Prevalence of mastitogenic pathogens in pasture and total mixed ration based dairies during 2008 and 2013

David JC Blignaut

i

PREVALENCE OF MASTITOGENIC PATHOGENS IN PASTURE AND TOTAL MIXED

RATION BASED DAIRIES DURING 2008 AND 2013

by

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Supervisor: Dr. I.M. Petzer

Co-supervisor: Prof. P.N. Thompson

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ii

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My wife Belinda and son Christiaan for your loving support through this long journey.

Soli Deo gloria

iii

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

BTA — Bovine blood trypose agar

CNS — Coagulase-negative *staphylococci*

CI — Confidence interval

CU — Contamination pathogens

E. coli — *Escherichia coli*

IMI — Intramammary Infection

MSD — Milk diagnostic system

OML — Onderstepoort milk laboratory

OR — Odds ratio

PMN — Polymorphoneuclear leucocytes

RAPD — Random amplified polymorphic DNA fingerprinting

SCC — Somatic cell count

S. aureus — *Staphylococcus aureus*

S. chromogenes — *Staphylococcus chromogenes*

S. epidermides — *Staphylococcus epidermides*

S. haemolyticus — *Staphylococcus haemolyticus*

S. simulans — *Staphylococcus simulans*

S. xylosus — *Staphylococcus xylosus*

Str. agalactiae — *Streptococcus agalactiae*

Str. dysgalactiae — *Streptoccoccus dysgalactiae*

Str. uberis — *Streptococcus uberis*

TMR — Total mixed ration

LIST OF TABLES

LIST OF FIGURES

DECLARATION

I was assisted by Dr. Inge-Marié Petzer and Prof. Peter Thompson (both from the Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, South Africa) in conceptualising and planning of this research project. Dr. Petzer as manager of the Onderstepoort Milk Laboratory, assisted in the transfer of available data from the Milk Sample Diagnostic system and her support staff received and processed all milk samples. Laboratory support staff assisted in gathering and listing contact details of all farmers. Prof. Thompson, as Veterinary Epidemiologist, assisted in the analysis of data.

With the exception of the abovementioned assistance this dissertation is the candidate's own original work. It is not submitted concurrently in candidature for any other degree.

SUMMARY

PREVALENCE OF MASTITOGENIC PATHOGENS IN PASTURE AND TOTAL MIXED

RATION BASED DAIRIES DURING 2008 AND 2013.

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DAVID JC BLIGNAUT

Supervisor: Dr. I.M. Petzer

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Degree: MMedVet (Med:Bovid)

Mastitis is one of the most economically important diseases in dairy cattle worldwide. Not only does it have a negative effect on milk production, it also is one of the main reasons for culling dairy cattle.

Pathogens causing mastitis in dairy cattle can be grouped into either contagious (or host adapted) or environmental pathogens. In different parts of the world it was shown that the prevalence of these differently grouped pathogens is dependent on various risk factors. Furthermore, it was shown that control measures implemented against contagious intramammary infections caused a relative shift over time towards a higher prevalence of environmental intramammary infections.

ix

In this study udder health data from the Onderstepoort Milk Laboratory (OML) was compared over two different years, 2008 and 2013, with regards to the prevalence of specified mastitogenic pathogens in total mixed ration (TMR) dairies and pasture-based dairies. Furthermore, the within-herd prevalence of *Streptococcus uberis* (*Str. uberis*) in *Str. uberis* positive herds was compared between the two years and the two management systems.

Statistically significant differences were found in the prevalence of most of the major contagious and environmental mastitogenic pathogens between 2008 and 2013 and between the TMR and pasture-based dairies. Coagulase-negative *staphylococci* (CNS) has the highest prevalence in both TMR and pasture-based for both 2008 and 2013. *Streptococcus uberis* overall showed an increase in prevalence from 2008 to 2013, with the highest prevalence in pasture-based dairies in 2013. *Staphylococcus aureus* (*S. aureus*) showed a statistically significant decrease in TMR and pasture-based dairies from 2008 to 2013.

The within-herd prevalence of *Str. uberis* increased from 2008 to 2013 with the highest within-herd prevalence in pasture-based dairies in 2013.

x

INTRODUCTION

Mastitis is defined as inflammation of the mammary gland. When pathogens transverse the streak canal of the teat-end and colonize the udder an inflammatory response is initiated and pending the degree of the inflammatory response it can be regarded as clinical or subclinical mastitis (Morin 2015). Mastitis is the most costly disease of dairy cows worldwide and it was estimated to cost the United States close to $ 2 billion in 1993 (Petrovski, Trajcev & Buneski 2006).

