	S2831.254E02535.156	7	Herringbone
34	Dealesville	100	S2827.229E02539.503	5	Stanchion
35	Dealesville	73	S2847.522E02551.972	3	Stanchion
36	Dealesville	67	S2824.723E02539.273	3	Herringbone
37	Dealesville	60	S2825.889E02548.198	3	Stanchion
38	De Brug	90	S2912.177E02555.472	4	Stanchion
39	De Brug	168	S2909.578E02547.816	4	Tandem
40	Tierpoort	82	S2927.572E02602.564	7	Herringbone
41	Tierpoort	52	S2928.816E02600.801	6	Stanchion
42	Tierpoort	60	S2926.490E02603.327	6	Stanchion

43	Tierpoort	50	S2928.939E0260.1762	1	Herringbone
44	Reddersburg	14	S2917.090E02613.572	4	Stanchion
45	Reddersburg	180	S2923.551E02615.745	14	Herringbone
46	Reddersburg	220	S2918.396E02612.695	6	Herringbone
47	Reddersburg	60	S2913.336E02612.475	6	Stanchion
48	Reddersburg	300	S2933.865E02611.266	10	Herringbone
49	Reddersburg	38	S2914.505E02613.169	4	Stanchion
50	Bultfontein	150	S2850.471E02611.227	15	Herringbone
51	Bultfontein	500	S2854.271E02608.235	12	Stanchion
52	Bultfontein	29	S2847.223E02613.470	5	Stanchion
53	Tweespruit	20	S2909.372E02627.239	2	Stanchion
54	Tweespruit	12	S2905.862E02703.921	3	Stanchion
55	Tweespruit	140	S2913.534E02703.499	7	Tandem
56	Dewetsdorp	70	S2950.081E02631.442	7	Stanchion
57	Dewetsdorp	35	S2945.295E02643.025	5	Herringbone
58	Dewetsdorp	130	S2930.133E02641.063	8	Stanchion
59	Dewetsdorp	38	S2934.299E02638.336	3	Stanchion
60	Dewetsdorp	50	S2942.091E02640.430	4	Stanchion
61	Dewetsdorp	15	S2942.481E02646.462	4	Herringbone
62	Dewetsdorp	80	S2931.509E02635.323	12	Herringbone
63	Dewetsdorp	240	S2930.473E02638.076	7	Herringbone
64	Dewetsdorp	80	S2925.522E02627.187	3	Stanchion
65	Dewetsdorp	450	S2937.590E02648.590	10	Herringbone
66	Dewetsdorp	27	S2933.401E02644.049	6	Herringbone
67	Brandfort	80	S2839.040E02620.401	3	Stanchion
68	Brandfort	44	S2844.138E02621.537	4	Stanchion
69	Brandfort	50	S2850.364E02636.175	2	Herringbone
70	Brandfort	120	S2845.569E02624.507	6	Stanchion
71	Brandfort	70	S2849.262E02614.087	6	Stanchion
72	Brandfort	250	S2839.199E02617.321	7	Herringbone
73	Brandfort	30	S2843.321E02625.056	4	Stanchion
74	Brandfort	110	S2847.166E02629.508	4	Herringbone
75	Verkeerdevlei	64	S2903.394E02630.080	9	Stanchion
76	Verkeerdevlei	70	S290047.2E0263210.3	5	Stanchion
77	Glen	60	S2856.183E02619.430	7	Stanchion
78	Roodewal	60	S2905.248E02622.176	12	Stanchion
79	Roodewal	220	S2906.507E02619.042	5	Herringbone
80	Vaalbank Zuid	60	S2903.205E02619.262	5	Stanchion
81	Riverside	25	S2905.353E02620.222	2	Tandem
82	Theunissen	35	S2835.292E02630.507	2	Stanchion
83	Theunissen	85	S2830.431E02638.469	4	Stanchion

3.2 Assessment of milk quality

3.2.1 Collection of milk samples

Each dairy farm was visited twice during the period August 2007 and November 2008. During the first visit to each farm, milk samples were taken from bulk milk tanks. On farms with more than one bulk milk tank, milk was sampled from all bulk tanks in operation. Before sampling was done, the temperature of the milk in the bulk storage tanks was measured with a sterile thermometer. Immersion samples were then taken from the milk in the bulk tanks, with sterile immersion sample bottles. The milk samples were then immediately placed in a cooler box at a temperature not exceeding 4ºC. Thereafter the samples were transported to the Mangaung Local Municipality Microbiology laboratory in Bloemfontein in the Free State for analyses.

3.2.2 Quantification of *E. coli*

Quantification of *E. coli* was executed according to the modified Eijkmann test for *E. coli*, prescribed by the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972). All steps were carried out in a manner to prevent contamination.

1. Firstly, the milk sample was thoroughly mixed by shaking the milk sample bottle.
2. For the detection of gas, three tubes fitted with an inverted Durham fermentation tube containing 10 ml of brilliant green bile broth (Oxoid) were inoculated with 0.01 ml of the milk sample.
3. For the measurement of the 0.01 ml quantities tested, decimal dilutions were prepared in accordance with the standard plate count method described in paragraph 3.2.4 mentioned below.
4. The inoculated brilliant green bile broth was then incubated for 48 hours in a water bath by keeping the temperature of the water bath at 44ºC ± 0.15ºC.
5. In cases where the incubation prescribed in paragraph (4) led to the formation of gas as seen in the Durham tube, an inoculum of 0.2 ml from each brilliant green bile broth tube in which gas has formed were transferred to a separate tube of tryptone water (Oxoid).

6. The tryptone water tubes were then incubated in a water bath at 44 ºC ± 0.25ºC for 24 hours ± 2 hours.
7. After the 24 hours ± 2 hours, the tryptone water in the tubes were tested for indole production by adding 0.5 ml of Kovac's reagent.
8. The formation of a rose-coloured ring at the interface of the two liquids indicated the presence of indole.
9. A positive result for gas and indole in any of the three tubes inoculated with the prescribed volume of the same milk were taken to indicate the presence of *E. coli*.

The Kovac's reagent used in the above-mentioned test for *E. coli* was prepared in the following manner:

a. 5 g paradimethylaminobenzaldehyde were dissolved in 75 ml amyl alcohol (pyridine free) to which 25 ml concentrated hydrochloric acid were added.
b. The mixture then turned yellow in colour.
c. The mixture was placed in an amber-coloured glass stoppered vessel and stored in a cool, dark place.
d. The mixture was then stored for at least 24 hours before it was used.

3.2.3 Quantification of coliforms

Quantification of coliform bacteria in milk was executed according to the dry rehydrated film method for coliform and *E. coli* count, using Petrifilm™, as prescribed in the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972), in the following manner:

1. Firstly, the milk sample was mixed thoroughly by shaking the milk sample bottle.
2. Decimal dilutions were prepared in accordance with the standard plate count method described in paragraph 3.2.4 mentioned below.
3. The Petrifilm™ for *E. coli* and coliform counting was placed on a flat surface and was labeled. The top film was lifted and 1 ml of each of the dilutions was transferred to the centre of the bottom film, by holding the pipette perpendicular to the film, beginning with the highest concentration and ending with the lowest.

4. The top film was then slowly rolled onto the sample to prevent air bubbles being trapped under the top film.
5. The sample was then distributed evenly on the film by applying gentle downward pressure with a spreader.
6. The film was then left undisturbed for one minute to solidify.
7. The films were then stacked in piles of not more than 20 and incubated at 30ºC ± 1 ºC for 24 ± 2 hours with the clear sides up.
8. The films were removed from the incubator at the end of the incubation period, and the colonies counted with the aid of magnification under uniform artificial illumination as follows:

    a. Blue colonies associated with gas indicated the presence of *E. coli,* while red colonies associated with gas indicated the presence of coliform colonies. Colonies that were not associated with gas were not counted as coliform colonies. All the red and blue colonies with gas represented the total coliform colony count.
    b. Films with 15 – 150 colonies were counted. In some cases an estimated count was made on films where the colonies exceeded 150. The growth area of the Petrifilm™ is divided into 1 cm^2 blocks. At least 4 squares or 20% of the growth area were counted. The number of viable coliform colonies per milliliter of milk was calculated and reported as an "estimated" total coliform colony count.

3.2.4 Quantification of total bacterial count

Quantification of total bacterial count in milk was performed according to the dry rehydrated film method for aerobic plate count, using Petrifilm™, as prescribed in the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972), in the following manner:

1. Firstly, the milk sample was thoroughly mixed by shaking the milk sample bottle.
2. A 1:10 dilution was prepared by adding 1 ml of the milk to 9 ml sterile diluent (phosphate buffer or peptone saline solution).
3. Decimal dilutions were then prepared.

4. With a sterile pipette, 1 ml of each of the dilutions was transferred, in duplicate, to sterile Petrifilm™, beginning with the highest concentration and ending with the lowest.
5. The Petrifilm™ for aerobic counts was placed on a flat surface and labeled. The top film was lifted and 1 ml of milk dilution was transferred to the centre of the bottom film by holding the pipette perpendicular to the film.
6. The top film was then slowly rolled onto the sample to prevent air bubbles being trapped under the top film.
7. The sample was then evenly distributed on the film by applying gentle downward pressure with a spreader.
8. The film was left undisturbed for one minute to solidify.
9. In piles of no more than 20, the films were incubated at 32ºC ± 1 ºC for 48 ± 3 hours with the clear sides up.
10. At the end of the incubation period the films were removed from the incubator. The colonies were then counted with the aid of magnification under uniform artificial illumination.

3.2.5 Quantification of somatic cells

Somatic cells were quantified using a Coulter Counter. Before any measurements were done, the Coulter Counter was calibrated according to the method prescribed in the manufacturer's instruction manual.

Processing of samples was conducted in the following manner:

1. One drop of Somafix was placed in a clean glass tube.
2. The milk sample was inverted 25 times before sub-sampling.
3. 3.3 ml milk was then forcibly expelled into the glass tube. The mixture was then shaken thoroughly.
4. The sample was heated in a water bath for 6.5 minutes at 60ºC ± 1ºC.
5. Each sample was then mixed for three to five seconds on a vortex mixer prior to dilution.
6. Each sample was then inverted two to three times immediately before dilution.
7. 0.1 ml of the milk sample was drawn into an automatic diluter.
8. With the edge of the tube, the milk drop which remained on the diluter tip was picked off.
9. 0.1 ml milk and 9.9 ml diluent was expelled from the diluter into a clean glass tube.

10. The diluter tip was kept in such a way that it never touched the tube and never projected under the surface of the solution.
11. The tube was then capped and placed in a water bath heated to 80ºC for 10 minutes.
12. The level of liquid in the tubes were always kept below the surface of the water bath.
13. The sample was then cooled in ice water for three minutes, removed and left to reach room temperature.
14. The sample was analyzed within one hour. Each sample was inverted four times before it was transferred to a cuvette.

Counting of somatic cells was executed as follows:

1. Before counting was done, the following were confirmed:
    a. The system was filled with diluent.
    b. The correct aperture tube was fitted.
    c. The diluent jar was filled.
    d. Waste water jar was empty.
    e. The instrument was switched on.
    f. The cuvette containing distilled water was removed and replaced by a cuvette filled with diluent.

2. Setup was pressed until the S1 screen appeared. The following were confirmed:
    a. The aperture tube and reference letter were 100 µm C.
    b. Kd = 59.49.
    c. The units were selected as µm.
    d. The Upper Size was set as Tu.
    e. The Lower Size was set as Tl at 4.3 µm.
    f. Count mode was at above Tl.

3. Setup was pressed and screen S2 appeared.
   The Coulter counter automatically measures the aperture characteristics and determines the optimum instrument settings for the sizes entered.

4. Output was pressed and screen A1 appeared. The following were confirmed:
    a. The next test was at the number required.

b. The result type was at Count.
c. Dilution factor was at 1.
d. Switch units was at µm.
e. Resolution was at 256.

5. The sample was placed on the platform and raised until the aperture tube and electrode were immersed.
6. Analyses were initiated by pressing Start.
7. A measurement was displayed and recorded.

3.3 Assessment of dairy surfaces

At each of the 83 dairy farms included in the study, two surface swabs were taken. The collection of surface swabs was done during the second visit to the dairy farms. In-line sampling was performed after washing and sanitizing of the dairy equipment. One sample was taken in the cluster collection chamber (pulsator) and one sample was taken in the milk pipeline. The samples were taken per 1 cm^2 with a sterile swab and were placed in a sterile transport medium of Ringer's solution (commercially prepared and supplied by Oxoid). The samples were then immediately placed in a cooler box where they were kept at a temperature not exceeding 4ºC. Thereafter the samples were transported to the Mangaung Local Municipality Microbiology laboratory in Bloemfontein in the Free State for analyses.

Quantification of coliform bacteria on dairy contact surfaces, was carried out according to the dry rehydrated film method for coliform and *E. coli* count, using Petrifilm™, as prescribed in the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972), and described in 3.2.3.

Quantification of total bacterial count on dairy contact surfaces was performed according to the dry rehydrated film method for aerobic plate count, using Petrifilm™, as prescribed in the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972), and described in 3.2.4.

3.4 Assessment of water quality

3.4.1 Collection of water samples

At each of the 83 dairy farms included in the study, one borehole water sample was taken at the point of use in the dairy. Sampling of the water was carried out during the first visit to each dairy farm. The opening of the tap was first disinfected with a portable gas flame. The water was then allowed to run for a reasonable time, approximately two minutes, before a 150 ml sample was collected in a sterile water sampling bottle. The water sample was placed in a cooler box with a temperature not exceeding 4ºC. Thereafter the samples were transported to the Mangaung Local Municipality Microbiology laboratory in Bloemfontein in the Free State for analyses.

3.4.2 Quantification of *E. coli* and coliforms

Quantification of *E. coli* and coliforms were carried out using the Colilert method as per manufacturer's (IDEXX) instructions.

1. The water sample was mixed thoroughly and excess water was poured out until 100 ml remained.
2. Colilert 18 reagent was then added to the water sample.
3. The sample was again mixed gently.
4. The mixture was allowed to stand for a while until the reagent dissolved completely.
5. The mixture turned to a light yellow colour.
6. The Quanti-tray was opened carefully using the tab, while all measures were taken to prevent contamination of the inside of the tray.
7. The mixture was emptied into the Quanti-tray and the tray was closed.
8. The Quanti-tray was placed on the rubber Quanti-tray holder, gently pressed to fit properly into all the holes.
9. The Quanti-tray sealer machine was switched on and warmed up and when the light indicated that the machine was ready, the Quanti-tray rubber holder containing the Quanti-tray was place in the machine with closed end entering first. With the wells facing downwards and the white part facing upwards the Quanti-tray was moved through the machine to be sealed.

10. The sealed Quanti-tray was then incubated for 18 hours at 35ºC.
11. The Quanti-tray was removed from the incubator at the end of the incubation period, and wells were counted as follows:
    a. All the wells that turned any shade of yellow were counted.
    b. Yellow wells indicates a positive reaction for total coliforms.
    c. The large wells and the small wells were counted separately.

       By using IDEXX Quanti-tray / 2000 MPN Table (per 100 ml) the most probable number (MPN) of coliforms were recorded by reading the large wells as well as the small wells. The example in Table 3.2 indicates that the MPN for total coliforms is 22 per 100 ml. Only part of the IDEXX Quanti-Tray / 2000 MPN™ table was included in this example.
    d. The Quanti-tray was then placed under an ultra violet light.
    e. All the wells that fluoresced under the ultra violet light were counted and recorded. Wells that fluoresced under ultra violet light indicated the presence of *E. coli*. In this study only presence or absence of *E. coli* was recorded.

Table 3.2 IDEXX Quanti-Tray / 2000 MPN™ coliforms per 100 ml.

# Large wells positive	# Small wells positive																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.6	17.6	18.6	19.7	20.8	21.8
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0

3.4.3 Quantification of total bacterial count

Quantification of total bacteria was performed using Aerobic count plate Petrifilm™, described in 3.2.4.

3.5 Analyses of data

3.5.1 Statistical analyses

Summary statistics were calculated for all the different measurements. Pearson Chi-square tests were also performed to ascertain the role of the different variables on the presence of *E. coli* in the milk. The Pearson Chi-square tests that were performed tested whether significant differences existed between observed and expected values for the presence or absence of *E. coli* in the following pairs:

1. Milk versus water.
2. Milk versus pulsator surface.
3. Milk versus surface pipe.
4. Milk versus positioning of the cows in the dairy shed.

3.5.2 Indexes

Milk quality indexes (MQIs) were calculated for the different milk samples. A MQI was calculated from the point of view of suitability of raw milk for further processing and for human consumption. The calculation of a milk quality index was based on the water quality index as described by Ramakrishnaiah *et al*. (2009). Three steps were followed to compute the MQI. The parameters used to calculate a MQI were total bacterial count (TBC), coliforms and somatic cell count (SCC) in milk. For *E. coli* only the presence was tested for, thus no values for *E. coli* were included in the calculations. In the first step, each of the three parameters, TBC, coliforms and SCC, was assigned a weighting (w_i) of relative importance in the overall quality of milk. In step 2 the relative weight (W_i) was calculated with the following calculation:

$$W_i = w_i / \sum_{i=1}^{n} w_i \qquad (1)$$

Where, W_i is the relative weight, w_i is the weight for each parameter and n is the number of parameters. In the last step, a quality rating scale (q_i) was calculated for each parameter by dividing its concentration in the milk sample by its legal standard. This legal standard was then multiplied by 100:

$$q_i = (C_i / S_i) \times 100 \quad (2)$$

Where q_i is the quality rating, C_i is the concentration of each parameter of each milk sample, and S_i the legal standard. The MQI was computed by calculating the S_i for each parameter, which was then used to determine the MQI as follows:

$$SI_i = W_i \times q_i \quad (3)$$

$$MQI = \Sigma SI_i \quad (4)$$

SI_i is the sub index of the i^{th} parameter; q_i is the rating based on the concentration of the i^{th} parameter and n is the number of parameters. The MQI values were classified into two types, excellent or poor. The milk quality index boundaries were standardized as 0 and 100, where milk with a MQI of less than 100 were classified as excellent and milk with a MQI of more than 100 were classified as poor. Farms with a MQI of less than 100, but that demonstrated *E. coli* in the milk were also classified as poor.

Bacteriological water quality indexes (BWQIs) were calculated from the point of view of suitability of borehole water for use in dairies that produce milk intended for human consumption. The calculation of a bacteriological water quality index was based on the water quality index as described by Ramakrishnaiah *et al*. (2009). Three steps were followed to compute the BWQI. The parameters used to calculate a BWQI were TBC and coliforms in water. For *E. coli* only the presence was tested for, thus no values for *E. coli* were included in the calculations. In the first step, each of the two parameters, TBC and coliforms, was assigned a weighting (w_i) of relative importance in the overall bacteriological quality of the borehole water at the point of use in the dairy. In step 2 the relative weight (W_i) was calculated with the following calculation:

$$W_i = w_i / \Sigma_{i=1}^{n} w_i \quad (5)$$

Where, W_i is the relative weight, w_i is the weight for each parameter and n is the number of parameters. In the last step, a quality rating scale (q_i) was calculated for each parameter by dividing its concentration in the water sample by its legal standard. This value was then multiplied by 100:

$$q_i = (C_i / S_i) \times 100 \quad (6)$$

Where q_i is the quality rating, C_i is the concentration of each parameter in each water sample, and S_i the legal standard for each of the parameters. The BWQI was computed, by calculating the S_i for each parameter, which was then used to determine the BWQI as follows:

$$SI_i = W_i \times q_i \quad (7)$$

$$BWQI = \Sigma SI_i \quad (8)$$

SI_i is the sub index of the i^{th} parameter; q_i is the rating based on the concentration of the i^{th} parameter and n is the number of parameters. The BWQI values were classified into two types, excellent or poor. The bacteriological water quality index boundaries were standardized as 0 and 100, where dairy farms with a BWQI of less than 100 were classified as excellent and dairy farms with a BWQI of more than 100 were classified as poor. Farms with a BWQI of less than 100, but that demonstrated *E. coli* in the water were also classified as poor.

Hygiene quality indexes (HQIs) were calculated from the point of view of suitability of dairies to produce milk intended for human consumption. The calculation of a hygiene quality index was based on the water quality index as described by Ramakrishnaiah *et al*. (2009). Three steps were followed to compute the HQI. The parameters used to calculate a HQI were TBC and coliforms in water and on pulsator and milk pipeline surfaces. For *E. coli* only the presence was tested for, thus no values for *E. coli* were included in the calculations. In the first step, each of the six parameters, which exclude *E. coli*, was assigned a weighting (w_i) of relative importance in the overall hygiene of the milk system. In step 2 the relative weight (W_i) was calculated with the following calculation:

$$W_i = w_i / \Sigma_{i=1}^{n} w_i \quad (5)$$

Where, W_i is the relative weight, w_i is the weight for each parameter and n is the number of parameters. In the last step, a quality rating scale (q_i) was calculated for each parameter by dividing its concentration in the water-, pulsator- and pipeline surface sample by its legal standard. This value was then multiplied by 100:

$$q_i = (C_i / S_i) \times 100 \quad (6)$$

Where q_i is the quality rating, C_i is the concentration of each parameter in each water-, pulsator surface-, and pipeline surface sample, and S_i the legal standard for each of the parameters. The HQI was computed, by calculating the S_i for each parameter, which was then used to determine the HQI as follows:

$$SI_i = W_i \times q_i \quad (7)$$

$$HQI = \Sigma SI_i \quad (8)$$

SI_i is the sub index of the i^{th} parameter; q_i is the rating based on the concentration of the i^{th} parameter and n is the number of parameters. The HQI values were classified into two types, excellent or poor. The hygiene quality index boundaries were standardized as 0 and 100, where dairy farms with a HQI of less than 100 were classified as excellent and dairy farms with a HQI of more than 100 were classified as poor. Farms with a HQI of less than 100, but that demonstrated *E. coli* in the water or on the dairy contact surfaces were also classified as poor.

Chapter 4
Results of milk parameters

4.1 Introduction

Eighty-three dairy farms that supply milk to the Mangaung region revealed data about four different milk parameters. These parameters comprised of the presence or absence of *E. coli* and the quantification of coliforms, total bacterial count and somatic cell count. Somatic cells were quantified to obtain information about the health status of the dairy herd of a particular farm. Although 83 dairy farms were studied, 12 of the farms had more than one storage tank therefore more than one measurement (up to four) was taken at these farms, making up a total of 102 measurements per parameter.

When representing the data graphically, the extreme values (outliers) caused the bunching of the less extreme values to the lower region of the graphs, lowering the impact of the representation of the data. It was then decided to remove these extreme values to ease the interpretation of the data. This was achieved by excluding values that were at least 1.5 interquartile below the first quartile or at least 1.5 interquartile above the third quartile.

4.2 Quantification of *E. coli* in milk

The presence of *E. coli* was ascertained for bulk milk collected on the 83 farms studied. According to the regulations relating to milk and dairy products, Regulation R.1555 of 1997, promulgated under the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972) milk may not contain any *E. coli.* The milk data revealed a high number of farms (93%) displaying *E. coli* in their bulk milk containers, thus 95 of 102 measurements did not comply with the legal standard because of the presence of *E. coli* (Figure 4.1).

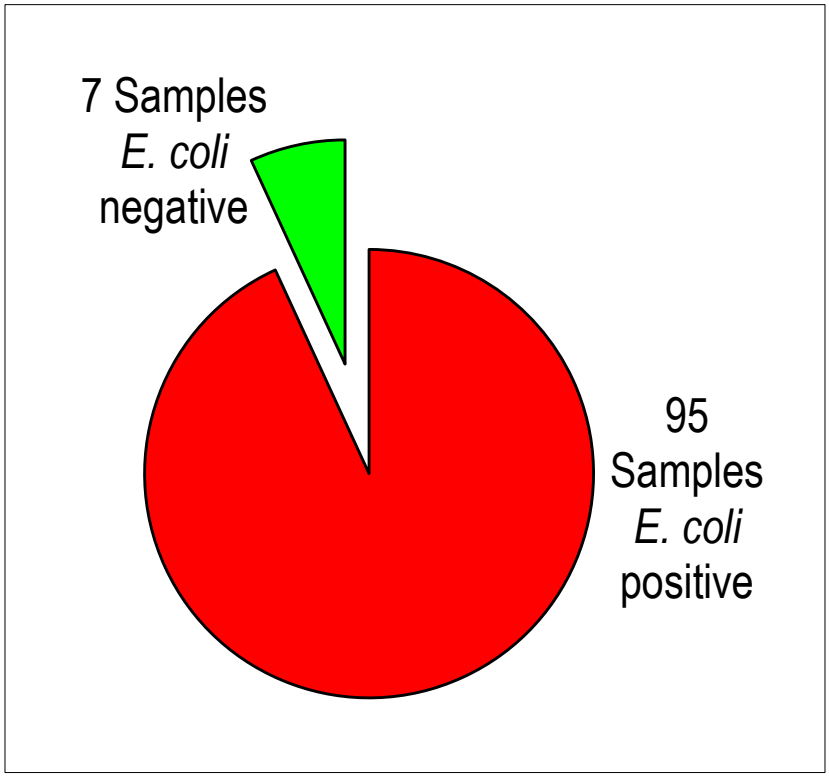
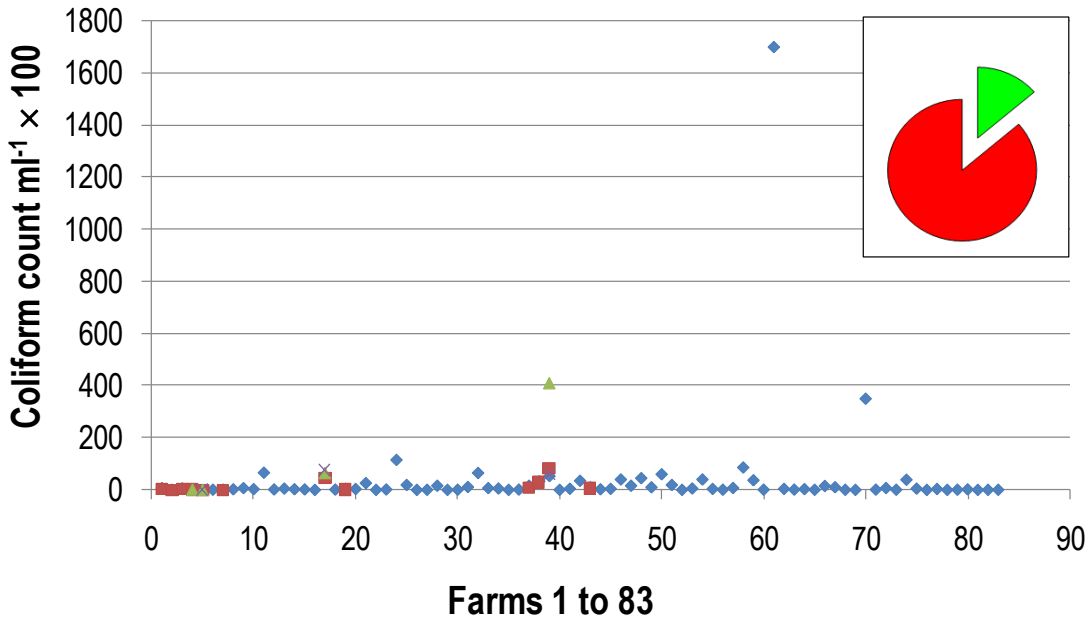


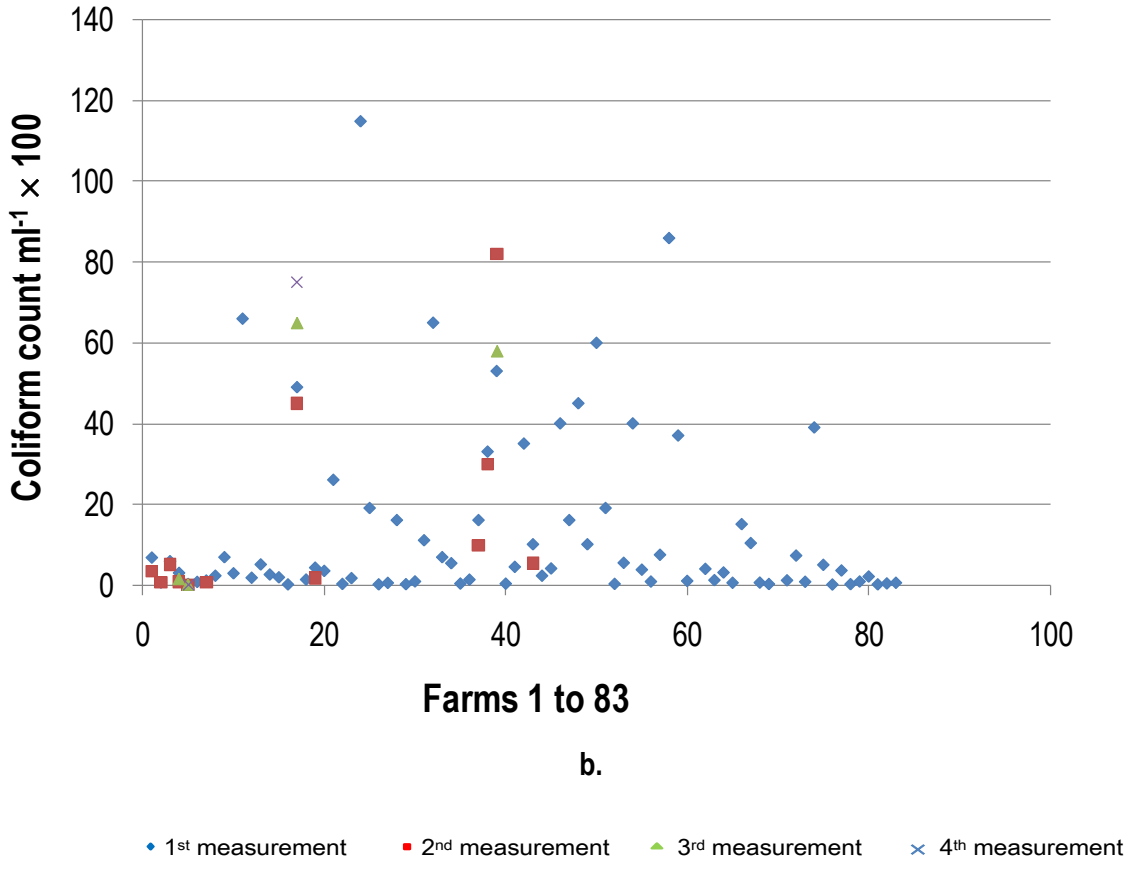
Figure 4.1 Presence/absence of *E. coli* in bulk milk of the 83 farms studied. Green represents compliance and red non-compliance with the legal standard (R. 1555; Act No 54 of 1972).

4.3 Quantification of coliforms in milk

The quantification of coliform bacteria in milk was ascertained for bulk tank milk on the 83 dairy farms studied. Even though three measurements, ranging from 35×10^3 to 17×10^4 CFU ml^{-1}, were substantially higher than the rest of the measurements (Figure 4.2-a), the milk data revealed that 86% of the measurements did not meet the legal standard of <20 CFU ml^{-1} as prescribe in Act No 54 of 1972 (Figure 4.2-a, pie graph). When the three outliers with measurements greater than 3×10^5 CFU ml^{-1} were removed from the data, the graph (Figure 4.2-b) showed that a substantial number of data points lied outside the prescribed legal standard of < 20 CFU ml^{-1} (Figure 4.2-b).



a.

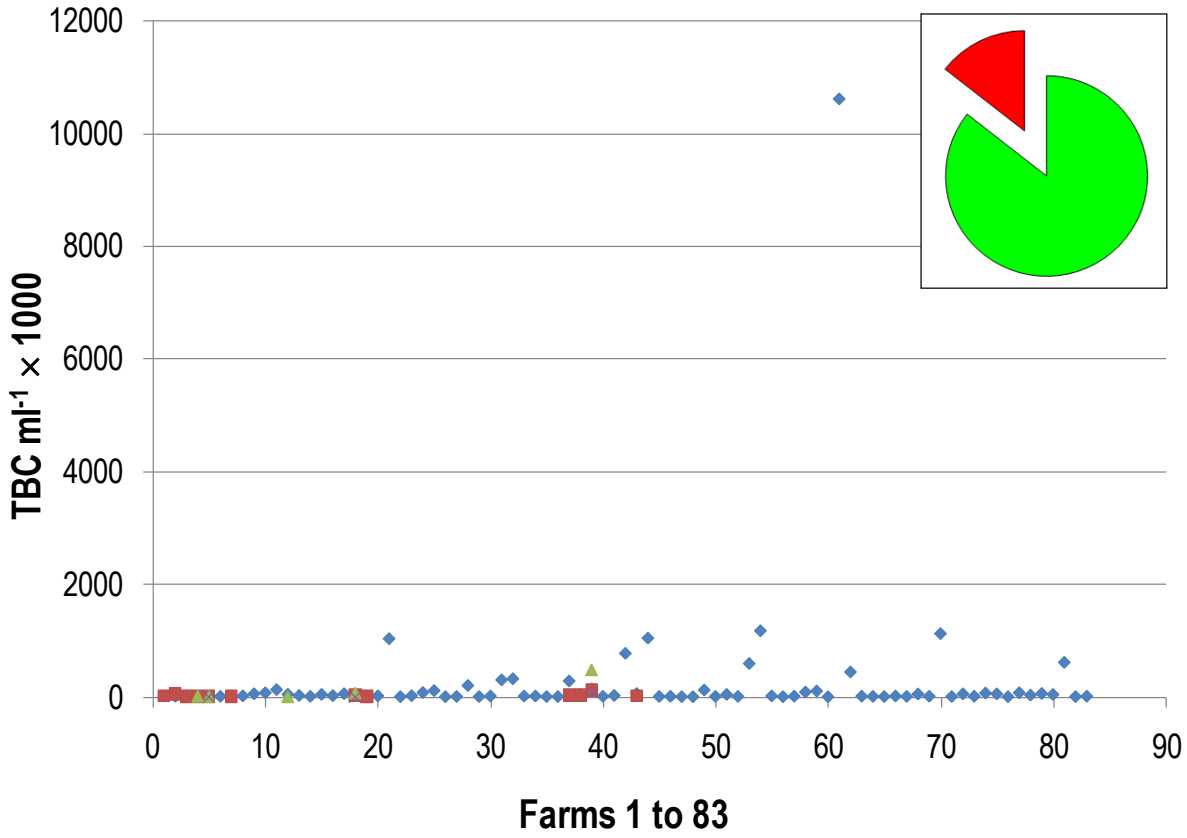


b.

Figure 4.2 Coliform counts in bulk milk of the 83 dairy farms studied. **a.** Including outliers, and **b.** With outliers removed. Small pie graph of coliform count includes all measurements; where green indicates compliance and red non-compliance to legal standards (R. 1555; Act No 54 of 1972).

4.4 Total bacterial count in milk

Total bacteria were quantified for bulk tank milk for the 83 dairy farms studied. One farm demonstrated an exceptionally high number of bacteria in the bulk milk of 10.6 $\times 10^6$ CFU ml^{-1} (Figure 4.3-a). The milk data also revealed that a large proportion (85%) of the measurements did comply with the legal standard of <2 $\times 10^5$ CFU ml^{-1} for TBC (Act No 54 of 1972) (Figure 4.3-a, pie graph). When the outlier of 10.6 $\times 10^6$ CFU ml^{-1} was removed from the data, Figure 4.3-b gives a better representation of all the other data points. Although a large portion of the measurements complied with the legal standard (R.1555; Act No 54 of 1972), a number of data points, with measurements between 2.8 $\times$ 10 and 11.7 $\times 10^5$ CFU ml^{-1} were substantially higher than the standard of < 2 $\times 10^5$ CFU ml^{-1} (R. 1555; Act No 54 of 1972) (Figure 4.3-b).



a.

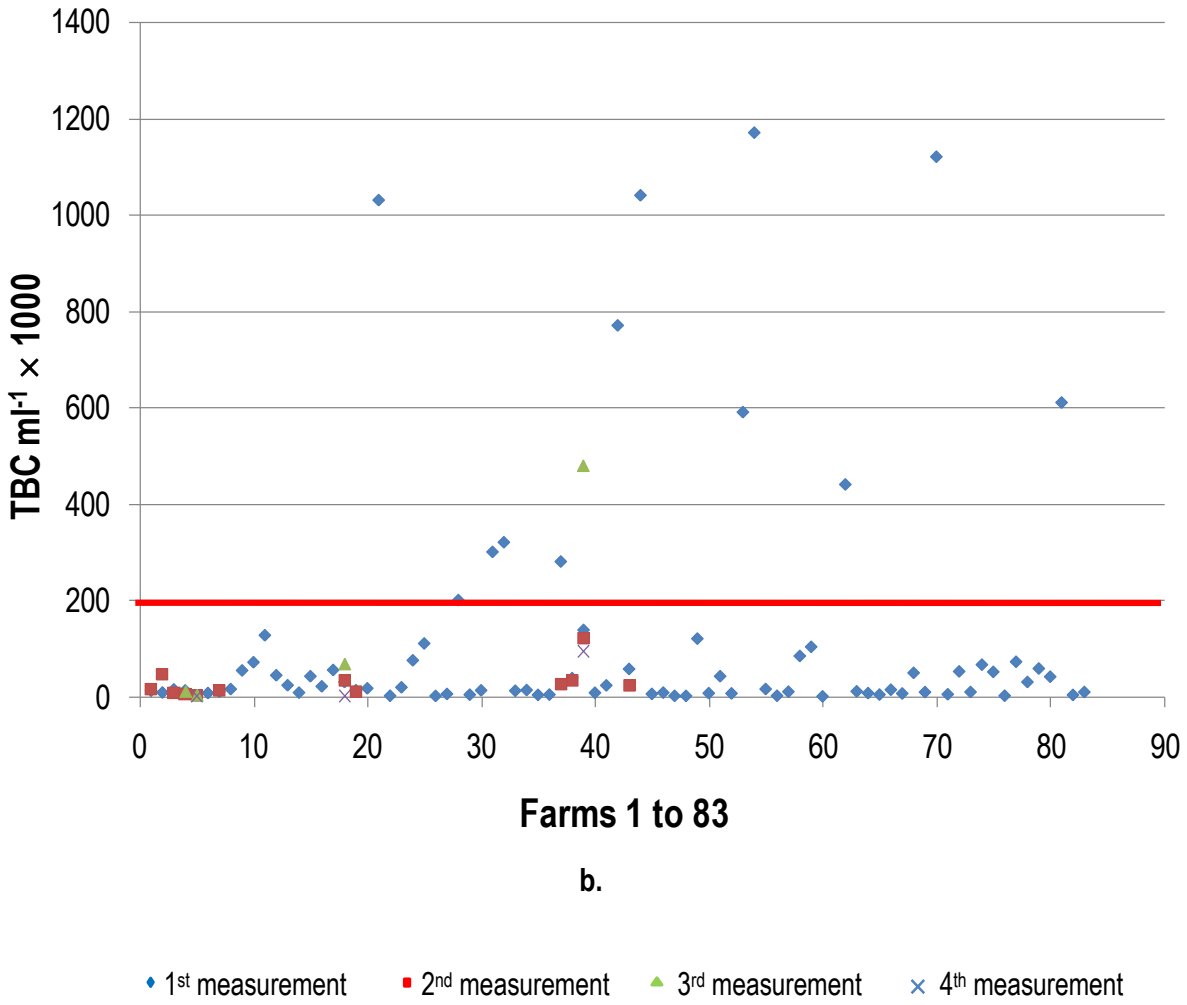


Figure 4.3 Total bacterial count (TBC) in bulk milk of the 83 dairy farms studied. **a.** Without outliers removed, and **b.** With outliers removed. Small pie graph of TBC includes all measurements; green indicates compliance and red non-compliance to legal standards (R.1555; Act No 54 of 1972).

4.5 Somatic cell count in milk

The somatic cell count (SCC) of bulk milk on the 83 dairy farms studied was quantified and ranged from 20 600 to 4 056 300 ml^{-1}. Approximately half of the farms (58%) revealed data that conformed to the legal standard of 5×10^5 ml^{-1} (Act No 54 of 1972) (Figure 4.4, pie graph); however, four of the farms demonstrated exceptionally high SCCs in their bulk milk, which ranged from 2 659 700 to 4 056 300 ml^{-1} (Figure 4.4). 58% of the measurements complied with the legal standard of 5×10^5 ml^{-1} (Act No 54 of 1972) (Figure 4.4, pie graph).

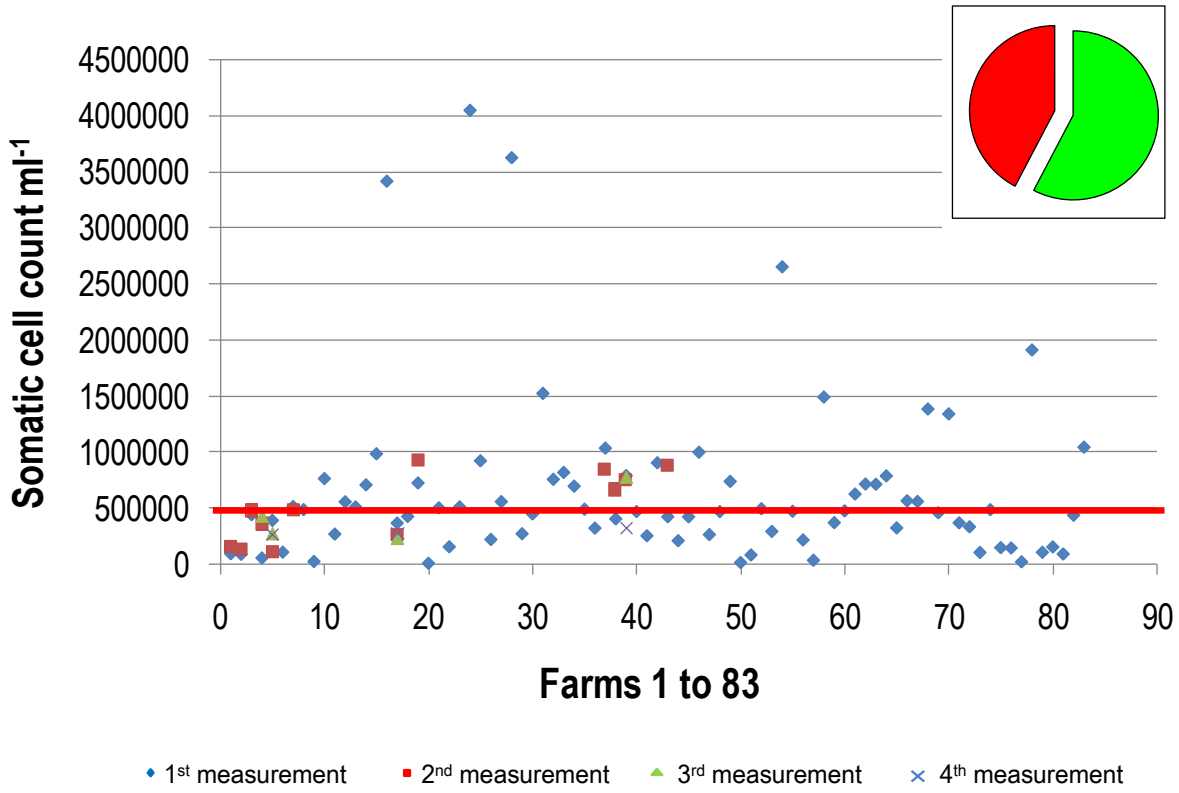


Figure 4.4 Somatic cell count (SCC) in bulk milk of the 83 dairy farms studied. Small pie graph of SCC; green indicates compliance and red non-compliance to legal standards (R.1555; Act No 54 of 1972).

4.6 Summary of milk parameters data

All parameters demonstrated that the measurements varied considerably (Table 4.1). The means of the measurements were all outside of the legal standards (R.1555; Act 54 of 1972). The median of the coliform measurements was considerably higher than the legal standard of < 20 CFU ml^{-1}, whereas the median of TBC and SCC showed to be within the legal standard of 2 × 10^5 CFU and 5 × 10^5 ml^{-1} respectively (R.1555; Act 54 of1972). The ranges of all parameters demonstrated a very large difference between the smallest and the largest values demonstrated by the standard deviation (SD).