Clinical mastitis, is defined as mastitis accompanied by visible changes in the milk, with or without secondary systemic signs in the cow and udder. Abnormal visual changes include the presence of clots and serum in the milk (Biggs 2009, Morin 2015). Inflammatory signs present in the udder include oedema, painful to the touch and discoloration in a number of cases. In severe cases the cow might show systemic signs including: a raised rectal temperature, agalactia, decreased rumination time as well as mild to moderate anorexia (Biggs 2009, Fogsgaard *et al.* 2012, Morin 2015). The prevalence of clinical mastitis from 180,486 quarter milk samples was between 8.1% and 15.4% in a South African study during the period 2002 to 2006 *(*Petzer *et al*. 2009a). In England the incidence of clinical mastitis was measured at between 47 and 65 cases per 100 cows per year during 2004 and 2005 (Bradley *et al*. 2007).

Subclinical mastitis is defined as an inflammatory response in the udder that does not result in any visible changes in the milk, the udder nor any noticeable systemic effects in the cow. It is recognized   by reduced milk production and alteration in milk composition, of which an increase in the somatic cell count (SCC) can be regarded as a consistent finding (Kehrli & Harp 2001, Morin 2015, Tizard 2013). The average herd prevalence of subclinical mastitis in an Australian study conducted on dairy farms in New South Wales was 29% and it was apparent that management practices such as wearing gloves, using towel paper and feeding

1

cows directly after milking were associated with lowered herd prevalence of subclinical mastitis (Plozza *et al.* 2011).

It is well known that intramammary infections (IMI) can be caused by pathogens originating either from the environment or from infected udders as the original reservoir. Traditionally pathogens that originate from a chronically subclinical infected udder and spread to a healthy udder, usually through contaminated milk during the milking process, are considered contagious pathogens. (Keefe 2012, Morin 2015, Roberson *et al.* 1994, Ruegg 2012, Smith, Todhunter & Schoenberger 1985a) The most prevalent contagious pathogens identified in dairies include *Streptococcus agalactiae* (*Str. agalactiae*) (RSA, Brazil, Germany), *S. aureus* (USA, RSA, Norway, Germany, Netherlands) and *Mycoplasma* spp. (USA)(Barkema *et al.* 1998, Duarte *et al.* 2004, Morin 2015, Petzer *et al.* 2009a, Tenhagen *et al.* 2006, USDA 2008, Østerås, Sølverød & Reksen 2006).

Opportunistic pathogens found in the surroundings of the cow are considered environmental pathogens. The most frequently isolated environmental pathogens include *Escherichia coli* (*E.coli*) (UK, Canada), *Klebsiella* spp. (USA, Netherlands), *Str. uberis* (RSA, Australia, New Zealand, UK), CNS (RSA, Finland, Norway) and *Streptococcus dysgalactiae* (*Str. dysgalactiae*) (Norway) (Barkema *et al.* 1998, Milne *et al.* 2002, Morin 2015, Munoz *et al.* 2007, Olde Riekerink *et al.* 2008, Petzer *et al.* 2009a, Pitkala *et al.* 2004, Shum *et al.* 2009, Tenhagen *et al.* 2006, Østerås, Sølverød & Reksen 2006).

Molecular strain typing techniques of mastitogenic pathogens (*E.coli, Klebsiella pneumoniae, Str. agalactiae, St. uberis* and *S. aureus*) have been developed over recent years and used in molecular epidemiological studies to demonstrate mechanisms of host-adaptation and evolution of these mastitogenic pathogens (Munoz *et al.* 2007, Zadoks *et al.* 2011, Zadoks & Schukken 2006). Studies making use of molecular strain typing techniques have shown that different strains of *Str. uberis* and *Klebsiella pneumoniae,* previously thought to be

2

environmental pathogens, showed the characteristics of contagious pathogens and are therefore not classic environmental pathogens, but rather more adapted to survive in the cow's udder and infect healthy udders by between-cow transmission (Munoz *et al.* 2007, Zadoks *et al.* 2003).

Over recent years there was an increase in the prevalence of environmental pathogens (Bradley 2002, Milne *et al.* 2002, Petzer *et al.* 2009a). This increase in prevalence could be ascribed to the improved control methods for contagious pathogens, difficulties in controlling pathogens from an environmental reservoir and the ability of *Str. uberis* and *E. coli* to persist in the udder (Abureema *et al.* 2014, Leigh 1999, Matthews, Almeida & Oliver 1994). *Streptococcus uberis*, being one of the most important cultured mastitogenic pathogens in Australian dairies (Abureema *et al.* 2014, Charman *et al.* 2012) showed an increase in incidence in South African dairies (Petzer *et al.* 2009a).