Table 4.1 Summary statistical data of the milk parameters studied for the 83 dairy farms.

Parameters	Statistical data of milk parameters					
	Legal standard ml^{-1}	Mean	Median	Range	SD	% Compliant
E. coli	0	*	*	*	*	7
Coliform count	<20 CFU	3 659	295	1 – 170 000	9.30	14
Total bacterial count (TBC)	<200 000 CFU	432 799	18300	1050 – 10 600 000	1.26	85
Somatic cell count (SCC)	500 000	622 287	475 400	20 600 – 4 056 300	21.71	58

SD=Standard deviation; * =No mean, median, range or standard deviation for *E. coli*, as only the presence/absence was tested for; CFU=Colony forming units.

All milk parameters demonstrated different levels of compliance and non-compliance with the legal standard (R.1555; Act No 54 of 1972). For the two parameters presence or absence of *E. coli* and coliforms, compliance was low, less than 20% of the farms were compliant, while for the two parameters TBC and SCC compliance was above 50%.

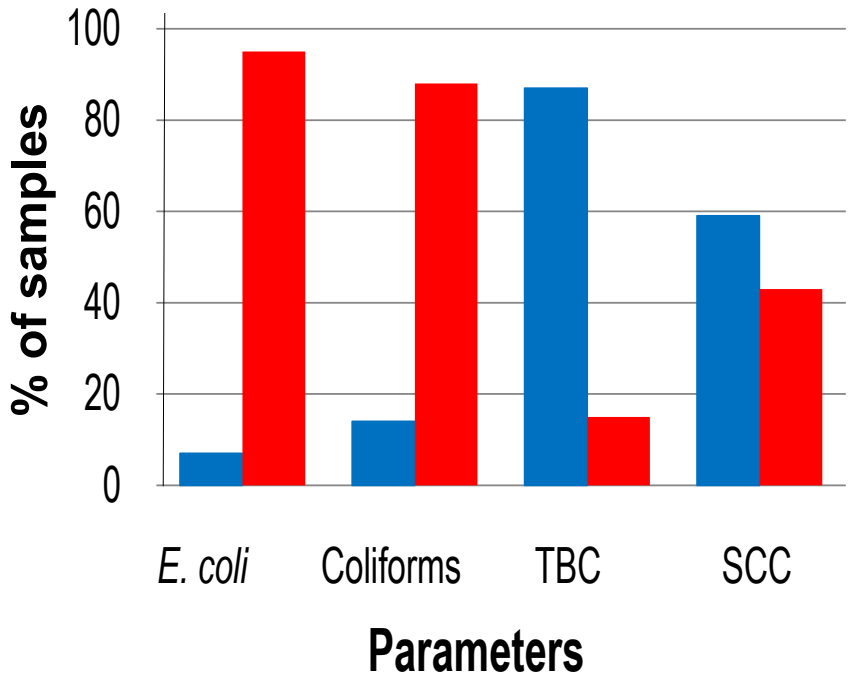


Figure 4.5 Percentages of compliance (blue bars) and non-compliance (red bars) for all milk parameters studied (Regulation R. 1555 of 1997; Act No 54 of 1972).

Chapter 5
Results of surface parameters

5.1 Introduction

The bacteriological content of milk contact surfaces revealed data of three different parameters. These parameters comprised of the presence or absence of *E. coli* as well as the quantification of coliforms and total bacterial count. Two measurements were taken at each of the 83 dairy farms studied. One measurement was taken on the pulsator surface and one measurement was taken on the inside of the milk pipeline.

When representing the data graphically, the extreme values (outliers) caused the bunching of the less extreme values to the lower region of the graphs, lowering the impact of the representation of the data. It was then decided to remove these extreme values to ease the interpretation of the data. This was achieved by excluding values that were at least 1.5 interquartile below the first quartile or at least 1.5 interquartile above the third quartile.

5.2 Pulsator data

5.2.1 Quantification of *E. coli* on pulsator surfaces

The presence of *E. coli* was ascertained for surface swabs taken on the pulsator surfaces of the 83 dairy farms studied. According to the regulations Relating to Milk and Dairy products, Regulation R.1555 of 1997, promulgated under the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972) milk may not contain any *E. coli* and therefore milk contact surfaces may also not contain any *E. coli.* The surface data revealed that only a few farms (13%) displayed *E. coli* on their pulsator surfaces, thus a large majority of the farms did comply with the legal standard because of the absence of *E. coli* (Figure 5.1).

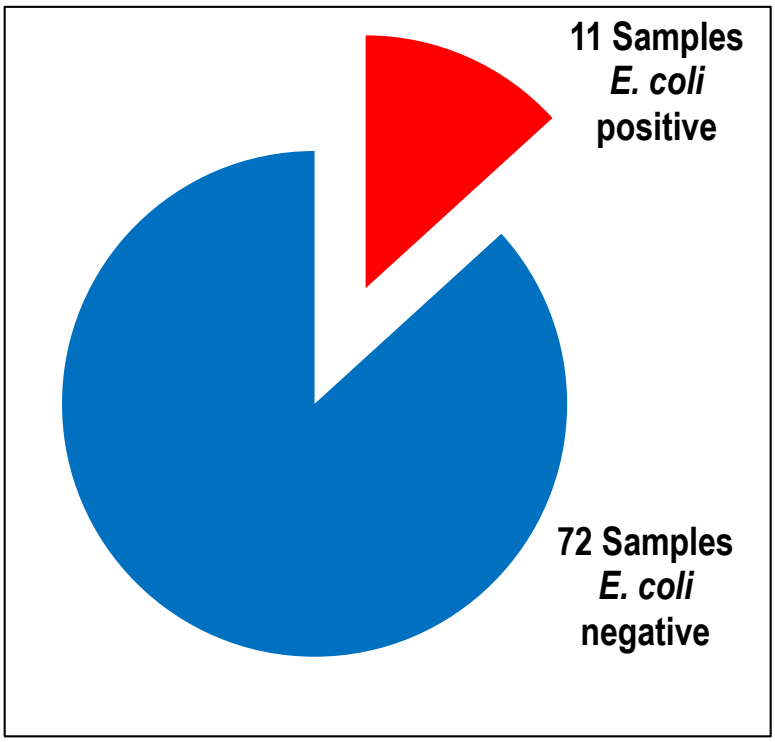
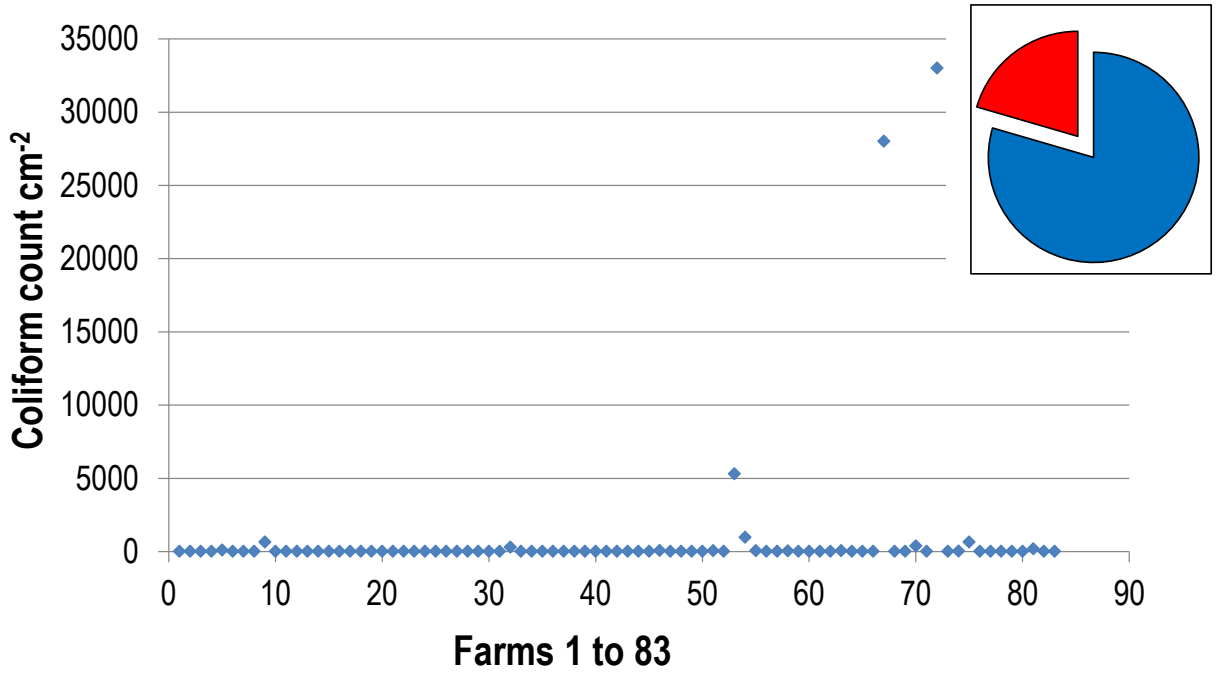


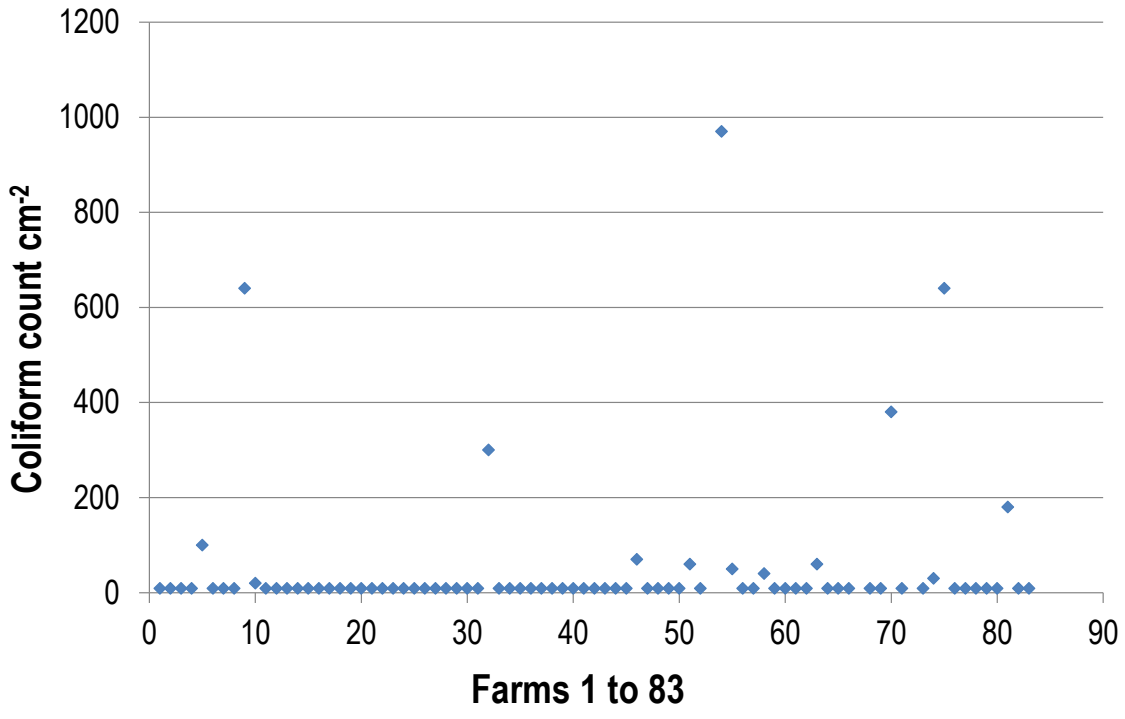
Figure 5.1 Presence/absence of *E. coli* on pulsator surfaces of 83 dairy farms studied. Blue represents compliance and red non-compliance to the legal standard (Regulation R. 1555; Act No 54 of 1972).

5.2.2 Quantification of coliforms on the pulsator surfaces

The quantification of coliform bacteria on milk contact surfaces was ascertained for pulsator surfaces on the 83 dairy farms studied. According to the regulations Relating to Milk and Dairy products, Regulation R.1555 of 1997, promulgated under the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972) milk may not contain more than 20 coliform bacteria per 1.0 ml and therefore milk contact surfaces may also not contain more coliforms than the legal standard of < 20 CFU cm^{-2}. Three measurements, ranging from 5.3×10^3 to 33×10^3 CFU cm^{-2}, were substantially higher than the rest of the measurements (Figure 5.2-a). The pulsator surface data furthermore revealed that 80% of the measurements did meet the legal standard of Act No 54 of 1972 (Figure 5.2-a, pie graph). When the three outliers with measurements greater than 5×10^3 CFU cm^{-2} were removed from the data, the graph (Figure 5.2-b) showed that only a few data points lied outside the prescribed legal standard of < 20 CFU cm^{-2} (R. 1555; Act No 54 of 1972) (Figure 5.2-b).



a.

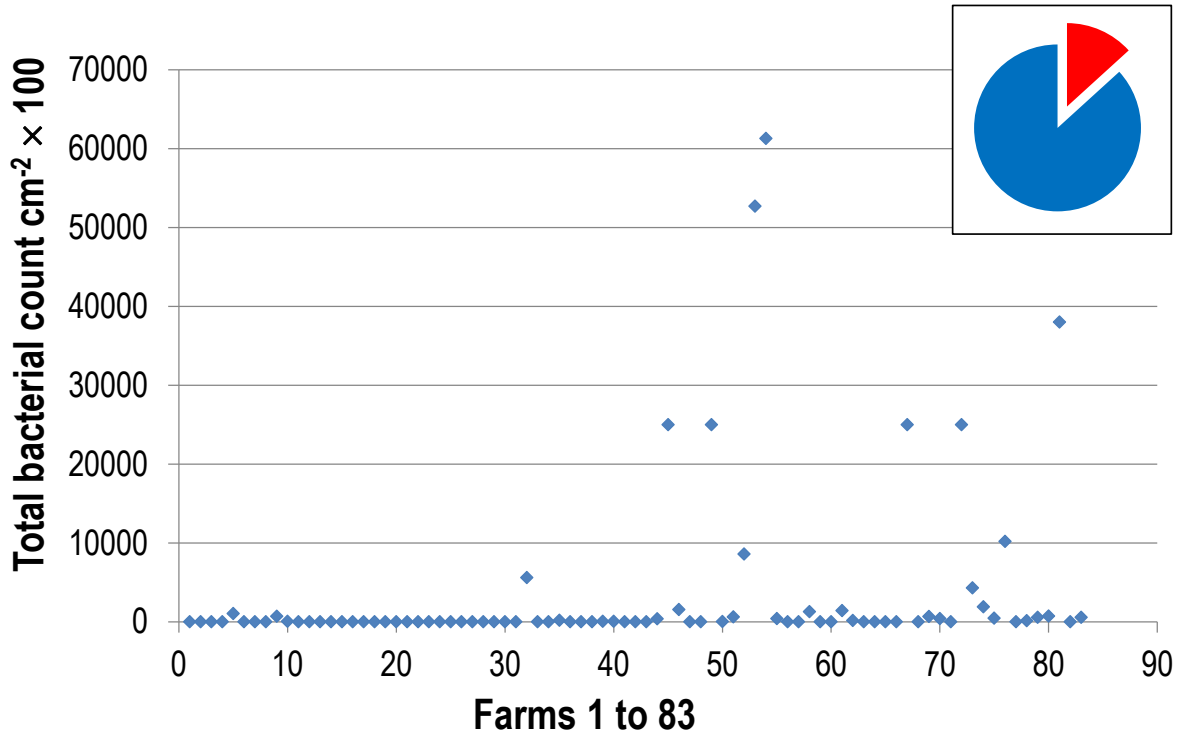


b.

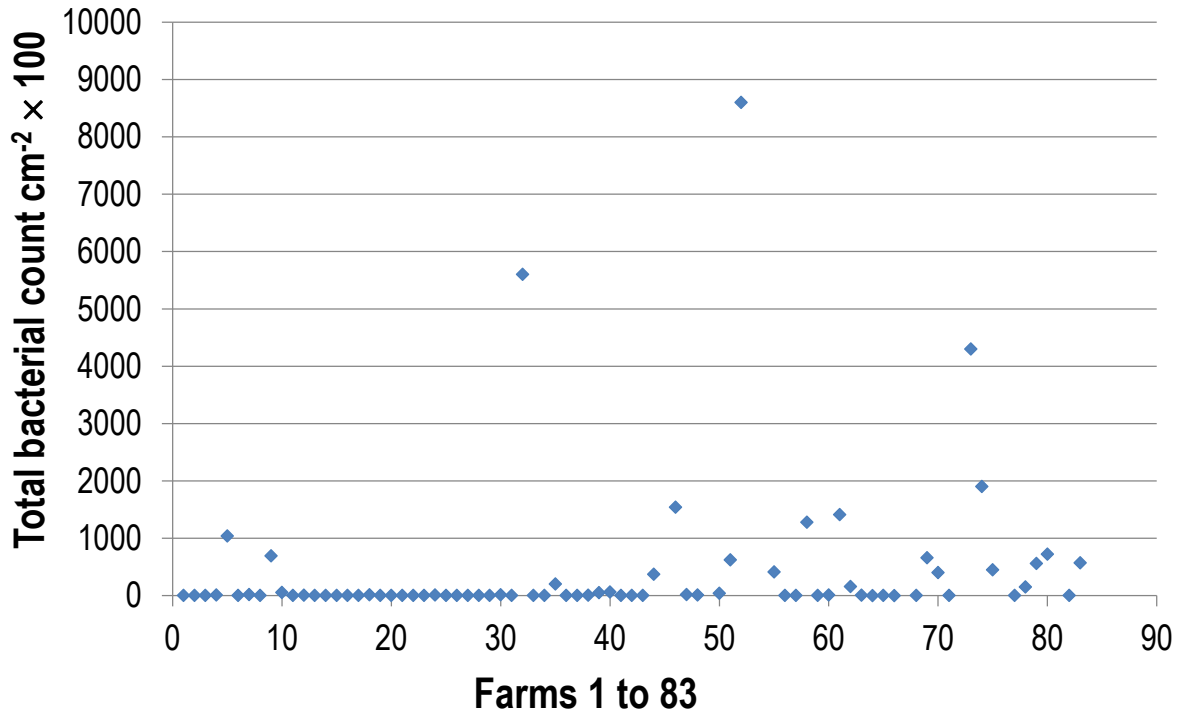
Figure 5.2 Coliform counts on the pulsator surfaces of the 83 dairy farms studied. a. Including outliers, and b. With outliers removed. Small pie graph of coliform count includes all measurements; blue indicates compliance and red non-compliance to legal standards (Regulation R. 1555; Act No 54 of 1972).

5.2.3 Total bacterial count (TBC) on the pulsator surfaces

Total bacteria were quantified for pulsator surfaces of the 83 dairy farms studied. Eight farms demonstrated exceptionally high numbers of bacteria on their pulsator surfaces. These measurements ranged between 10.2 $\times$ 10^5 and 61.3 $\times$ 10^5 CFU cm^{-2} (Figure 5.3-a). The pulsator surface data also revealed that a large proportion (81%) of the measurements did not comply with the legal standard of < 10 CFU cm^{-2} for TBC (Regulations relating to hygiene requirements for milking sheds, the transport of milk and related matters, Regulation R. 961 of 2012, promulgated under the Foodstuffs, Cosmetics and Disinfectants Act, Act 54 of 1972) (Figure 5.3-a, pie graph). When the outliers were removed from the data, Figure 5.3-b gives a better representation of all the other data points.



a.



b.

Figure 5.3 Total bacterial count (TBC) on the pulsator surfaces of the 83 dairy farms studied. **a.** Including outliers, and **b.** With outliers removed. Small pie graph of TBA includes all measurements; blue indicates compliance and red non-compliance to legal standards (Regulation R. 961; Act No 54 of 1972).

5.2.4 Summary of pulsator data

The measurements of all the parameters studied varied considerably. The means of the measurements were all outside of the legal standards (Regulation R. 1555 of 1997 and Regulation R. 961 of 2012) (Table 5.1). The median of the coliform measurements was within the legal standard of 20 (Regulation R. 1555 of 1997); whereas the median of TBC showed to be substantially higher than the legal standard of 1 × 10 CFU cm^{-2} (Regulation R. 961 of 2012). The ranges of all parameters demonstrated a very large difference between the smallest and the largest values demonstrated by the standard deviation (SD).

Table 5.1 Summary statistical data of the pulsator surface parameters for the 83 dairy farms studied.

Parameters	Statistical data of pulsator surface parameters					
	Legal standard cm^2	Mean	Median	Range	SD	% Compliant
E. coli	0 #	*	*	*	*	87
Coliform count	<20 CFU #	848.602	9	9 – 33000	4.93	80
Total bacterial count (TBC)	10 CFU ♣	354419	690	10 – 6130000	1.35	19

SD=Standard deviation; * =No mean, median, range or standard deviation for *E. coli*, as only the presence/absence was tested for. # = Legal standard as per Regulation R.1555 of 1997 (Act 54 of 1972). ♣ = Legal standard as per Regulation R.961 of 2012 (Act 54 of 1972); CFU=Colony forming units.

All pulsator surface parameters demonstrated different levels of compliance and non-compliance with the legal standard (Regulation R. 1555 of 1997 and Regulation R. 961 of 2012; Act No 54 of 1972). For the two parameters presence or absence of *E. coli* and coliforms, compliance was high, with 80% and 87% of the farms being compliant, while for the parameter TBC compliance was below 20%.

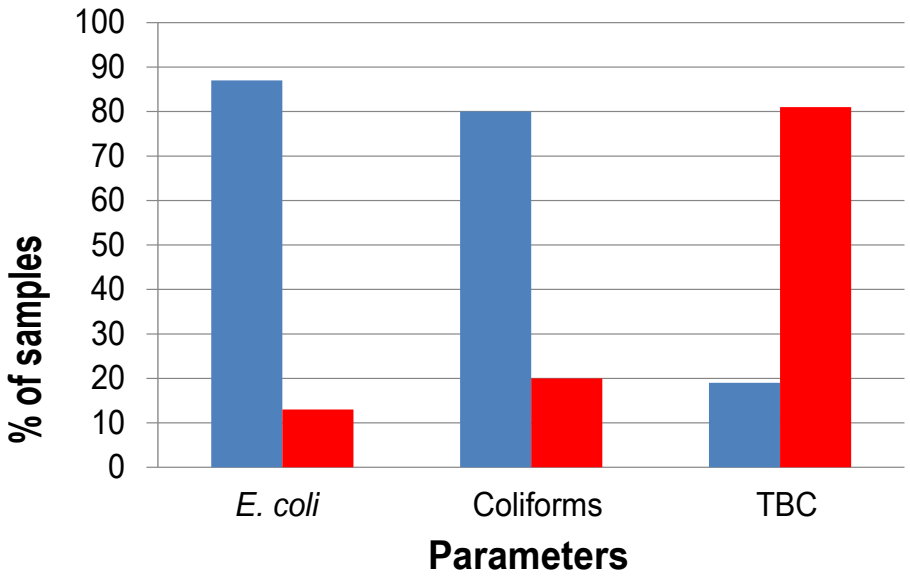


Figure 5.4 Percentages of compliance (blue bars) and non-compliance (red bars) for all pulsator surface parameters studied (Regulation R. 1555 of 1997 and Regulation R. 961 of 2012; Act No 54 of 1972).

5.3 Milk pipeline data

5.3.1 Quantification of *E. coli* on the milk pipeline surfaces

The presence of *E. coli* was ascertained for surface swabs taken on the milk pipeline surfaces of the 83 dairy farms studied. According to the regulations relating to milk and dairy products, Regulation R.1555 of 1997, promulgated under the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972) milk may not contain any *E. coli* and therefore milk contact surfaces may also not contain any *E. coli.* The surface data revealed that only a few farms displayed *E. coli* on their milk pipeline surfaces, 13%, thus a large majority of the farms did comply with the legal standard because of the absence of *E. coli* (Figure 5.5).

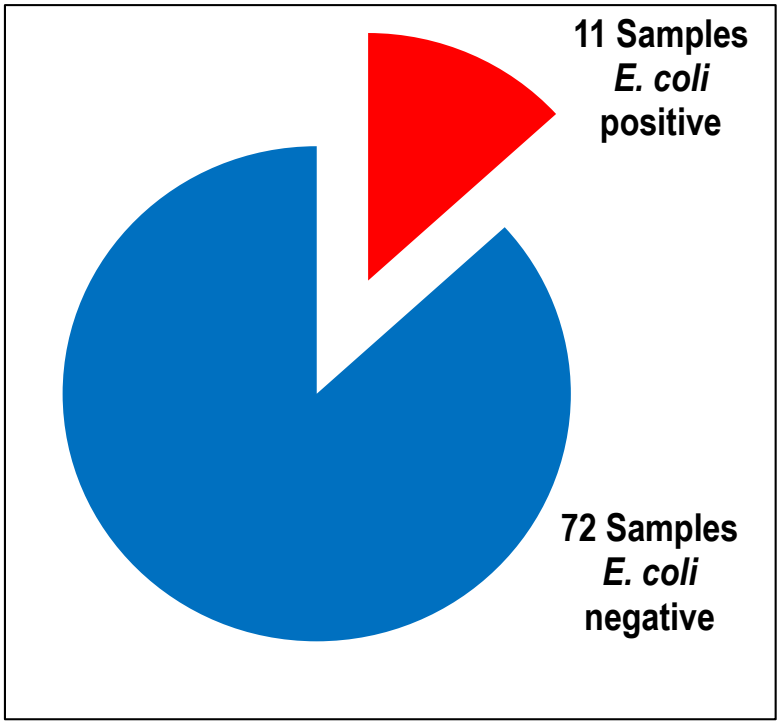
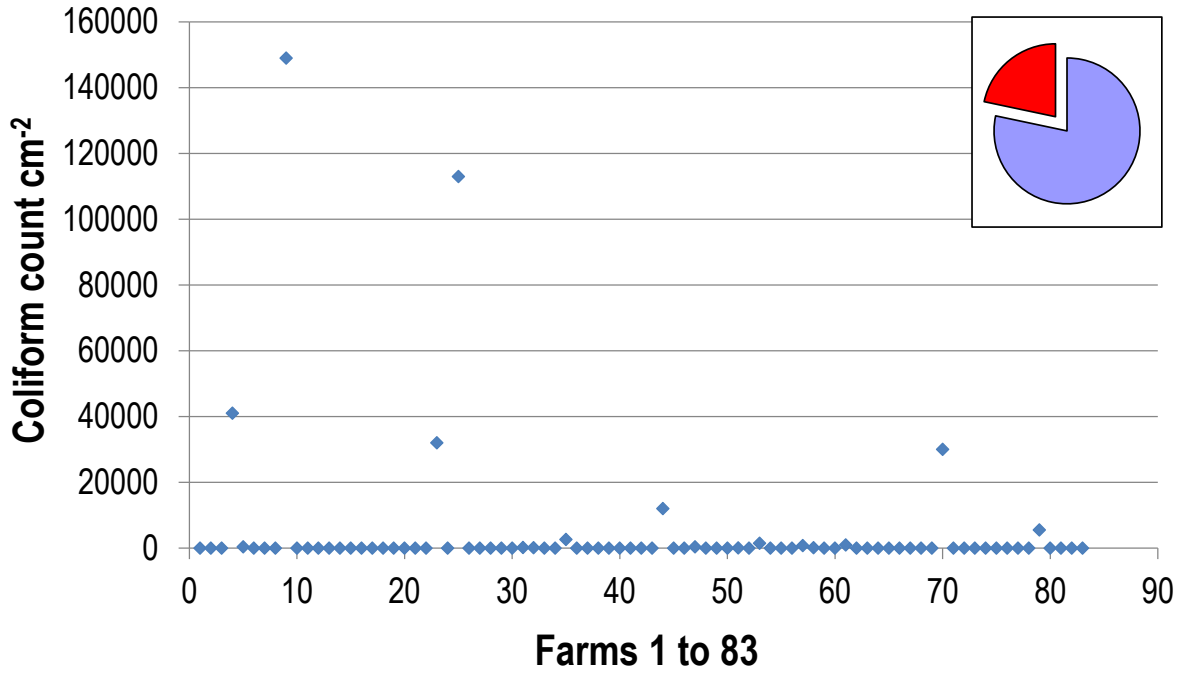


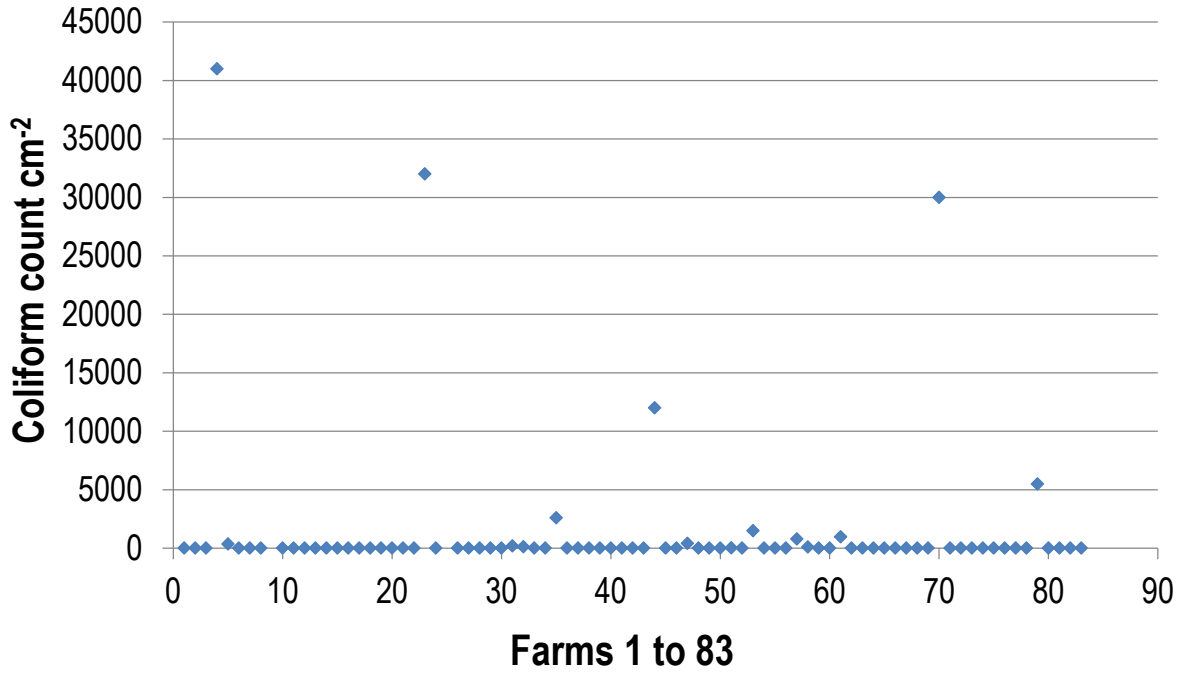
Figure 5.5 Presence/absence of *E. coli* on milk pipeline surfaces of 83 dairy farms studied. Blue represents compliance and red non-compliance to the legal standard (Regulation R. 1555; Act No 54 of 1972).

5.3.2 Quantification of coliforms on the milk pipeline surfaces

The quantification of coliform bacteria on milk contact surfaces was ascertained for milk pipeline surfaces on the 83 dairy farms studied. According to the regulations relating to milk and dairy products, Regulation R.1555 of 1997, promulgated under the Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act No 54 of 1972) milk may not contain more than 20 coliform bacteria per 1.0 ml and therefore milk contact surfaces may also not contain more coliforms than the legal standard of < 20 CFU cm^{-2}. Two measurements of 11.3 $\times$ 10^3 CFU cm^{-2} and 14.9 $\times$ 10^3 CFU cm^{-2} were substantially higher than the rest of the measurements (Figure 5.2-a). The milk pipeline surface data furthermore revealed that 78% of the measurements did meet the legal standard of Act No 54 of 1972 (Figure 5.6-a, pie graph). When the two outliers with measurements greater than 10 $\times$ 10^4 CFU cm^{-2} were removed from the data, the graph (Figure 5.6-b) showed that only a few data points lied outside the prescribed legal standard of < 20 CFU cm^{-2} (Figure 5.6-b).



a.

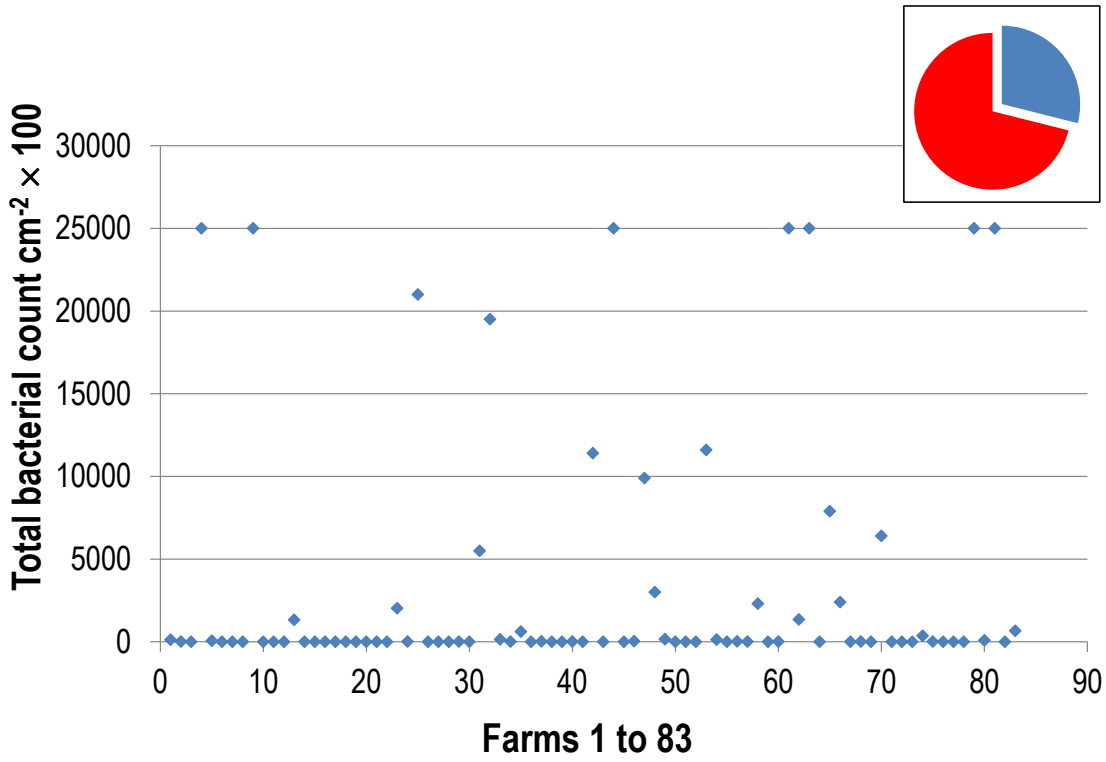


b.

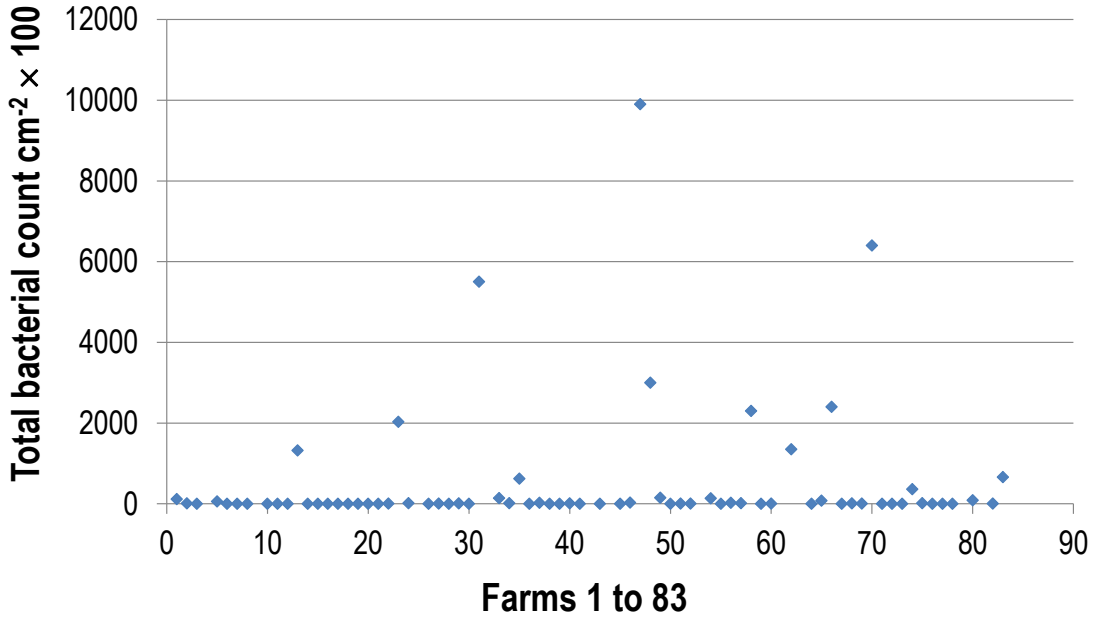
Figure 5.6 Coliform counts on the milk pipeline surfaces of the 83 dairy farms studied. **a.** Including outliers, and **b.** With outliers removed. Small pie graph of coliform count includes all measurements; blue indicates compliance and red non-compliance to legal standards (Regulation R. 1555; Act No 54 of 1972).

5.3.3 Total bacterial count (TBC) on the milk pipeline surfaces

Total bacteria were quantified for milk pipeline surfaces of the 83 dairy farms studied. Eleven farms demonstrated exceptionally high numbers of bacteria on their milk pipeline surfaces. These measurements ranged between 11.4×10^5 and 25×10^5 CFU cm^{-2} (Figure 5.7-a). The milk pipeline surface data also revealed that a large proportion (71%) of the measurements did not comply with the legal standard of < 10 CFU cm^{-2} for TBC (Regulations relating to hygiene requirements for milking sheds, the transport of milk and related matters, R. 961 of 2012, promulgated under the Foodstuffs, Cosmetics and Disinfectants Act, Act 54 of 1972) (Figure 5.7-a, pie graph). When the outliers were removed from the data, Figure 5.7-b gives a better representation of all the other data points.



a.



b.

Figure 5.7 Total bacterial count (TBC) on the milk pipeline surfaces of the 83 dairy farms studied. **a.** Including outliers, and **b.** With outliers removed. Small pie graph of TBA includes all measurements; blue indicates compliance and red non-compliance to legal standards (Regulation R. 961 of 2012; Act 54 of 1972).

5.3.4 Summary of milk pipeline data

The measurements of all parameters studied varied considerably. The means of the measurements were all outside of the legal standards (Regulation R. 1555 of 1997 and Regulation R. 961 of 2012; Act 54 of 1972) (Table 5.2). The median of the coliform measurements was within the legal standard of < 20 (Regulation R. 1555 of 1997; Act No 54 of 1972); whereas the median of TBC showed to be substantially higher than the legal standard of 1 × 10 CFU cm^{-2} (Regulation R. 961 of 2012). The ranges of all parameters demonstrated a very large difference between the smallest and the largest values demonstrated by the standard deviation (SD).

Table 5.2 Summary statistical data of the milk pipeline surface parameters studied for the 83 dairy farms.

Parameters	Statistical data of milk pipeline surface parameters					
	Legal standard cm^2	**Mean**	**Median**	**Range**	**SD**	**% Compliant**
E. coli	0 #	*	*	*	*	87
Coliform count	<20 CFU #	4701.698	9	9 – 149 000	1.97	78
Total bacterial count (TBC)	<10 CFU ♣	331700	630	0 – 2 500 000	32.68	29

SD=Standard deviation; * =No mean, median, range or standard deviation for *E. coli*, as only the presence/absence was tested for. # = Legal standard as per Regulation R.1555 of 1997 (Act 54 of 1972). ♣ = Legal standard as per Regulation R.961 of 2012 (Act 54 of 1972); CFU=Colony forming units.

All milk pipeline surface parameters demonstrated different levels of compliance and non-compliance with the legal standard (Regulation R. 1555 of 1997 and Regulation R. 961 of 2012; Act No 54 of 1972). For the two parameters presence or absence of *E. coli* and coliforms, compliance was high, with 87% and 78% of the farms being compliant, while for the parameter TBC, compliance was below 30%.

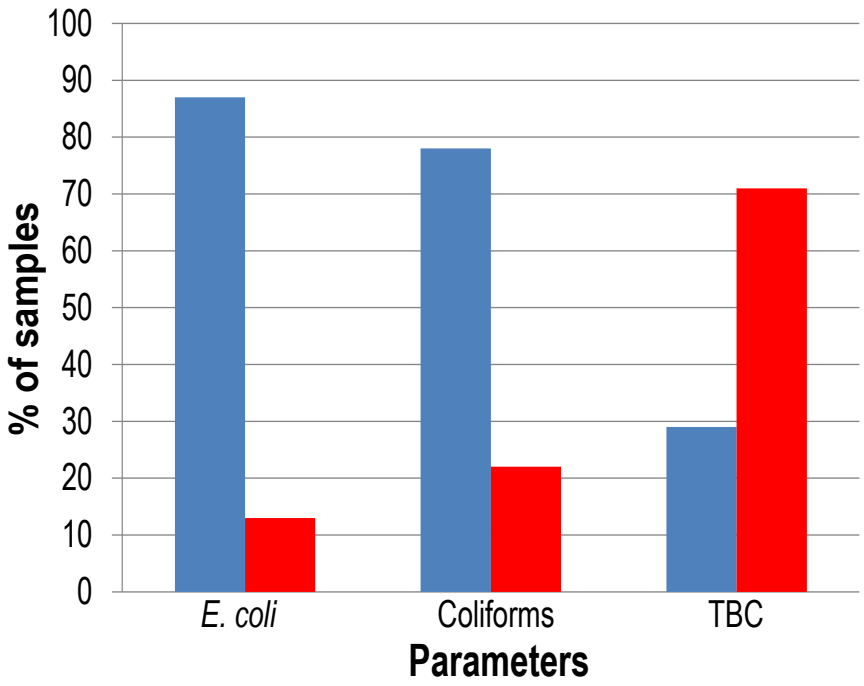


Figure 5.8 Percentages of compliance (blue bars) and non-compliance (red bars) for all milk pipeline surface parameters studied (Regulation R. 1555 of 1997 and Regulation R. 961 of 2012; Act No 54 of 1972).

Chapter 6
Results of water parameters

6.1 Introduction

The bacterial content of the borehole water used on the 83 dairy farms that supply milk to the Mangaung region revealed data about three different water parameters. These parameters comprised of the presence or absence of *E. coli* as well as the quantification of coliforms and total bacterial count. One measurement was taken per farm, making up a total of 83 measurements per parameter.

When representing the data graphically, the extreme values (outliers) caused the bunching of the less extreme values to the lower region of the graphs, lowering the impact of the representation of the data. It was then decided to remove these extreme values to ease the interpretation of the data. This was achieved by excluding values that were at least 1.5 interquartile below the first quartile or at least 1.5 interquartile above the third quartile.

6.2 Quantification of *E. coli* in water

The presence of *E. coli* was ascertained for the water used in the dairies of the 83 farms studied. According to the South African national standard for drinking water SANS 241 of 2011, drinking water may not contain any *E. coli.* The water data revealed a substantial number of farms (31%) displaying *E. coli* in their water used in the dairies, thus 26 of 83 measurements did not comply with the legal standard because of the presence of *E. coli* (Figure 6.1).