During the same period there was an overall increase in pasture-based dairies compared to TMR in South Africa (Lactodata, 2013). Future research can be directed to find out what the potential impact of this change has on South African dairies with regards to the prevalence and associated risk factors of contagious and environmental pathogens.

LITERATURE REVIEW

Mastitis and udder immunity

Udder immunity can be regarded as a complex cohort of defence mechanisms that is continuously reducing the risks for mastitis in the cow. The teat canal is protected by a keratin plug between milkings and during the dry period (Kehrli & Harp 2001). This keratin plug can be regarded as the first line of defense against the potential introduction of pathogens into the teat canal. Although components within the keratin plug have known antimicrobial activity, the major function of the plug is to serve as a physical and mechanical barrier to invading pathogens (Kehrli & Harp 2001).

Should pathogens traverse the teat canal, the innate and adaptive immune responses are triggered to act against further infection of the mammary gland. Firstly, the innate immunity is activated immediately after pathogen invasion (Kehrli & Harp 2001, Tizard 2013). It can be regarded as a combination of lacteal secretions (including lactoperoxidases, lactoferrin and complement) and a cellular immune response of which neutrophils are the dominant cell (Tizard 2013).

Following activation signals from the innate immune system, the adaptive immune system is activated (Tizard 2013). One component of the adaptive immune system includes the recruitment of antigen-specific T and B lymphocytes responsible for an antibody-mediated response. Another component of the adaptive immune system is a cell-mediated immune response directed against intracellular pathogens (Morin 2015, Tizard 2013). Should the infection be cleared rapidly with little compromise to the gland tissue, this infection can be regarded as acute, while an infection that continue and resist the host defense mechanisms for an extended period of time, can be regarded as chronic (Tizard 2013).

4

The leucocytic cellular response is measured in practice utilizing the SCC (Morin 2015). Somatic cells consist of mammary epithelium cells, polymorphonuclear (PMN) leucocytes, lymphocytes and macrophages (Burton & Erskine 2003, Morin 2015, Sharif & Muhammad 2008). Somatic cell count is a general indicator of udder health and is influenced by various factors including: stage of lactation, faulty milking technique, intramammary infections, stress, age and season (Dohoo & Meek 1982). A SCC threshold of 200 X10^3 cells/ml in quarter milk samples of individual cows is a sensitive and specific measurement to distinguish between infected and non-infected quarters (Dohoo & Leslie 1991, Olechnowicz & Jaskowski 2012, Schukken *et al*. 2003). For food safety regulations in European countries, Australia and New Zealand SCC above 400 000 cells/ml milk is not safe for human consumption, the USA it is above 750 000 cells/ml milk and Canada and South Africa 500 000 cells/ml milk (Department of Health 1997, Olechnowicz & Jaskowski 2012, Schukken *et al.* 2003).

Environmental pathogens

Environmental pathogens are regarded as pathogens that are predominantly found in the cows' immediate surroundings. Pathogens are transmitted into the udder during exposure of the teat-ends to contaminated surroundings (Hogan & Smith 2012, Morin 2015).

Streptococcus uberis was cultured from feces, rumen contents, genital tract and teats of normal healthy cows (Hogan & Smith 2012, Zadoks *et al.* 2003). Infection of the mammary gland occurs through exposure of the teat to a contaminated environmental source. This can occur during the milking process, between milkings, during the dry period and prior to first calving in heifers (De Vliegher *et al.* 2012). Although regarded as an environmental pathogen, a study done by Tamilselvam *et al.* (2006) showed that *Str. uberis* has the potential to survive within mammary epithelial cells for up to 120 hours. This intracellular survival mechanism of *Str. uberis* might be evidence for persistent IMI as a result of this pathogen.

5

Coagulase-negative s*taphylococci* are becoming an important group of bacteria as they are frequently isolated from milk samples in herds with good control over the contagious group of mastitogenic pathogens. This group consists of over 24 *Staphylococcus* strains, with *S. chromogenes, S. haemolyticus, S. epidermidis, S. simulans* and *S. xylosus* being the most commonly isolated from bovine milk (Vanderhaeghen *et al.* 2015). Coagulase-negative *staphylococci* were shown to be the most prevalent pathogens isolated from dairies in South Africa and Finland (Petzer *et al.* 2009a, Pitkala *et al.* 2004). Although it was thought that the CNS group consisted of purely environmental pathogens, recent studies showed that a few of the strains, especially *S. chromogenes,* can persist within the udder after initial intramammary infection (De Visscher *et al.* 2014, Vanderhaeghen *et al.* 2015). Persistence within the udder is regarded as host-adaptation. In the study done by De Visscher *et al.* (2014), they concluded that *S. stimulans* and *S. xylosus* also associated with the udder, even though *S. stimulans* was isolated from the environment in previous studies (Piessens *et al.* 2011). The distribution of CNS in the udder and environment as primary niches can differ between different farms (Piessens *et al.* 2011).