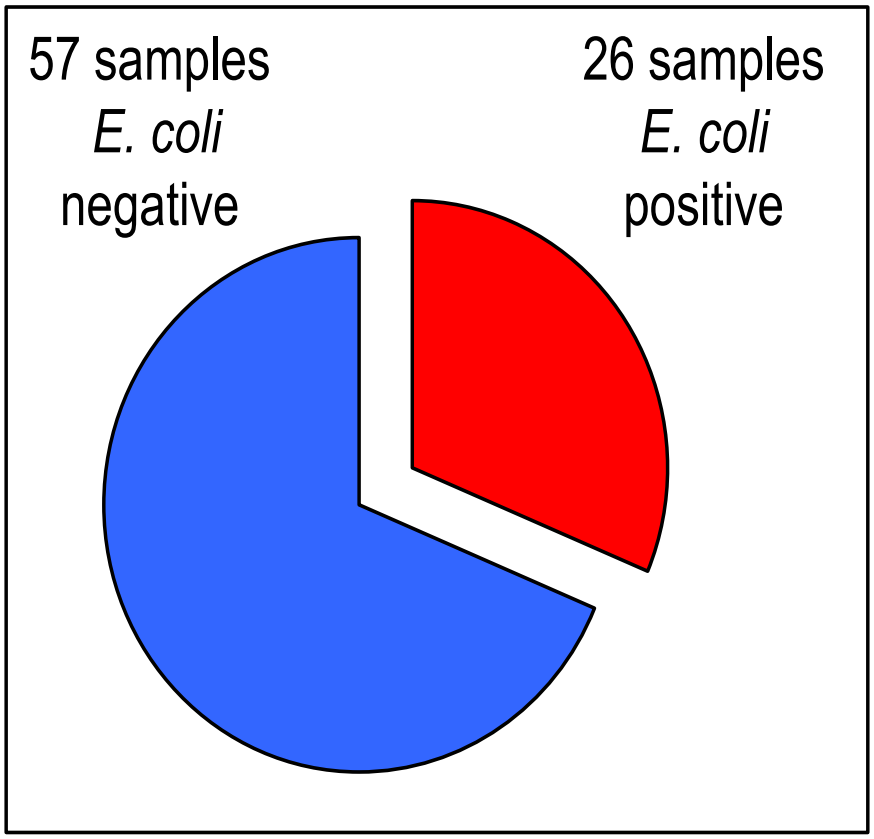
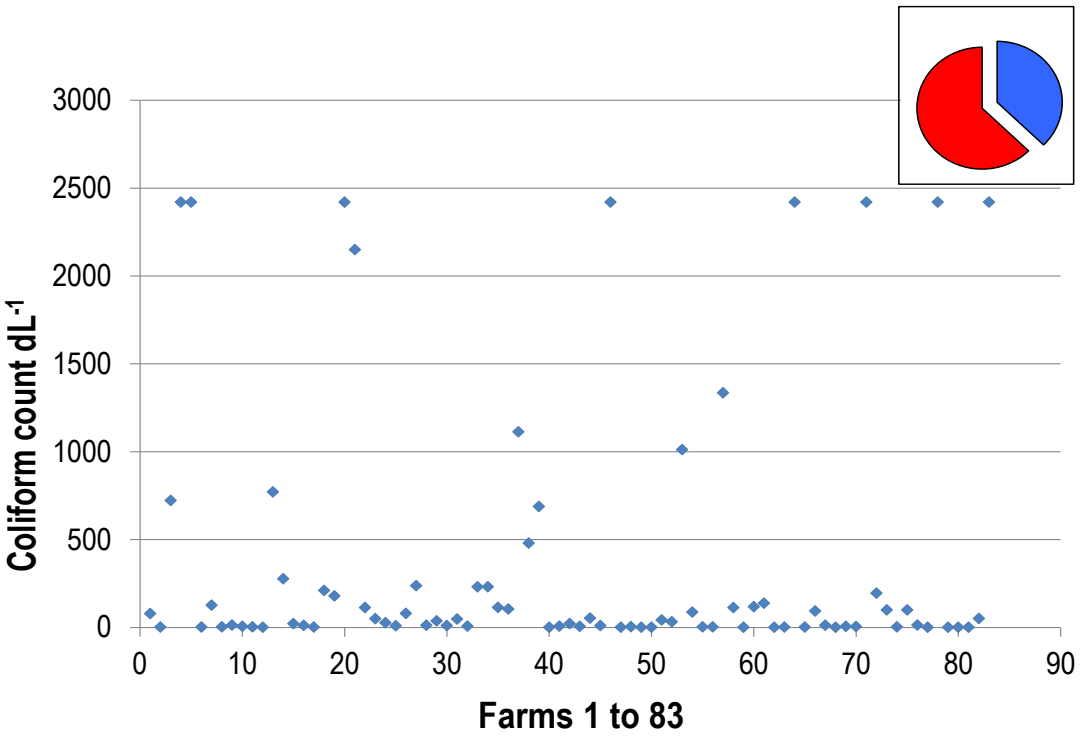


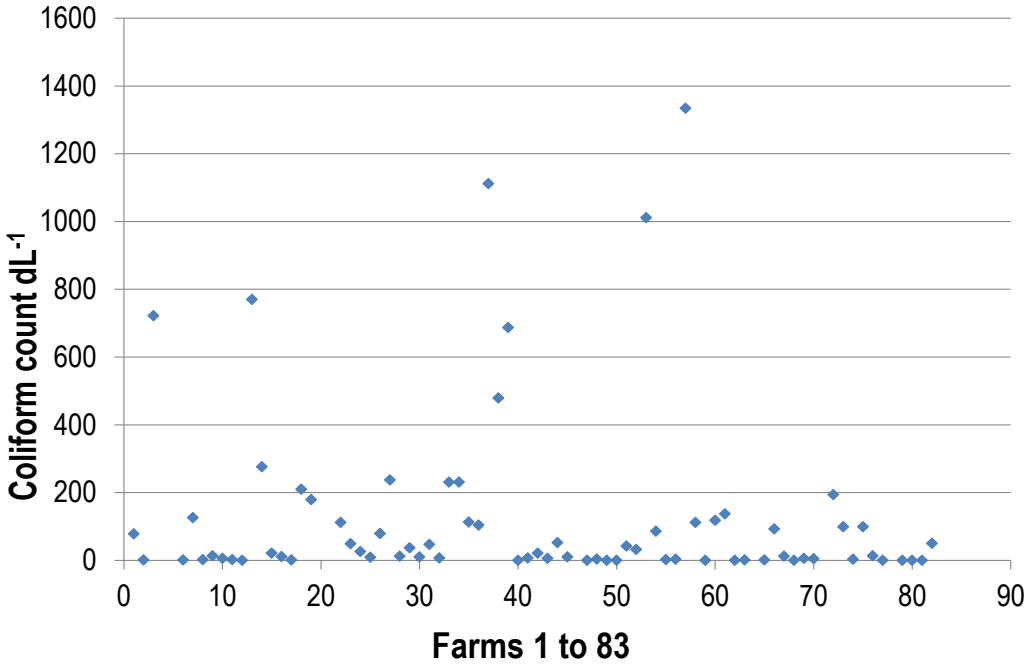
Figure 6.1 Presence/absence of *E. coli* in water of the 83 dairy farms studied. Blue represents compliance and red non-compliance with the legal standard (SANS 241 of 2011).

6.3 Quantification of coliforms in water

The quantification of coliform bacteria in water was ascertained for water used in the dairies of the 83 farms studied. Even though nine measurements of approximately 24×10^2 dL^{-1}, were substantially higher than the rest of the measurements (Figure 6.2-a), the water data revealed that 37% of the measurements did meet the legal standard of less than 10 dL^{-1} SANS 241 of 2011 (Figure 6.2-a, pie graph). When the nine outliers with measurements greater than 24×10^2 dL^{-1} were removed from the data, the graph (Figure 6.2-b) showed that a substantial number of data points lied outside the prescribed SANS 241 (2011) legal standard of < 10 dL^{-1} (Figure 6.2-b).



a.

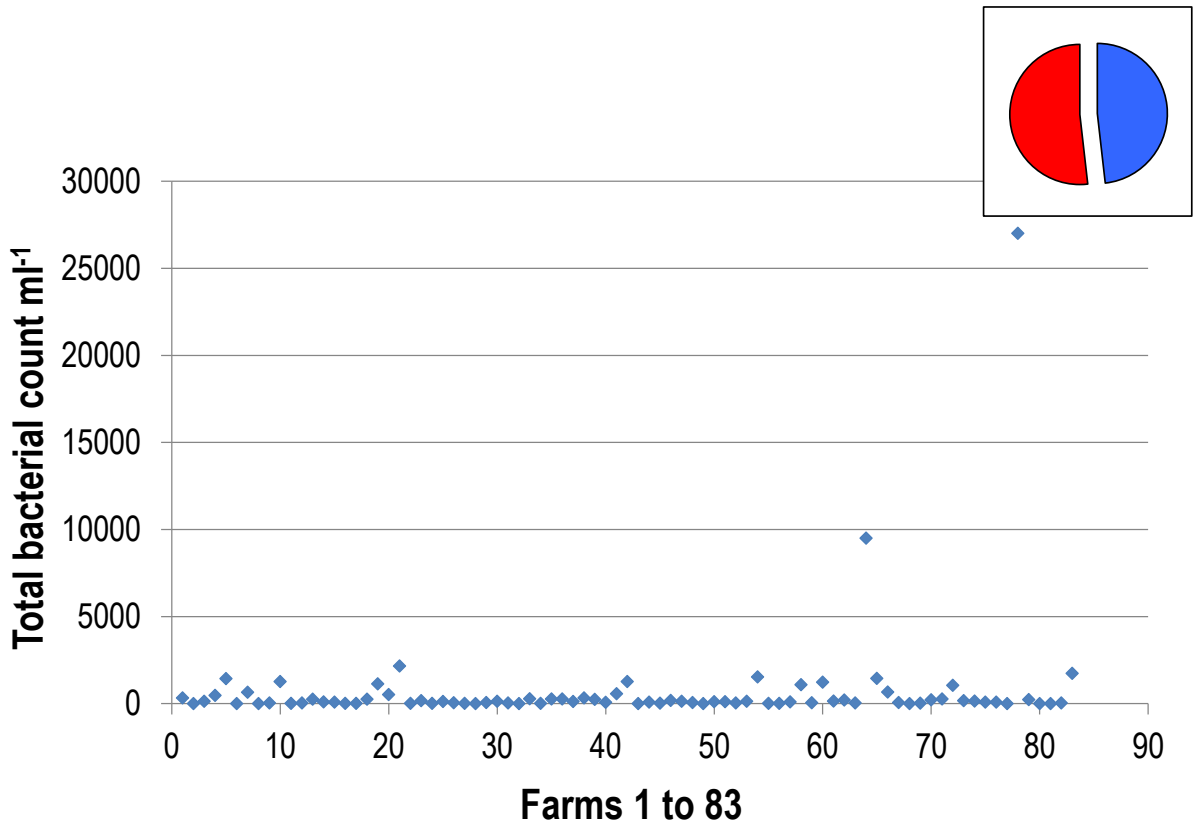


b.

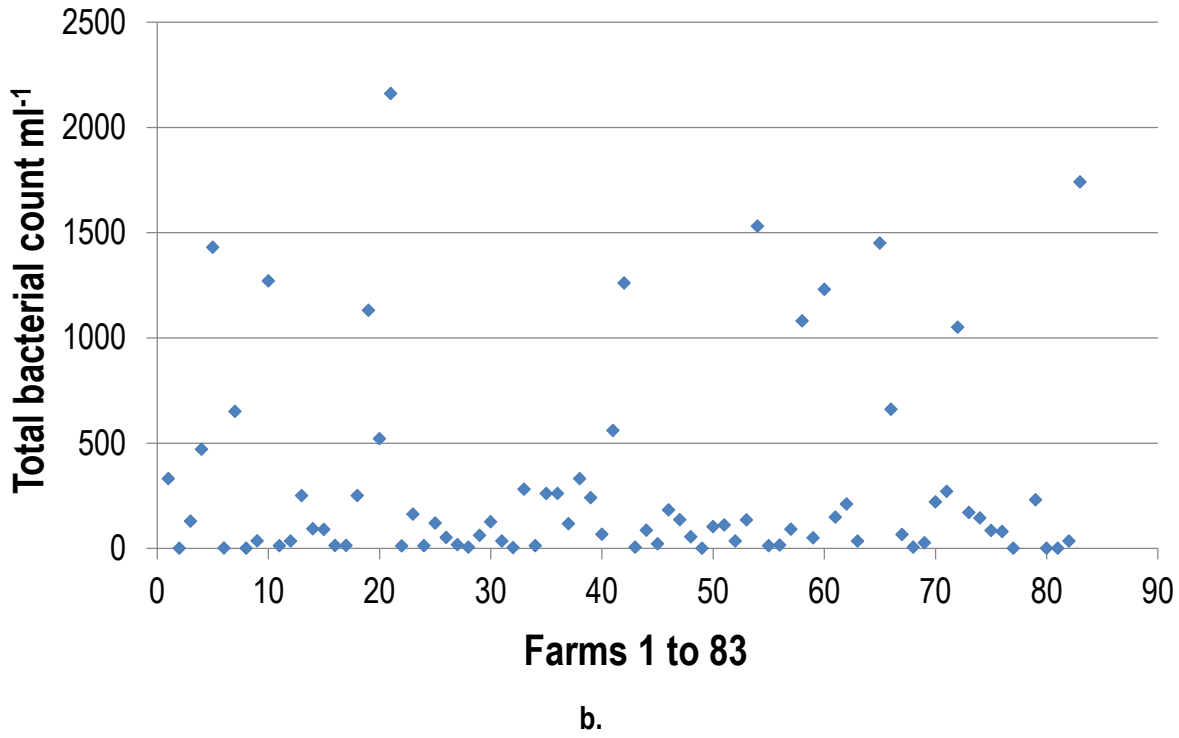
Figure 6.2 Coliform counts in water of the 83 dairy farms studied. **a.** Including outliers, and **b.** With outliers removed. Small pie graph of coliform count includes all measurements; where blue indicates compliance and red non-compliance to legal standards (SANS 241 of 2011).

6.4 Total bacterial count (TBC) in water

Total bacteria were quantified for water used in the dairies of the 83 farms studied. Two farms demonstrated exceptional high numbers of bacteria in the water, 9.5×10^3 ml^{-1} and 2.7×10^4 ml^{-1} (Figure 6.3-a). The water data also revealed that a substantial proportion (52%) of the measurements did not comply with the guideline standard of $<1 \times 10^2$ ml^{-1} for TBC as set out in the Mangaung water action plan (Potgieter *et al*., 2007) (Figure 6.3-a, pie graph). When the outliers of 9.5×10^3 ml^{-1} and 2.7×10^4 ml^{-1} were removed from the data, Figure 6.3-b gives a better representation of all the other data points. Although 48% of the measurements complied with the Mangaung water action plan guideline standard (Potgieter *et al*., 2007), a number of data points, with measurements between 11.2×10^1 and 21.6×10^2 ml^{-1} were substantially higher than the Mangaung water action plan guideline standard of $< 1 \times 10^2$ ml^{-1} (Potgieter *et al*., 2007) (Figure 6.3-b).



a.



b.

Figure 6.3 Total bacterial count (TBC) in water of the 83 dairy farms studied. **a.** Without outliers removed, and **b.** With outliers removed. Small pie graph of TBA includes all measurements; blue indicates compliance and red non-compliance to Mangaung water action plan guideline standards (Potgieter *et al*., 2007).

6.5 Summary of water parameters data

All parameters demonstrated that the measurements varied considerably (Table 6.1). The means of the measurements were all outside of the legal and Mangaung water action plan guideline standards (SANS 241 of 2011; Potgieter *et al*., 2007). The median of the coliforms as well as the TBC measurements were higher than the legal standard of 10 dL^{-1} (SANS 241 of 2011) and 1×10^{2} ml^{-1} (Potgieter *et al*., 2007) respectively. The ranges of all parameters demonstrated a very large difference between the smallest and the largest values demonstrated by the standard deviation (SD).

Table 6.1 Summary statistical data of the water parameters studied for the 83 dairy farms.

Parameters	Statistical data of water parameters					
	Legal standard	Mean	Median	Range	SD	% Compliant
E. coli	0 dL^{-1}#	*	*	*	*	69
Coliform count	< 10 dL^{-1}#	374.15	37	1 - 2419	4.93	37
Total bacterial count (TBC)	< 100 ml^{-1}♣	733	110	0 – 27 000	3.095×10^{11}	48

SD=Standard deviation; * = No mean, median, range or standard deviation for *E. coli*, as only the presence/absence was tested for. # = Standard as per SANS 241 of 2011; ♣ = Standard as per Mangaung water action plan (Potgieter *et al*., 2007).

All water parameters demonstrated different levels of compliance and non-compliance with the legal standard (SANS 241 of 2011; Potgieter *et al*., 2007). For the two parameters coliforms and TBC compliance was less than 50%, while for the presence or absence of *E. coli* compliance was above 60%.

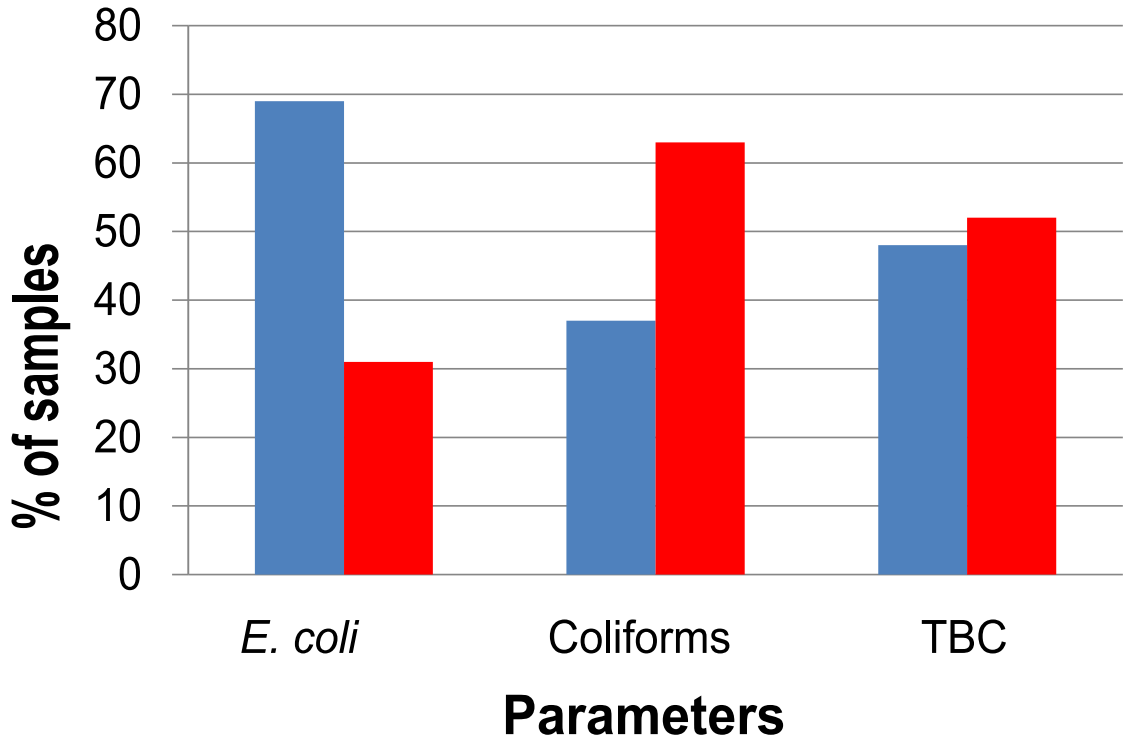


Figure 6.4 Percentages of compliance (blue bars) and non-compliance (red bars) for all water parameters studied (SANS 241 of 2011; Mangaung water action plan – Potgieter *et al*., 2007).

Chapter 7

Comparative analyses of milk, water and surface data

7.1 Introduction

A holistic view of the results was generated by calculating the percentage compliance and non-compliance for all the parameters investigated. Figure 7.1 indicates that the parameters in all the areas studied displayed different levels of compliance to applicable legislation. For the two parameters, presence of *E. coli* and coliforms in milk, compliance was low, with less than 20% of the farms being compliant, while for the two parameters TBC and SCC compliance was above 50%. The two parameters, coliforms and TBC in water, displayed compliance of less than 50%, while for *E. coli* compliance was greater than 60%. The two hygiene parameters pulsator and pipeline surfaces demonstrated similar results, whereas for *E. coli* and coliforms compliance was more than 70%, while for TBC compliance was less than 30%.

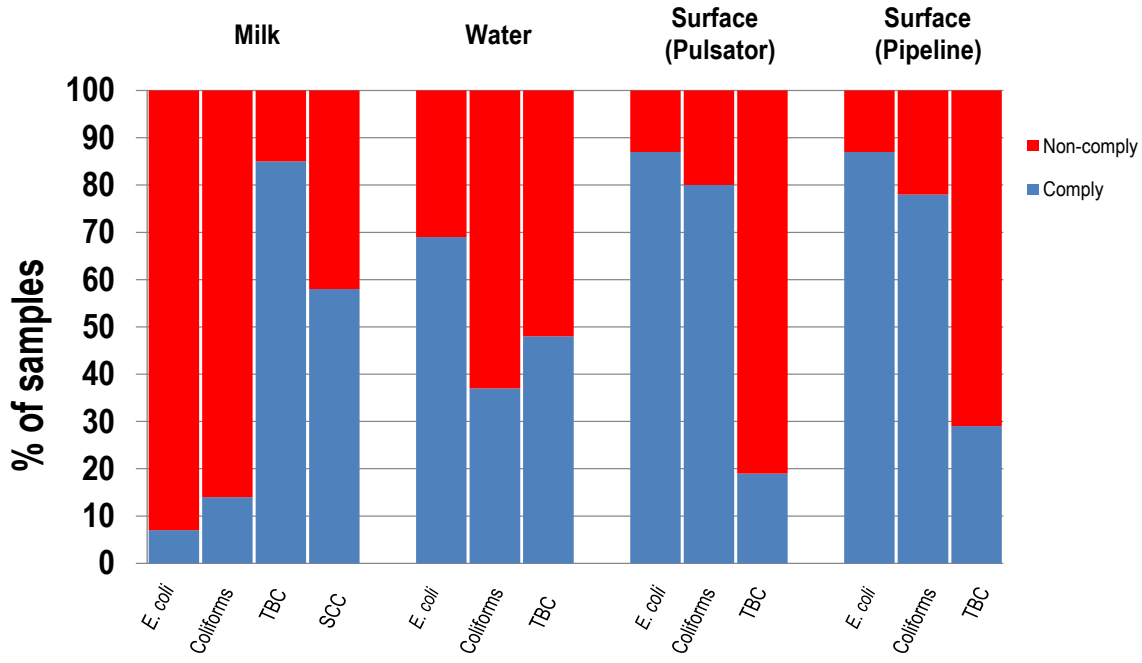


Figure 7.1 Percentage compliance and non-compliance for milk, water and surfaces.

7.2 Milking shed layout

Three layout types describing the positioning of the dairy cows in the milking shed were identified in this investigation. In the herringbone layout the cows were arranged around the milk pulsator points in the shape of a herringbone. In the stanchion, also referred to as a line arrangement, the cows were arranged parallel next to one another, while in the tandem arrangement the cows were positioned head-to-tail (Figure 7.2). Of the 83 dairy farms studied, 25 of the dairy farms used the herringbone layout, 51 the stanchion and seven the tandem type.

A. B. C.

Figure 7.2 Positioning of cows in a milking shed. A – Herringbone; B – Stanchion; C-Tandem.

7.3 Comparative analyses of data

A number of comparative analyses were performed to ascertain if significant differences existed between the variables on the presence of *E. coli* in milk using Pearson Chi-square tests. The tests that were performed compared the effects in terms of the presence of *E. coli* in milk versus water; milk versus pulsator surface; milk versus surface pipe, and the presence of *E. coli* and positioning of the cows in the milking shed.

7.3.1 Test 1: *E. coli* in milk vs. *E. coli in* water

The following hypotheses were tested:

H_0: The proportion of *E. coli* in milk is not affected by water.
H_a: The proportion of *E. coli* in milk is affected by water.

A 2 × 2 Chi-square contingency table was constructed to ascertain if significant differences existed for the presence/absence of *E. coli* in milk versus water (Table 7.1). The Chi-square test revealed that the observed and the expected numbers of the different categories differed significantly, therefore the H_0 was rejected at a level of significance level of $\alpha = 0.05$ ($\chi^2 = 63.5723$; df = 1; $p = 0.0000$).

Table 7.1 2 × 2 Chi-square contingency table for the presence or absence of *E. coli* in milk and water.

Variable	Category		Total
	***E. coli* + Observed (Expected)**	***E. coli* – Observed (Expected)**	
Milk	76 (83×102/166=51)	7 (83×64/166=32)	83
Water	26(83×102/166=51)	57 (83×64/166=32)	83
Total	102	64	166

7.3.2 Test 2: *E. coli* in milk vs. *E. coli in* pipeline surface

The following hypotheses were tested:

H_0: The proportion of *E. coli* in milk is not affected by pipeline surface.
H_a: The proportion of *E. coli* in milk is affected by pipeline surface.

A 2 × 2 Chi-square contingency table was constructed to ascertain if significant differences existed for the presence/absence of *E. coli* in milk versus pipeline surface (Table 7.2). The Chi-square test revealed that the observed and the expected numbers of the different categories differed significantly therefore, the H_0 was rejected at a significance level of $\alpha = 0.05$ ($\chi^2 = 102.0442$; df = 1; $p = 0.0000$).

Table 7.2 2 × 2 Chi-square contingency table for the presence or absence of *E. coli* in milk and pipeline surface.

Variable	Category		Total
	***E. coli* + Observed (Expected)**	***E. coli* – Observed (Expected)**	
Milk	76 (83×87/166=43.5)	7 (83×79/166=39.5)	83
Pipeline surface	11(83×87/166=43.5)	72 (83×79/166=39.5)	83
Total	87	79	166

7.3.3 Test 3: *E. coli* in milk vs. *E. coli* in pulsator surface

The following hypotheses were tested:

H_0: The proportion of *E. coli* in milk is not affected by pulsator surface.
H_a: The proportion of *E. coli* in milk is affected by pulsator surface.

A 2 × 2 Chi-square contingency table was constructed to ascertain if significant differences existed for the presence/absence of *E. coli* in milk versus pulsator surfaces (Table 7.2). The Chi-square test revealed that the observed and the expected numbers of the different categories differed significantly therefore, the H_0 was rejected at a significance level of $\alpha = 0.05$ ($\chi^2 = 102.0442$; df = 1; $p = 0.0000$).

Table 7.3 2 × 2 Chi-square contingency table for the presence or absence of *E. coli* in milk and pulsator surface.

Variable	Category		Total
	***E. coli* + Observed (Expected)**	***E. coli* – Observed (Expected)**	
Milk	76(83×87/166=43.5)	7 (83×79/166=39.5)	83
Pulsator surface	11(83×87/166=43.5)	72 (83×79/166=39.5)	83
Total	87	79	166

7.3.4 Test 4: *E. coli* in milk vs. dairy layout (positioning of the cows)

The following hypotheses were tested:

H_0: The proportion of *E. coli* in milk is not affected by the positioning of the cows.
H_a: The proportion of *E. coli* in milk is affected by the positioning of the cows.

A 2 × 3 Chi-square contingency table was constructed to ascertain if significant differences existed for the presence/absence of *E. coli* in milk versus dairy layout (Table 7.2). The Chi-square test revealed that the observed and the expected numbers of the different categories did not differ significantly, therefore the H_0 was not rejected at a significance level of $\alpha = 0.05$ ($\chi^2 = 0.7482$; df = 2; $p = 0.6878$) indicating that the expected numbers of *E. coli* in the milk was probably not influenced by the positioning of the cows in the dairy.

Table 7.4 2 × 3 Chi-square contingency table for the presence or absence of *E. coli* in milk and the three different dairy cow arrangements.

Variable	Category		Total
	***E. coli* +** **Observed (Expected)**	***E. coli* –** **Observed (Expected)**	
Herringbone	27 (28×94/101=26.06)	1 (28×7/101=1.94)	28
Stanchion (line)	53 (58×94/101=54)	5 (58×7/101=4.02)	58
Tandem	14 (15×94/101=14)	1 (15×7/101=1.04)	15
Total	94	7	101

7.4 Indexes

Milk, bacteriological water and hygiene quality indexes were computed to describe the overall milk and water quality as well as the status of hygiene practices on each of the 83 dairy farms studied. These indexes were developed by modifying the formulas used for the calculation of water quality devised by Ramakrishnaiah *et al.* (2009).

7.4.1 Milk quality index (MQI)

MQIs were calculated to ascertain what the overall quality of the milk on the respective farms was. The MQI values were used to classify the quality of the milk into two categories, namely, poor or excellent. Farms with a MQI less than 100 were classified as excellent and farms with a MQI more than 100 were classified as poor. Farms with a MQI of less than 100, but that demonstrated *E. coli* in the milk were also classified as poor. The data revealed that only one farm could be classified as being excellent with a MQI value of less than 100 and with no *E. coli* present in the milk (Table 7.5).

Table 7.5 Milk quality index for the 83 dairy farms studied.

Farm	Wi	TBC CFU ml^{-1}	CF CFU ml^{-1}	SCC ml^{-1}	TBC Std CFU ml^{-1}	qTBC	CF Std CFU ml^{-1}	qCF	SCC Std ml^{-1}	qSCC	S(TBC)	S(CF)	S(SCC)	MQI	*E. coli*	Milk quality
61	0.5	10.6×10^{6}	1.7×10^{5}	632300	2×10^{5}	5300	20	8.5×10^{5}	5×10^{5}	126.46	2650	425000	63.23	427713.2	pos	Poor
70	0.5	11.2×10^{5}	3.5×10^{4}	1347100	2×10^{5}	560	20	17.5×10^{4}	5×10^{5}	269.42	280	87500	134.71	87914.71	pos	Poor
39	0.5	208500	15075	662600	2×10^{5}	104.25	20	75375	5×10^{5}	132.52	52.12	37687.5	66.26	37805.89	pos	Poor
24	0.5	7.5×10^{4}	11500	4056300	2×10^{5}	37.5	20	57500	5×10^{5}	811.26	18.75	28750	405.63	29174.38	pos	Poor
58	0.5	8.4×10^{4}	8600	1498000	2×10^{5}	42	20	4.3×10^{4}	5×10^{5}	299.60	21	21500	149.80	21670.8	pos	Poor
11	0.5	12.7×10^{4}	6600	274900	2×10^{5}	63.5	20	3.3×10^{4}	5×10^{5}	54.98	31.75	16500	27.49	16559.24	pos	Poor
32	0.5	3.2×10^{5}	6500	763500	2×10^{5}	160	20	32500	5×10^{5}	152.70	80	16250	76.35	16406.35	pos	Poor
17	0.5	46500	5850	284300	2×10^{5}	23.25	20	29250	5×10^{5}	56.86	11.62	14625	28.43	14665.06	pos	Poor
54	0.5	1.17×10^{6}	4000	2659700	2×10^{5}	585	20	2×10^{4}	5×10^{5}	531.94	292.5	10000	265.97	10558.47	pos	Poor
74	0.5	6.6×10^{4}	3900	492300	2×10^{5}	33	20	19500	5×10^{5}	98.46	16.5	9750	49.23	9815.73	pos	Poor
59	0.5	10.3×10^{4}	3700	376800	2×10^{5}	51.5	20	18500	5×10^{5}	75.36	25.75	9250	37.68	9313.43	pos	Poor
42	0.5	7.7×10^{5}	3500	910800	2×10^{5}	385	20	17500	5×10^{5}	182.16	192.5	8750	91.08	9033.58	pos	Poor
38	0.5	36500	3150	540550	2×10^{5}	18.25	20	15750	5×10^{5}	108.11	9.12	7875	54.05	7938.18	pos	Poor
21	0.5	10.3×10^{5}	2600	509200	2×10^{5}	515	20	1.3×10^{4}	5×10^{5}	101.84	257.5	6500	50.92	6808.42	pos	Poor
25	0.5	1.1×10^{5}	1900	928700	2×10^{5}	55	20	9500	5×10^{5}	185.74	27.5	4750	92.87	4870.37	pos	Poor
51	0.5	4.2×10^{4}	1900	88300	2×10^{5}	21	20	9500	5×10^{5}	17.66	10.5	4750	8.83	4769.33	pos	Poor
28	0.5	2×10^{5}	1600	3633100	2×10^{5}	100	20	8000	5×10^{5}	726.62	50	4000	363.31	4413.31	pos	Poor
66	0.5	14.3×10^{4}	1500	571800	2×10^{5}	71.5	20	7500	5×10^{5}	114.36	35.75	3750	57.18	3842.93	pos	Poor
37	0.5	15.3×10^{4}	1295	945000	2×10^{5}	76.5	20	6475	5×10^{5}	189	38.25	3237.5	94.5	3370.25	pos	Poor

31	0.5	3×10^5	1100	1530500	2×10^5	150	20	5500	5×10^5	306.10	75	2750	153.05	2978.05	pos	Poor
67	0.5	6200	1030	565800	2×10^5	3.1	20	5150	5×10^5	113.16	1.55	2575	56.58	2633.13	neg	Poor
57	0.5	9900	740	41300	2×10^5	4.95	20	3700	5×10^5	8.26	2.47	1850	4.13	1856.60	neg	Poor
72	0.5	5.2×10^4	720	339800	2×10^5	26	20	3600	5×10^5	67.96	13	1800	33.98	1846.98	pos	Poor
33	0.5	11900	680	824700	2×10^5	5.95	20	3400	5×10^5	164.94	2.97	1700	82.47	1785.44	pos	Poor
9	0.5	5.4×10^4	680	30300	2×10^5	27	20	3400	5×10^5	6.06	13.5	1700	3.03	1716.53	pos	Poor
53	0.5	5.9×10^5	540	298700	2×10^5	295	20	2700	5×10^5	59.74	147.5	1350	29.87	1527.37	neg	Poor
3	0.5	10950	545	464550	2×10^5	5.475	20	2725	5×10^5	92.91	2.73	1362.5	46.45	1411.69	pos	Poor
34	0.5	13300	530	702400	2×10^5	6.65	20	2650	5×10^5	140.48	3.32	1325	70.24	1398.56	pos	Poor
13	0.5	23300	500	517000	2×10^5	11.65	20	2500	5×10^5	103.4	5.82	1250	51.7	1307.52	pos	Poor
41	0.5	23000	490	259700	2×10^5	11.5	20	2450	5×10^5	51.94	5.75	1225	25.97	1256.72	pos	Poor
75	0.5	5.1×10^4	490	153600	2×10^5	25.5	20	2450	5×10^5	30.72	12.75	1225	15.36	1253.11	pos	Poor
62	0.5	4.4×10^5	390	721200	2×10^5	220	20	1950	5×10^5	144.24	110	975	72.12	1157.12	pos	Poor
45	0.5	5300	400	429000	2×10^5	2.65	20	2000	5×10^5	85.8	1.32	1000	42.9	1044.22	pos	Poor
55	0.5	15400	370	478100	2×10^5	7.7	20	1850	5×10^5	95.62	3.85	925	47.81	976.66	pos	Poor
1	0.5	13750	352	127950	2×10^5	6.875	20	1760	5×10^5	25.59	3.43	880	12.79	896.23	pos	Poor
77	0.5	7.2×10^4	350	27200	2×10^5	36	20	1750	5×10^5	5.44	18	875	2.72	895.72	pos	Poor
20	0.5	16900	340	144400	2×10^5	8.45	20	1700	5×10^5	28.88	4.22	850	14.44	868.66	neg	Poor
19	0.5	12050	295	930500	2×10^5	6.025	20	1475	5×10^5	186.1	3.01	737.5	93.05	833.56	pos	Poor
44	0.5	10.4×10^5	220	215900	2×10^5	520	20	1100	5×10^5	43.18	260	550	21.59	831.59	pos	Poor
64	0.5	6900	300	794200	2×10^5	3.45	20	1500	5×10^5	158.84	1.72	750	79.42	831.145	pos	Poor
10	0.5	7.1×10^4	280	770100	2×10^5	35.5	20	1400	5×10^5	154.02	17.75	700	77.01	794.76	pos	Poor
14	0.5	8000	250	714100	2×10^5	4	20	1250	5×10^5	142.82	2	625	71.41	698.41	pos	Poor
8	0.5	15300	220	493000	2×10^5	7.65	20	1100	5×10^5	98.6	3.825	550	49.3	603.12	pos	Poor
4	0.5	9400	207	469633.3	2×10^5	4.7	20	1035	5×10^5	93.92	2.35	517.5	46.96	566.81	pos	Poor
15	0.5	4.2×10^4	180	991100	2×10^5	21	20	900	5×10^5	198.22	10.5	450	99.11	559.61	pos	Poor
80	0.5	4.1×10^4	200	160000	2×10^5	20.5	20	1000	5×10^5	32	10.25	500	16	526.25	pos	Poor
12	0.5	4.4×10^4	170	562600	2×10^5	22	20	850	5×10^5	112.52	11	425	56.26	492.26	pos	Poor
23	0.5	1.9×10^4	160	517800	2×10^5	9.5	20	800	5×10^5	103.56	4.75	400	51.78	456.53	pos	Poor
68	0.5	4.9×10^5	49	1390900	2×10^5	245	20	245	5×10^5	278.18	122.5	122.5	139.09	384.09	neg	Poor
16	0.5	2.1×10^4	4	3423900	2×10^5	10.5	20	20	5×10^5	684.78	5.25	10	342.39	357.64	neg	Poor
18	0.5	2080	125	431700	2×10^5	1.04	20	625	5×10^5	86.34	0.52	312.5	43.17	356.19	pos	Poor
63	0.5	10600	110	719700	2×10^5	5.3	20	550	5×10^5	143.94	2.65	275	71.97	349.62	pos	Poor
36	0.5	3900	123	327800	2×10^5	1.95	20	615	5×10^5	65.56	0.97	307.5	32.78	341.25	pos	Poor
43	0.5	40550	100	657850	2×10^5	20.275	20	500	5×10^5	131.57	10.13	250	65.78	325.92	pos	Poor
71	0.5	4500	105	375100	2×10^5	2.25	20	525	5×10^5	75.02	1.12	262.5	37.51	301.13	pos	Poor
60	0.5	17600	91	481900	2×10^5	8.8	20	455	5×10^5	96.38	4.4	227.5	48.19	280.09	pos	Poor
7	0.5	12502	80	494750	2×10^5	6.251	20	400	5×10^5	98.95	3.12	200	49.47	252.60	pos	Poor
30	0.5	12700	78	454700	2×10^5	6.35	20	390	5×10^5	90.94	3.17	195	45.47	243.64	pos	Poor
78	0.5	3×10^4	10	1917600	2×10^5	15	20	50	5×10^5	383.52	7.5	25	191.76	224.26	pos	Poor
83	0.5	9200	45	1050500	2×10^5	4.6	20	225	5×10^5	210.1	2.3	112.5	105.05	219.85	pos	Poor
79	0.5	58000	76	112200	2×10^5	29	20	380	5×10^5	22.44	14.5	190	11.22	215.72	pos	Poor
56	0.5	1510	75	222400	2×10^5	0.755	20	375	5×10^5	44.48	0.37	187.5	22.24	210.11	pos	Poor
46	0.5	8000	40	1005300	2×10^5	4	20	200	5×10^5	201.06	2	100	100.53	202.53	pos	Poor
73	0.5	9300	70	110600	2×10^5	4.65	20	350	5×10^5	22.12	2.32	175	11.06	188.38	pos	Poor
6	0.5	7100	66	113200	2×10^5	3.55	20	330	5×10^5	22.64	1.77	165	11.32	178.09	pos	Poor

81	0.5	610000	5	97000	2×10^5	305	20	25	5×10^5	19.4	152.5	12.5	9.7	174.7	pos	Poor
27	0.5	5200	42	564100	2×10^5	2.6	20	210	5×10^5	112.82	1.3	105	56.41	162.71	pos	Poor
48	0.5	1200	45	475100	2×10^5	0.6	20	225	5×10^5	95.02	0.3	112.5	47.51	160.31	pos	Poor
2	0.5	25100	55	113750	2×10^5	12.55	20	275	5×10^5	22.75	6.27	137.5	11.37	155.15	pos	Poor
50	0.5	6800	60	20600	2×10^5	3.4	20	300	5×10^5	4.12	1.7	150	2.06	153.76	pos	Poor
65	0.5	3900	45	329700	2×10^5	1.95	20	225	5×10^5	65.94	0.97	112.5	32.97	146.44	pos	Poor
49	0.5	120000	10	745300	2×10^5	60	20	50	5×10^5	149.06	30	25	74.53	129.53	pos	Poor
82	0.5	3200	28	442800	2×10^5	1.6	20	140	5×10^5	88.56	0.8	70	44.28	115.08	pos	Poor
35	0.5	3300	25	496800	2×10^5	1.65	20	125	5×10^5	99.36	0.82	62.5	49.68	113	pos	Poor
40	0.5	7700	23	475700	2×10^5	3.85	20	115	5×10^5	95.14	1.92	57.5	47.57	106.99	pos	Poor
52	0.5	6400	17	501100	2×10^5	3.2	20	85	5×10^5	100.22	1.6	42.5	50.11	94.21	pos	Poor
69	0.5	9000	14	464700	2×10^5	4.5	20	70	5×10^5	92.94	2.25	35	46.47	83.72	pos	Poor
47	0.5	1280	16	270300	2×10^5	0.64	20	80	5×10^5	54.06	0.32	40	27.03	67.35	pos	Poor
5	0.5	2487.5	14	261625	2×10^5	1.24375	20	70	5×10^5	52.32	0.62	35	26.16	61.78	pos	Poor
22	0.5	1460	16	161100	2×10^5	0.73	20	80	5×10^5	32.22	0.36	40	16.11	56.47	pos	Poor
29	0.5	3700	9	277900	2×10^5	1.85	20	45	5×10^5	55.58	0.92	22.5	27.79	51.21	pos	Poor
26	0.5	1050	5	226600	2×10^5	0.525	20	25	5×10^5	45.32	0.26	12.5	22.66	35.42	neg	Excellent
76	0.5	1530	1	150600	2×10^5	0.765	20	5	5×10^5	30.12	0.38	2.5	15.06	17.94	pos	Poor

Wi=Relative weight; TBC CFU=Total bacterial count Colony forming units; CF CFU=Coliforms Colony forming units; SCC=Somatic cell count; TBC Std=Total bacterial count legal standard; qTBC=Quality rating for Total bacterial count; CF Std=Coliform legal standard; qCF=Quality rating for Coliforms; SCC Std=Somatic cell count legal standard; qSCC=Quality rating for Somatic cell count; S (TBC)=Sub index for Total bacterial count; S (CF)=Sub index for Coliforms; S (SCC)=Sub index for Somatic cell count; MQI=Milk quality index.

7.4.2 Bacteriological water quality index (BWQI)

BWQIs were calculated to ascertain what the overall quality of the water used in the dairies were on the respective farms. An equal weighting of 0.5 was awarded to each of the two parameters, namely, TBC and coliforms. The legal standard for each of the parameters were included in the calculation of the BWQI. The BWQI values were used to classify the quality of the water into two categories, namely, poor or excellent. Farms with a BWQI less than 100 were classified as excellent and farms with a BWQI more than 100 were classified as poor. Farms with a BWQI of less than 100, but that also demonstrated *E. coli* in the water were classified as poor. The BWQI data revealed that 43% of the dairy farms used poor quality water for washing of dairy equipment (Table 7.6).

Table 7.6 Bacteriological water quality index for the 83 dairy farms studied.

Farm	Wi	Coliform Water dL^{-1}	TBC Water 1×10^2 ml^{-1}	CF Std Water < 10 dL^{-1}	qColiform Water	TBC Std Water $<1\times10^2$ ml^{-1}	qTBC Water	S(CF) Water	S(TBC) Water	BWQI	*E. coli* Water	Water Quality
78	0.5	24	27 000	10	241.9	100	27000	48.38	8100	8148.38	Pos	Poor
64	0.5	24	9 500	10	241.9	100	9500	48.38	2850	2898.38	Pos	Poor
21	0.5	24	2 160	10	241.9	100	2160	48.38	648	696.38	Pos	Poor
83	0.5	24	1 740	10	241.9	100	1740	48.38	522	570.38	Pos	Poor
5	0.5	24	1 430	10	241.9	100	1430	48.38	429	477.38	Neg	Poor
54	0.5	1	1 530	10	8.6	100	1530	1.72	459	460.72	Neg	Poor
65	0.5	0	1 450	10	0.1	100	1450	0.02	435	435.02	Neg	Poor
10	0.5	0	1 270	10	0.6	100	1270	0.12	381	381.12	Neg	Poor
42	0.5	0	1 260	10	2.1	100	1260	0.42	378	378.42	Neg	Poor
60	0.5	1	1230	10	11.8	100	1230	2.36	369	371.36	Neg	Poor
19	0.5	2	1 130	10	17.9	100	1130	3.58	339	342.58	Pos	Poor
58	0.5	1	1080	10	11.2	100	1080	2.24	324	326.24	Neg	Poor
72	0.5	2	1050	10	19.4	100	1050	3.88	315	318.88	Pos	Poor
20	0.5	24	520	10	241.9	100	520	48.38	156	204.38	Pos	Poor
66	0.5	1	660	10	9.3	100	660	1.86	198	199.86	Pos	Poor
7	0.5	1	650	10	12.6	100	650	2.52	195	197.52	Pos	Poor
4	0.5	24	470	10	241.9	100	470	48.38	141	189.38	Neg	Poor
41	0.5	0	560	10	0.7	100	560	0.14	168	168.14	Neg	Poor
71	0.5	24	270	10	241.9	100	270	48.38	81	129.38	Pos	Poor
38	0.5	5	330	10	47.9	100	330	9.58	99	108.58	Pos	Poor
46	0.5	24	182	10	241.9	100	182	48.38	54.6	102.98	Neg	Poor
1	0.5	1	330	10	7.8	100	330	1.56	99	100.56	Pos	Poor
13	0.5	8	250	10	77	100	250	15.4	75	90.4	Neg	Excellent
33	0.5	2	280	10	23.1	100	280	4.62	84	88.62	Pos	Poor
39	0.5	7	240	10	68.7	100	240	13.74	72	85.74	Pos	Poor
35	0.5	1	260	10	11.3	100	260	2.26	78	80.26	Neg	Excellent
36	0.5	1	260	10	10.4	100	260	2.08	78	80.08	Pos	Poor
18	0.5	2	250	10	21	100	250	4.2	75	79.2	Pos	Poor
79	0.5	0	230	10	0	100	230	0	69	69	Neg	Excellent
70	0.5	0	220	10	0.5	100	220	0.1	66	66.1	Neg	Excellent
62	0.5	0	210	10	0	100	210	0	63	63	Neg	Excellent
53	0.5	10	135	10	101.1	100	135	20.22	40.5	60.72	Pos	Poor
37	0.5	11	116	10	111.2	100	116	22.24	34.8	57.04	Neg	Excellent
57	0.5	13	90	10	133.4	100	90	26.68	27	53.68	Neg	Excellent
3	0.5	7	129	10	72.2	100	129	14.44	38.7	53.14	Pos	Poor
73	0.5	1	170	10	9.9	100	170	1.98	51	52.98	Pos	Poor
23	0.5	0	162	10	4.9	100	162	0.98	48.6	49.58	Pos	Poor
61	0.5	1	148	10	13.7	100	148	2.74	44.4	47.14	Neg	Excellent
74	0.5	0	144	10	0.3	100	144	0.06	43.2	43.26	Neg	Excellent
47	0.5	0	136	10	0	100	136	0	40.8	40.8	Neg	Excellent
30	0.5	0	126	10	1	100	126	0.2	37.8	38	Neg	Excellent
25	0.5	0	119	10	0.9	100	119	0.18	35.7	35.88	Neg	Excellent
51	0.5	0	110	10	4.2	100	110	0.84	33	33.84	Neg	Excellent
14	0.5	3	92	10	27.6	100	92	5.52	27.6	33.12	Neg	Excellent
50	0.5	0	102	10	0	100	102	0	30.6	30.6	Neg	Excellent
75	0.5	1	84	10	9.9	100	84	1.98	25.2	27.18	Neg	Excellent
15	0.5	0	89	10	2.1	100	89	0.42	26.7	27.12	Pos	Poor

44	0.5	1	85	10	5.2	100	85	1.04	25.5	26.54	Pos	Poor
76	0.5	0	79	10	1.3	100	79	0.26	23.7	23.96	Pos	Poor
40	0.5	0	66	10	0	100	66	0	19.8	19.8	Neg	Excellent
67	0.5	0	65	10	1.2	100	65	0.24	19.5	19.74	Neg	Excellent
29	0.5	0	61	10	3.7	100	61	0.74	18.3	19.04	Neg	Excellent
26	0.5	1	51	10	7.9	100	51	1.58	15.3	16.88	Neg	Excellent
48	0.5	0	54	10	0.4	100	54	0.08	16.2	16.28	Neg	Excellent
59	0.5	0	49	10	0	100	49	0	14.7	14.7	Neg	Excellent
82	0.5	1	34	10	5	100	34	1	10.2	11.2	Pos	Poor
31	0.5	0	34	10	4.7	100	34	0.94	10.2	11.14	Neg	Excellent
52	0.5	0	34	10	3.2	100	34	0.64	10.2	10.84	Neg	Excellent
9	0.5	0	35	10	1.3	100	35	0.26	10.5	10.76	Neg	Excellent
63	0.5	0	34	10	0.1	100	34	0.02	10.2	10.22	Neg	Excellent
12	0.5	0	34	10	0	100	34	0	10.2	10.2	Neg	Excellent
27	0.5	2	17	10	23.7	100	17	4.74	5.1	9.84	Neg	Excellent
34	0.5	2	12	10	23.1	100	12	4.62	3.6	8.22	Neg	Excellent
69	0.5	0	26	10	0.6	100	26	0.12	7.8	7.92	Neg	Excellent
45	0.5	0	21	10	1	100	21	0.2	6.3	6.5	Neg	Excellent
22	0.5	1	11	10	11.2	100	11	2.24	3.3	5.54	Neg	Excellent
56	0.5	0	15	10	0.3	100	15	0.06	4.5	4.56	Neg	Excellent
24	0.5	0	12	10	2.6	100	12	0.52	3.6	4.12	Pos	Poor
16	0.5	0	13	10	1.1	100	13	0.22	3.9	4.12	Neg	Excellent
17	0.5	0	13	10	0.1	100	13	0.02	3.9	3.92	Neg	Excellent
11	0.5	0	12	10	0.2	100	12	0.04	3.6	3.64	Neg	Excellent
55	0.5	0	12	10	0	100	12	0	3.6	3.6	Neg	Excellent
28	0.5	0	5	10	1.2	100	5	0.24	1.5	1.74	Neg	Excellent
43	0.5	0	5	10	0.6	100	5	0.12	1.5	1.62	Pos	Poor
68	0.5	0	5	10	0	100	5	0	1.5	1.5	Neg	Excellent
32	0.5	0	3	10	0.7	100	3	0.14	0.9	1.04	Neg	Excellent
6	0.5	0	1	10	0.1	100	1	0.02	0.3	0.32	Neg	Excellent
8	0.5	0	0	10	0.2	100	0	0.04	0	0.04	Neg	Excellent
2	0.5	0	0	10	0.1	100	0	0.02	0	0.02	Neg	Excellent
81	0.5	0	0	10	0	100	0	0	0	0	Neg	Excellent
49	0.5	0	0	10	0	100	0	0	0	0	Neg	Excellent
80	0.5	0	0	10	0	100	0	0	0	0	Neg	Excellent
77	0.5	0	0	10	0	100	0	0	0	0	Neg	Excellent

Wi=Relative weight; TBC=Total bacterial count; TBC Std=Total bacterial count legal standard; qTBC=Quality rating for Total bacterial count; CF Std=Coliform legal standard; qCF=Quality rating for Coliforms; S (CF)=Sub index for Coliforms; S (TBC)=Sub index for Total bacterial count; BWQI=Bacteriological water quality index.

7.4.3 Hygiene quality index (HQI)

HQIs were calculated to ascertain what the overall quality of the hygiene practices were on the respective farms. An equal weighting of 0.2 was awarded to each of the six parameters, namely, TBC and coliforms in water and on pulsator and pipeline surfaces. The legal standard for each of the parameters were included in the calculation of the HQI. The HQI values were used to classify the

quality of hygiene practices into two categories, namely, poor or excellent. Farms with a HQI less than 100 were classified as excellent and farms with a HQI more than 100 were classified as poor. Farms with a HQI of less than 100, but that also demonstrated *E. coli* in the water and or milk contact surfaces were classified as poor. The data revealed that the hygiene practices on 80 of the 83 dairy farms were not up to standard for the production of milk for human consumption (Table 7.7).

Table 7.7 Hygiene quality index for the 83 dairy farms studied.

Farm	Wi	CF Puls CFU cm^{-2}	CF Pipe CFU cm^{-2}	CF Water < 10 dL^{-1}	TBC Puls CFU cm^{-2}	TBC Pipe CFU cm^{-2}	TBC Water 1×10^{2} ml^{-1}	CF Std Surf < 20 CFU cm^{-2}	CF Std Water < 10 dL^{-1}	qCF Puls	qCF Pipe	qCF Water	TBC Std Surf < 10 CFU cm^{-2}	TBC Std Water $<1\times10^{2}$ ml^{-1}	qTBC Puls	qTBC Pipe	qTBC Water	S (CF) Puls	S (CF) Pipe	S (CF) Water	S (TBC) Puls	S (TBC) Pipe	S (TBC) Water	HQI	*E. coli* Water	*E. coli* Puls	*E. coli* Pipe
32	0.2	300	120	0	5.6×10^{6}	19.5×10^{5}	3	20	10	1500	600	0.7	100	100	5.6×10^{6}	19.5×10^{5}	3	300	120	0.14	8.4×10^{5}	292500	0.9	1132921.04	Neg	Pos	Pos
53	0.2	5300	1500	10	5.27×10^{6}	11.6×10^{5}	135	20	10	26500	7500	101.1	100	100	52.7×10^{5}	11.6×10^{5}	135	5300	1500	20.22	790500	17.4×10^{4}	40.5	971360.72	Pos	Neg	Neg
81	0.2	180	9	0	3.8×10^{6}	2.5×10^{6}	0	20	10	900	45	0	100	100	3.8×10^{6}	2.5×10^{6}	0	180	9	0	5.7×10^{5}	37.5×10^{4}	0	945189	Neg	Neg	Neg
54	0.2	970	9	1	6.13×10^{6}	13500	1530	20	10	4850	45	8.6	100	100	61.3×10^{5}	13500	1530	970	9	1.72	919500	2025	459	922964.72	Neg	Pos	Neg
9	0.2	640	14.9×10^{4}	0	6.9×10^{4}	2.5×10^{6}	35	20	10	3200	74.5×10^{4}	1.3	100	100	6.9×10^{4}	2.5×10^{6}	35	640	14.9×10^{4}	0.26	10350	37.5×10^{4}	10.5	535000.76	Neg	Neg	Neg
25	0.2	9	11.3×10^{4}	0	9	2.1×10^{6}	119	20	10	45	56.5×10^{4}	0.9	100	100	9	2.1×10^{6}	119	9	11.3×10^{4}	0.18	1.35	31.5×10^{4}	35.7	428046.23	Neg	Neg	Neg
4	0.2	9	4.1×10^{4}	24	800	2.5×10^{6}	470	20	10	45	20.5×10^{4}	241.9	100	100	800	2.5×10^{6}	470	9	4.1×10^{4}	48.38	120	37.5×10^{4}	141	416318.38	Neg	Neg	Neg
72	0.2	3.3×10^{4}	9	2	2.5×10^{6}	70	1050	20	10	16.5×10^{4}	45	19.4	100	100	2.5×10^{6}	70	1050	3.3×10^{4}	9	3.88	37.5×10^{4}	10.5	315	408338.38	Pos	Neg	Neg
67	0.2	2.8×10^{4}	9	0	25×10^{6}	10	65	20	10	14×10^{4}	45	1.2	100	100	2.5×10^{6}	10	65	2.8×10^{4}	9	0.24	37.5×10^{4}	1.5	19.5	403030.24	Neg	Pos	Neg
61	0.2	9	980	1	14.1×10^{4}	2.5×10^{6}	148	20	10	45	4900	13.7	100	100	14.1×10^{4}	2.5×10^{6}	148	9	980	2.74	21150	37.5×10^{4}	44.4	397186.14	Neg	Neg	Neg
44	0.2	9	1.2×10^{4}	1	3.7×10^{4}	2.5×10^{6}	85	20	10	45	6×10^{4}	5.2	100	100	3.7×10^{4}	2.5×10^{6}	85	9	1.2×10^{4}	1.04	5550	37.5×10^{4}	25.5	392585.54	Pos	Neg	Neg
79	0.2	9	5500	0	5.6×10^{4}	2.5×10^{6}	230	20	10	45	27500	0	100	100	5.6×10^{4}	2.5×10^{6}	230	9	5500	0	8400	37.5×10^{4}	69	388978	Neg	Neg	Neg
49	0.2	9	9	0	2.5×10^{6}	1.5×10^{4}	0	20	10	45	45	0	100	100	2.5×10^{6}	1.5×10^{4}	0	9	9	0	37.5×10^{4}	2250	0	377268	Neg	Neg	Neg
63	0.2	60	9	0	570	2.5×10^{6}	34	20	10	300	45	0.1	100	100	570	2.5×10^{6}	34	60	9	0.02	85.5	37.5×10^{4}	10.2	375164.72	Neg	Neg	Neg
45	0.2	9	9	0	2.5×10^{6}	9	21	20	10	45	45	1	100	100	2.5×10^{6}	9	21	9	9	0.2	37.5×10^{4}	1.35	6.3	375025.85	Neg	Neg	Neg
42	0.2	9	20	0	270	1.14×10^{6}	1260	20	10	45	100	2.1	100	100	270	11.4×10^{5}	1260	9	20	0.42	40.5	17.1×10^{4}	378	171447.92	Neg	Pos	Neg
76	0.2	9	9	0	1.02×10^{6}	9	79	20	10	45	45	1.3	100	100	10.2×10^{5}	9	79	9	9	0.26	15.3×10^{4}	1.35	23.7	153043.31	Pos	Neg	Neg
47	0.2	9	410	0	1510	9.9×10^{5}	136	20	10	45	2050	0	100	100	1510	9.9×10^{5}	136	9	410	0	226.5	148500	40.8	149186.3	Neg	Neg	Neg
70	0.2	380	3×10^{4}	0	4×10^{4}	6.4×10^{5}	220	20	10	1900	1.5×10^{5}	0.5	100	100	4×10^{4}	6.4×10^{5}	220	380	3×10^{4}	0.1	6000	9.6×10^{4}	66	132446.1	Neg	Neg	Neg
52	0.2	9	9	0	8.6×10^{5}	410	34	20	10	45	45	3.2	100	100	8.6×10^{5}	410	34	9	9	0.64	12.9×10^{4}	61.5	10.2	129090.34	Neg	Neg	Pos
31	0.2	9	200	0	30	5.5×10^{5}	34	20	10	45	1000	4.7	100	100	30	5.5×10^{5}	34	9	200	0.94	4.5	82500	10.2	82724.64	Neg	Neg	Neg
73	0.2	9	9	1	4.3×10^{5}	20	170	20	10	45	45	9.9	100	100	4.3×10^{5}	20	170	9	9	1.98	64500	3	51	64573.98	Pos	Neg	Neg
23	0.2	9	3.2×10^{4}	0	9	20.3×10^{4}	162	20	10	45	1.6×10^{5}	4.9	100	100	9	20.3×10^{4}	162	9	3.2×10^{4}	0.98	1.35	30450	48.6	62509.93	Pos	Neg	Neg

58	0.2	40	100	1	12.8×10^{4}	2.3×10^{5}	1080	20	10	200	500	11.2	100	100	12.8×10^{4}	2.3×10^{5}	1080	40	100	2.24	19200	34500	324	54166.24	Neg	Neg	Neg
48	0.2	9	9	0	870	3×10^{5}	54	20	10	45	45	0.4	100	100	870	3×10^{5}	54	9	9	0.08	130.5	4.5×10^{4}	16.2	45164.78	Neg	Neg	Neg
66	0.2	9	9	1	30	24×10^{4}	660	20	10	45	45	9.3	100	100	30	2.4×10^{5}	660	9	9	1.86	4.5	3.6×10^{4}	198	36222.36	Pos	Neg	Neg
74	0.2	30	9	0	1.9×10^{5}	3.6×10^{4}	144	20	10	150	45	0.3	100	100	1.9×10^{5}	3.6×10^{4}	144	30	9	0.06	28500	5400	43.2	33982.26	Neg	Neg	Neg
46	0.2	70	9	24	15.4×10^{4}	3000	182	20	10	350	45	241.9	100	100	15.4×10^{4}	3000	182	70	9	48.38	23100	450	54.6	23731.98	Neg	Pos	Pos
62	0.2	9	9	0	1.58×10^{4}	13.5×10^{4}	210	20	10	45	45	0	100	100	15800	13.5×10^{4}	210	9	9	0	2370	20250	63	22701	Neg	Neg	Neg
13	0.2	9	9	8	9	13.2×10^{4}	250	20	10	45	45	77	100	100	9	13.2×10^{4}	250	9	9	15.4	1.35	19800	75	19909.75	Neg	Neg	Neg
83	0.2	9	9	24	5.7×10^{4}	6.6×10^{4}	1740	20	10	45	45	241.9	100	100	5.7×10^{4}	6.6×10^{4}	1740	9	9	48.38	8550	9900	522	19038.38	Pos	Neg	Neg
5	0.2	100	360	24	10.4×10^{4}	5900	1430	20	10	500	1800	241.9	100	100	10.4×10^{4}	5900	1430	100	360	48.38	15600	885	429	17422.38	Neg	Neg	Neg
35	0.2	9	2600	1	2×10^{4}	6.2×10^{4}	260	20	10	45	1.3×10^{4}	11.3	100	100	2×10^{4}	6.2×10^{4}	260	9	2600	2.26	3000	9300	78	14989.26	Neg	Neg	Neg
80	0.2	9	9	0	7.2×10^{4}	8700	0	20	10	45	45	0	100	100	7.2×10^{4}	8700	0	9	9	0	10800	1305	0	12123	Neg	Neg	Neg
78	0.2	9	9	24	1.48×10^{4}	30	2.7×10^{4}	20	10	45	45	241.9	100	100	14800	30	27000	9	9	48.38	2220	4.5	8100	10390.88	Pos	Neg	Pos
69	0.2	9	9	0	6.6×10^{4}	260	26	20	10	45	45	0.6	100	100	6.6×10^{4}	260	26	9	9	0.12	9900	39	7.8	9964.92	Neg	Neg	Pos
51	0.2	60	40	0	6.2×10^{4}	510	110	20	10	300	200	4.2	100	100	6.2×10^{4}	510	110	60	40	0.84	9300	76.5	33	9510.34	Neg	Pos	Neg
75	0.2	640	9	1	4.5×10^{4}	1220	84	20	10	3200	45	9.9	100	100	4.5×10^{4}	1220	84	640	9	1.98	6750	183	25.2	7609.18	Neg	Pos	Neg
55	0.2	50	9	0	4.1×10^{4}	9	12	20	10	250	45	0	100	100	4.1×10^{4}	9	12	50	9	0	6150	1.35	3.6	6213.95	Neg	Neg	Neg
64	0.2	9	9	24	10	10	9500	20	10	45	45	241.9	100	100	10	10	9500	9	9	48.38	1.5	1.5	2850	2919.38	Pos	Neg	Neg
33	0.2	9	9	2	300	13800	280	20	10	45	45	23.1	100	100	300	13800	280	9	9	4.62	45	2070	84	2221.62	Pos	Neg	Neg
1	0.2	9	9	1	280	11500	330	20	10	45	45	7.8	100	100	280	11500	330	9	9	1.56	42	1725	99	1885.56	Pos	Neg	Neg
65	0.2	9	9	0	410	7900	1450	20	10	45	45	0.1	100	100	410	7900	1450	9	9	0.02	61.5	1185	435	1699.52	Neg	Neg	Neg
10	0.2	20	9	0	5400	100	1270	20	10	100	45	0.6	100	100	5400	100	1270	20	9	0.12	810	15	381	1235.12	Neg	Pos	Neg
40	0.2	9	9	0	6100	970	66	20	10	45	45	0	100	100	6100	970	66	9	9	0	915	145.5	19.8	1098.3	Neg	Pos	Neg
57	0.2	9	790	13	20	1320	90	20	10	45	3950	133.4	100	100	20	1320	90	9	790	26.68	3	198	27	1053.68	Neg	Neg	Neg
39	0.2	9	9	7	5100	9	240	20	10	45	45	68.7	100	100	5100	9	240	9	9	13.74	765	1.35	72	870.09	Pos	Neg	Neg
21	0.2	9	9	24	9	9	2160	20	10	45	45	241.9	100	100	9	9	2160	9	9	48.38	1.35	1.35	648	717.08	Pos	Neg	Neg
50	0.2	9	9	0	4000	50	102	20	10	45	45	0	100	100	4000	50	102	9	9	0	600	7.5	30.6	656.1	Neg	Neg	Neg
60	0.2	9	9	1	760	630	1230	20	10	45	45	11.8	100	100	760	630	1230	9	9	2.36	114	94.5	369	597.86	Neg	Neg	Neg
7	0.2	9	9	1	1800	10	650	20	10	45	45	12.6	100	100	1800	10	650	9	9	2.52	270	1.5	195	487.02	Pos	Neg	Neg

37	0.2	9	9	11	10	2600	116	20	10	45	45	111.2	100	100	10	2600	116	9	9	22.24	1.5	390	34.8	466.54	Neg	Neg	Neg
19	0.2	9	9	2	20	9	1130	20	10	45	45	17.9	100	100	20	9	1130	9	9	3.58	3	1.35	339	364.93	Pos	Neg	Neg
56	0.2	9	9	0	50	2200	15	20	10	45	45	0.3	100	100	50	2200	15	9	9	0.06	7.5	330	4.5	360.06	Neg	Neg	Pos
24	0.2	9	9	0	800	1420	12	20	10	45	45	2.6	100	100	800	1420	12	9	9	0.52	120	213	3.6	355.12	Pos	Neg	Pos
34	0.2	9	9	2	220	1500	12	20	10	45	45	23.1	100	100	220	1500	12	9	9	4.62	33	225	3.6	284.22	Neg	Neg	Pos
18	0.2	9	9	2	1210	9	250	20	10	45	45	21	100	100	1210	9	250	9	9	4.2	181.5	1.35	75	280.05	Pos	Neg	Neg
38	0.2	9	9	5	690	10	330	20	10	45	45	47.9	100	100	690	10	330	9	9	9.58	103.5	1.5	99	231.58	Pos	Pos	Neg
30	0.2	9	9	0	1140	9	126	20	10	45	45	1	100	100	1140	9	126	9	9	0.2	171	1.35	37.8	228.35	Neg	Neg	Pos
20	0.2	9	9	24	9	10	520	20	10	45	45	241.9	100	100	9	10	520	9	9	48.38	1.35	1.5	156	225.23	Pos	Neg	Neg
29	0.2	9	9	0	180	920	61	20	10	45	45	3.7	100	100	180	920	61	9	9	0.74	27	138	18.3	202.04	Neg	Pos	Neg
41	0.2	9	9	0	40	9	560	20	10	45	45	0.7	100	100	40	9	560	9	9	0.14	6	1.35	168	193.49	Neg	Neg	Pos
68	0.2	9	9	0	100	900	5	20	10	45	45	0	100	100	100	900	5	9	9	0	15	135	1.5	169.5	Neg	Neg	Neg
2	0.2	9	9	0	9	910	0	20	10	45	45	0.1	100	100	9	910	0	9	9	0.02	1.35	136.5	0	155.87	Neg	Neg	Neg
71	0.2	9	9	24	9	9	270	20	10	45	45	241.9	100	100	9	9	270	9	9	48.38	1.35	1.35	81	150.08	Pos	Neg	Neg
36	0.2	9	9	1	10	20	260	20	10	45	45	10.4	100	100	10	20	260	9	9	2.08	1.5	3	78	102.58	Pos	Neg	Neg
12	0.2	9	9	0	470	9	34	20	10	45	45	0	100	100	470	9	34	9	9	0	70.5	1.35	10.2	100.05	Neg	Neg	Neg
22	0.2	9	9	1	30	400	11	20	10	45	45	11.2	100	100	30	400	11	9	9	2.24	4.5	60	3.3	88.04	Neg	Neg	Neg
8	0.2	9	9	0	360	100	0	20	10	45	45	0.2	100	100	360	100	0	9	9	0.04	54	15	0	87.04	Neg	Neg	Neg
82	0.2	9	9	1	9	360	34	20	10	45	45	5	100	100	9	360	34	9	9	1	1.35	54	10.2	84.55	Pos	Neg	Neg
3	0.2	9	9	7	30	10	129	20	10	45	45	72.2	100	100	30	10	129	9	9	14.44	4.5	1.5	38.7	77.14	Pos	Neg	Pos
27	0.2	9	9	2	80	212	17	20	10	45	45	23.7	100	100	80	212	17	9	9	4.74	12	31.8	5.1	71.64	Neg	Neg	Neg
14	0.2	9	9	3	30	100	92	20	10	45	45	27.6	100	100	30	100	92	9	9	5.52	4.5	15	27.6	70.62	Neg	Neg	Neg
15	0.2	9	9	0	40	30	89	20	10	45	45	2.1	100	100	40	30	89	9	9	0.42	6	4.5	26.7	55.62	Pos	Neg	Neg
59	0.2	9	9	0	9	120	49	20	10	45	45	0	100	100	9	120	49	9	9	0	1.35	18	14.7	52.05	Neg	Neg	Neg
26	0.2	9	9	1	100	10	51	20	10	45	45	7.9	100	100	100	10	51	9	9	1.58	15	1.5	15.3	51.38	Neg	Neg	Neg
17	0.2	9	9	0	9	150	13	20	10	45	45	0.1	100	100	9	150	13	9	9	0.02	1.35	22.5	3.9	45.77	Neg	Neg	Neg
28	0.2	9	9	0	110	9	5	20	10	45	45	1.2	100	100	110	9	5	9	9	0.24	16.5	1.35	1.5	37.59	Neg	Neg	Neg
77	0.2	9	9	0	80	9	0	20	10	45	45	0	100	100	80	9	0	9	9	0	12	1.35	0	31.35	Neg	Neg	Neg

11	0.2	9	9	0	20	9	12	20	10	45	45	0.2	100	100	20	9	12	9	9	0.04	3	1.35	3.6	25.99	Neg	Neg	Neg
16	0.2	9	9	0	10	9	13	20	10	45	45	1.1	100	100	10	9	13	9	9	0.22	1.5	1.35	3.9	24.97	Neg	Neg	Neg
43	0.2	9	9	0	9	9	5	20	10	45	45	0.6	100	100	9	9	5	9	9	0.12	1.35	1.35	1.5	22.32	Pos	Neg	Neg
6	0.2	9	9	0	9	9	1	20	10	45	45	0.1	100	100	9	9	1	9	9	0.02	1.35	1.35	0.3	21.02	Neg	Neg	Neg

Wi=Relative weight; CF Puls=Coliforms on pulsator surface; CF Pipe=Coliforms on pipeline surface; CF Water=Coliforms in water; TBC Puls=Total bacterial count on pulsator surface; TBC Pipe=Total bacterial count on pipeline surface; TBC Water=Total bacterial count in water; CF Std Surf=Legal standard for Coliforms on surface; CF Std Water=Standard for coliforms in water; qCF Puls=Quality rating for coliforms on pulsator surface; qCF Pipe=Quality rating for coliforms on pipeline surface; qCF Water=Quality rating for coliforms in water; TBC Std Surf=Standard for Total bacteria on surfaces; TBC Std water=Standard for Total bacteria in water; qTBC Puls=Quality rating for Total bacteria on pulsator surfaces; qTBC Pipe=Quality rating for Total bacteria on pipeline surfaces; qTBC Water=quality rating for Total bacteria in water; S(CF)Puls=Sub index for coliforms on pulsator surface; S(CF)Pipe=Sub index for coliforms on pipeline surface; S(CF)Water=Sub index for coliforms in water; S(TBC)Puls=Sub index for total bacterial count on pulsator surface; S(TBC)Pipe=Sub index for total bacterial count on pipeline surface; S(TBC)Water=Sub index for total bacterial count in water; HQI=Hygiene quality index.

The parameters used to calculate a MQI were TBC, coliforms and SCC in milk. The standard for each parameter was used for the S_i value in each calculation. Each of the three parameters was assigned a weighting (w_i) of relative importance in the overall quality of milk. The relative weight (W_i) was calculated, where (W_i) is the relative weight, w_i is the weight for each parameter and n is the number of parameters (Table 7.8).

Table 7.8 Milk quality index parameters, standard, weight and relative weight.

Parameter	**S_i (per 1 mL)**	**w_i**	**W_i** $W_i = w_i / \Sigma^n_{i=1} w_i$
TBC	200 000	5	0.333333
Coliforms	20	5	0.333333
Somatic cell count	500 000	5	0.333333
Total = 3		Σ **w_i** = 15	Σ **W_i** = 1.000000

The parameters used to calculate a BWQI were TBC and coliforms in water. The standard for each parameter was used for the S_i value in each calculation. Each of the two parameters was assigned a weighting (w_i) of relative importance in the overall quality of milk. The relative weight (W_i) was calculated, where (W_i) is the relative weight, w_i is the weight for each parameter and n is the number of parameters (Table 7.9).

Table 7.9 Bacteriological water quality index parameters, standard, weight and relative weight.

Parameter	**S_i (per 1 mL)**	**w_i**	**W_i** $W_i = w_i / \Sigma^n_{i=1} w_i$
TBC	100	5	0.5
Coliforms	10	5	0.5
Total = 2		Σ **w_i** = 10	Σ **W_i** = 1.000000

The parameters used to calculate a HQI were TBC and coliforms in water as well as on pulsator and milk pipeline surfaces. The standard for each parameter was used for the S_i value in each calculation. Each of the

six parameters was assigned a weighting (w_i) of relative importance in the overall quality of milk. The relative weight (W_i) was calculated, where (W_i) is the relative weight, w_i is the weight for each parameter and n is the number of parameters (Table 7.10).

Table 7.10 Hygiene quality index parameters, standard, weight and relative weight.

Parameter	**S_i (per 1 mL / cm²)**	**w_i**	**W_i** $W_i = w_i / \Sigma^n_{i=1} w_i$
TBC - Pulsator	100	2	0.166666
TBC – Pipeline	100	2	0.166666
TBC – Water	100	2	0.166666
Coliforms – Pulsator	20	2	0.166666
Coliforms – Pipeline	20	2	0.166666
Coliforms - Water	10	2	0.166666
Total = 6		Σ **w_i** = 12	Σ **W_i** = 1.000000

Chapter 8
Discussion and conclusions

8.1 Discussion

Commercial dairy farming in South Africa is one of the key role players in the agricultural sector. It is a major contributor to the South African economy through employing a large number of workers and producing products for local and international markets. Many of the commercial milk producing dairies are situated in central South Africa. The milk quality and contributing factors of 83 commercial dairy farms in central South Africa were studied. Milk quality was tested for the presence of *E. coli*, coliform bacteria, total bacterial count and somatic cells. The standards according to Regulations relating to milk and dairy products (Regulation R. 1555 of 1997), as well as the Regulations relating to hygiene requirements for milking sheds, the transport of milk and related matters (Regulation R. 961 of 2012) were used to measure compliance of the milk produced, as well as the level of hygiene maintained on these dairy farms. The contributing factors included were surface hygiene and water quality. A number of hygiene indicators of pulsator and milk pipeline surfaces were analysed to ascertain to which extent these surfaces could contribute to the quality of milk produced in the dairy. Because dairies use water in all the steps of the dairy process, including cleaning, sanitization, heating, cooling and floor washing, the quality of water was also tested for total bacterial count, as well as the presence of *E. coli* and coliform bacteria. The standards according to the South African standard for drinking water SANS 241 of 2011 as well as the Mangaung water action plan of 2007 were used to measure compliance of the water used in the dairies.

8.2 Milk quality

Total bacterial count (TBC) is generally used as an indicator of the overall quality and safety of milk, and is also decisive of the suitability for further processing. It is therefore a widely used criterion in quality incentive programmes for the grading of raw milk intended for further processing (Elmoslemany *et al*., 2009a; Mhone *et al*., 2011). In this study the TBC measurements revealed that a large number of farms, 85%, produced milk within the legal standard pertaining to TBC. However, the exceptionally high prevalence of *E. coli* in milk; 93% of the farms, necessitated the development of another measure of milk quality. Therefore, a milk quality index (MQI) was derived from the water quality index of

Ramakrishnaiah *et al.* (2009) to produce a single value that describes the quality of the milk produced on a particular farm.

The MQI is calculated from all measurements of the different parameters and describes the quality of milk as either being of excellent or of poor quality. According to the MQIs, most (99%) of the farms in the study produced milk that was of poor bacteriological quality. The presence of *E. coli* in the milk of 93% of the dairy farms resulted in the milk of the vast majority of farms to fall into the category of poor quality. The presence of *E. coli* in milk is indicative of faecal contamination during the process of milk production on a dairy farm. The presence of *E. coli* also implicates the possible presence of enteropathogenic and toxigenic microorganisms, which constitute a health risk to consumers of contaminated milk (Altalhi & Hassan, 2009; Mhone *et al*., 2011). *E. coli* in milk is especially of public health concern because of an increase in consumption of unpasteurised milk and dairy products manufactured from unpasteurised milk (Oliver *et al*., 2009). Despite numerous epidemiological studies which show the health risks involved in the consumption of unpasteurised milk, there is an increase in the consumption of unpasteurised milk. The consumption continues even though people know that milk can be contaminated with various pathogens associated with disease in humans (Oliver *et al*., 2009). The most vulnerable are farm workers, the farmer and family, and their children who are known to consume unpasteurised milk produced on their farm (Personal experience).

Milk and dairy products contaminated with *E. coli* are known to cause diarrhoea after consumption (Kousta *et al.,* 2010). The presence of *E. coli* O157:H7, an enterohaemorrhagic strain of the bacterium *E. coli,* could cause illness through consumption of contaminated food and can lead to haemorrhagic colitis and the potentially lethal haemolytic-uremic syndrome (Schouten *et al*., 2004). Consumption of raw milk is considered to be one of the main causes of *E. coli* O157:H7 infections in humans. Shedding of the bacteria in the faeces of infected cows can lead to contamination of milk and surface water (Schouten *et al*., 2004). An outbreak of *E. coli* O157:H7 infections, although not serious, was caused by consumption of unpasteurised milk sold at Oregon grocery stores in the United States of America (Keene *et al*., 1997). A number of food poisoning outbreaks where *E. coli* was the causative agent were reported in the United States of America, France and Scotland after consumption of contaminated cheese such as Brie, Camembert and Gouda (Kousta *et al.*, 2010).

Although pasteurization was introduced to improve the shelf life and safety of milk by decreasing the bacterial content (Lund *et al*., 2002), it is not effective in eliminating all microorganisms because the thermal destruction process is logarithmic and eliminates bacteria at a rate that is proportional to the

number of bacteria present in raw milk (LeJeune & Rajala-Schultz, 2009; Oliver *et al*. 2009). Pasteurization is also not effective in eliminating the toxins and spores of microorganisms (LeJeune & Rajala-Schultz, 2009).

Coliforms are inhabitants of the intestinal tract of cows and are widely distributed in the farming environment, such as in manure, bedding material, soil and contaminated water (Elmoslemany *et al*., 2009c; Pantoja *et al*., 2011). An elevated coliform count in milk is generally caused by improperly cleaned dairy equipment as well as dirt on the exterior of the udder and teats of the cow (Elmoslemany *et al*., 2009b). There is also a possibility that the high coliform counts in milk could partially be caused by dairy equipment contaminated because of poor quality wash water (Perkins *et al*., 2009; Mhone *et al*., 2011). Other factors which could contribute to the contamination of raw milk include the cow itself, personnel engaged in milk processing or extraneous dirt (Altalhi & Hassan, 2009). Thus, the presence of coliform bacteria in milk is generally indicative of the standard of hygiene practices in a dairy. This study demonstrated a concerning low compliance for the presence of coliform bacteria in milk; less than 15% of the farms. The high percentage of measurements containing more coliforms than the legal standard signified poor hygiene maintenance and practices (Altalhi & Hassan, 2009; Mhone *et al*., 2011; Pantoja *et al*., 2011) on these dairy farms.

The presence of elevated levels of somatic cells (SCC) in milk signifies the health status of the dairy herd (Elmoslemany *et al*., 2009c). Elevated SCCs indicate the prevalence of mastitis in a herd (Le Maréchal *et al*., 2011), and is caused by pathogenic bacteria that enter the mammary gland via the teat canal. It is possible that pathogenic bacteria can enter the teat canal due to contaminated bedding and dirty stalls (Elmoslemany *et al*., 2009b). Studies have shown a significant association between dairy shed hygiene and cow and teat cleanliness, as well as a positive correlation between shed cleanliness and clinical mastitis (Elmoslemany *et al*., 2009b). In this study 42% of the farms demonstrated SCC values that were indicative of possible prevalence of mastitis in the herds. Concerning was the possibility of pathogenic bacteria being present, such as *Staphylococcus aureus, Listeria* spp*., Streptococcus* spp. and *E. coli*, which causes mastitis (Ruegg, 2003; Lindmark-Månsson *et al*., 2006; Sharif & Muhammad, 2008; Le Maréchal *et al*., 2011).

8.3 Factors that influence milk quality

High quality raw milk is important for the production of high quality pasteurised milk and dairy products. The production of high quality milk starts at the farm and is influenced by many hygiene practices related to the milking shed. Poor hygiene practices may result in the spoilage of milk and thereby products produced from milk, and could ultimately result in loss of income for the dairy farmer (Bonfoh *et al*., 2003). Because water is used throughout the dairy process, it is also important to consider the quality of water as a potential contributing source of poor quality milk. Assessing the milk pipeline and pulsator surfaces, as well as water for the presence of bacteria, provides a means to ascertain the status of hygiene practices in a diary shed.

It is generally expected that contact surfaces in a dairy shed demonstrate 100% compliance to hygiene standards, because of thorough washing and sanitizing of the dairy equipment. However, in this study many farms revealed contaminated surfaces. For the TBC on dairy contact surfaces, compliance was low with less than 30% of the farms with counts less than the legal standard. *E. coli* was present on more than 10% of the pulsator and milk pipeline surfaces, indicating faecal contamination, while coliform counts were higher than the legal standard in more than 20% of the farms. Significant differences ($p < 0.001$) were evident between the presence of *E. coli* in milk and *E. coli* on the dairy contact surfaces indicating that the contact surface could have contributed to the *E. coli* contamination of the milk. Clusters falling on the dairy parlour floor is associated with increased coliform counts, including *E. coli*, on dairy contact surfaces (Pantoja *et al*., 2011).

The presence of contaminating bacteria on dairy contact surfaces is concerning, because an important survival mechanism of microorganisms is the production of biofilms. Some produce biofilms to protect themselves from unfavourable environments. Such microbial biofilms are defined as microbial aggregates which are embedded in the matrix of exopolymers, which can attach it to biotic or abiotic surfaces, such as milk pipelines and pulsators. After adhesion to a wet surface, microorganisms secrete a complex extracellular matrix and then embed itself in it (Bayoumi *et al.,* 2012). Although *E. coli* adheres poorly to surfaces, it can become embedded in biofilms that were not removed during the washing and sanitising process (Salo *et al*., 2006).

The presence of biofilms and accompanying pathogens on dairy contact surfaces present a biotransfer potential, which increase the probability of cross contamination to the milk (Abban *et al*., 2012; Bodur &

Cagri-Mehmetoglu, 2012). Insufficient cleaning and sanitation of dairy equipment promotes cross contamination (Ortega *et al*., 2010). Although accumulation of microorganisms on milk contact surfaces may take several days to reach a point where the total bacterial count in milk is influenced, the presence of coliform bacteria in milk is an indication of persistent cleaning failure and poor equipment maintenance (Reinemann & Rasmussen, 2011).

Water quality was measured to determine whether water could contribute to the poor milk quality found on the farms under investigation. The properties of water on a dairy farm can affect the cleaning process and milk quality, and therefore water quality can be considered as a basic determinant of milk quality (Bonfoh *et al*., 2003). The water data of this study revealed that 31% of the dairy farms did not comply with the legal standard because of the presence of *E. coli* in the borehole water used in the dairies. The water data further revealed that 63% of the measurements contained more coliforms than the legal standard and 52% did not comply with the guideline standard for total bacterial count. A bacteriological water quality index (BWQI), also derived from Ramakrishnaiah's (2009) water quality index, was calculated for each of the dairy farms by taking all the bacteriological water parameters into consideration, namely, TBC, coliforms and *E. coli*. The BWQI revealed that 43% of the dairy farms used poor quality borehole water for washing and sanitising of dairy equipment.

The poor bacteriological quality of the borehole water in this study is worrying, because poor quality wash water does not only contaminate dairy contact surfaces (Elmoslemany *et al*., 2010), it can also contaminate milk through water droplets which accidently mix with the milk (Swai & Schoonman, 2011). Adulteration is the purposeful mixing of water with milk. This is accomplished by adding water with the intention of increasing volume for greater profit (Das *et al*., 2011). If the quality of the water used in adulteration is poor, there is a risk of introducing microbial health hazards as well as reducing the processing quality and marketing value of the milk (Swai & Schoonman, 2011). A study performed in Ontario, Canada, showed that wash water contaminated with *E. coli* can be associated with higher bacterial counts as well as the presence of *E. coli* in raw milk. *E. coli* in the water used to wash dairy equipment can contaminate dairy contact surfaces and subsequently contribute to the presence of *E. coli* in milk (Perkins *et al*., 2009). In this study significant differences ($p < 0.001$) were found between the presence of *E. coli* in milk and *E. coli* in water, strongly suggesting that water could also have contributed to the poor quality of milk produced on the farms studied. It is therefore important to use good quality water to prevent contamination of milking equipment and therefore also subsequent contamination of milk (Perkins *et al*., 2009). High coliform counts and the presence of other pathogens,

such as *Pseudomonas* spp. and other Gram-negative bacteria in raw milk could also be the result of contaminated wash water (Afif *et al.*, 2008; Elmoslemany *et al.*, 2009a).

By taking all the parameters relating to hygiene into consideration, a hygiene quality index (HQI), also derived from Ramakrishnaiah's (2009) water quality index, was calculated for each of the dairy farms. The parameters included in the HQI calculation were coliforms in water and on pulsator and milk pipeline surfaces, *E. coli* in water and on pulsator and milk pipeline surfaces and total bacterial count (TBC) in water and on pulsator and milk pipeline surfaces. The HQIs showed that less than 4% of the dairy farms in this study displayed acceptable levels of hygiene, with a HQI of less than 100 and with *E. coli* absent.

8.4 Concluding remarks

This study revealed that the milk contact surfaces as well as the water used in the dairies do have an influence on the quality of the milk produced on the 83 dairy farms included in this study. The surface and water data along with the HQI, clearly signifies ineffective cleaning and disinfection of milking equipment. Milk is contaminated by dirty milking equipment at the beginning of the milking process, causing deterioration in the quality, safety and shelf life of the milk produced (Ortega *et al*., 2010). To avoid the contamination of milk, it is of utmost importance that all contact areas should be cleaned and sanitized properly, because coliforms and other pathogenic microorganisms may incubate on residual films of improperly cleaned milking equipment (Elmoslemany *et al*., 2010).

A number of conclusions stem from this study. The results demonstrated that hygiene practices as well as maintenance of dairy equipment on dairy farms required upgrading; that the water used in dairy sheds should be treated so as to improve its quality; that the health of dairy herds be examined twice yearly by a veterinary surgeon, in order to prevent contamination of milk by infected or sick cows; that adulteration be monitored; and that a routine programme of milk and water quality testing be introduced by the regulatory authority. Furthermore, the introduction of an incentive scheme by smaller processing companies could motivate farmers to become more vigilant in the production of high quality milk. Currently, only large processing companies have implemented incentive schemes. Such schemes reimburse farmers according to the quality of the milk that they supply. Lastly it is suggested that a

comprehensive training programme is introduced to dairy farmers and personnel, to provide education on all aspects of producing high quality milk in a suitable manner.

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