Streptococcus dysgalactiae was cultured worldwide and the prevalence varies between different countries: South Africa (2.5%), Switzerland (1.3%), Tanzania (5%) and the Netherlands (0.9-1.5%) (Guelat-Brechbuehl *et al.* 2010, Kivaria & Noordhuizen 2007, Petzer *et al.* 2009a, Sampimon *et al*. 2009) The prevalence of *Str. dysgalactiae* as environmental pathogen was the second highest in herds with high SCC in the Netherlands (Barkema *et al.* 1998).

Environmental pathogen control

In principle the control measures for decreasing the prevalence of environmental IMI are based on reducing the pathogen load that is in contact with the teat-end (Smith & Hogan 1993). Pre-dipping with a teat disinfectant and keeping all housing areas as clean and dry

as possible have shown to reduce the teat-end pathogen load (Morin 2015, Smith & Hogan 1993).

Morton *et al.* (2014) concluded that pre-dipping did not reduce the incidence of IMI in Australian pasture-grazed herds to such a level where it was economically warranted to use this practice for mastitis control. However, the lactating cows in the herds they used had relatively clean and dry teats with a relatively low incidence of clinical mastitis. They suggested as a result of the reduction in clinical mastitis cases in other studies that investigated pre-dipping (Oliver *et al.* 1993, Oliver *et al.* 2001), it might be worthwhile to use this practice when the incidence of soiled teats or environmental clinical mastitis are high (Morton e*t al.* 2014)

The use of dry cow therapy for the control of environmental streptococcal and coliform IMI was evaluated by Smith *et al.* (1985b). They concluded that the use of dry cow therapy can be advantageous for the control of streptococcal IMI during the early dry period, but not immediately prior to the prepartum period. It was shown that the use of teat sealants and dry cow antibiotic preparations can reduce the occurrence of new IMI through the dry period (Berry & Hillerton 2002, Godden *et al.* 2003, Petzer *et al.* 2009b).

Measures used to control contagious IMI (see *Contagious pathogen control* below) are not effective for the control of environmental IMI (Smith & Hogan 1993).

Risk factors influencing environmental IMI

Although various risk factors can influence the incidence of environmental IMI within a dairy only the most common and relevant will be discussed within the following paragraphs:

7

STAGE OF LACTATION

The stage of lactation plays a role in the incidence of environmental IMI. It was shown that cows are more susceptible to environmental IMI during the dry period, especially immediately after drying off and on average two weeks prior to calving (Hogan & Smith 2012, Smith, Todhunter & Schoenberger 1985b, Zadoks *et al.* 2003). Coliform clinical mastitis is more prevalent in early lactation with the initial infection during the dry period prior to parturition (Hogan & Smith 2012, Smith, Todhunter & Schoenberger 1985a). The rate of coliform IMI decreases as the days in milk increases (Hogan & Smith 2003).

STALL BEDDING

Housing of dry cows and lactating cows plays a major role in the transmission of environmental pathogens. Environmental pathogen load is much lower on inorganic matter such as washed sand, compared to organic matter such as straw, dried manure and sawdust (Hogan & Smith 2012, Smith & Hogan 1993). Kristula *et al.* (2005) investigated the use of recycled sand compared to clean sand as bedding in dairies. It was found that the total number of pathogens (Coliform and *Klebsiella* spp.) were similar for clean sand and recycled sand up to 7 days after the sand was placed (Kristula *et al.* 2005). Kristula *et al.* (2005) recommended that if either clean or recycled sand it used, it should be changed at least twice a week due to the rapid increase of *Streptococcus* spp. numbers in both these beddings. Hogan *et al.* (1989) indicated that all bacterial population numbers were lower in sand compared to organic bedding. Sand should contain less than 5% organic particles to effectively have a lower pathogen load (Hogan & Smith 2012).

SEASONALITY

A study done in New Zealand (Petrovski *et al.* 2011) showed that seasonal patterns for the incidence of IMI can occur. The incidence of *Str. uberis* IMI was higher during late winter and early spring, but did not differ much for both *Str. uberis* and S. *aureus* for the rest of the year (Petrovski *et al.* 2011). The occurrence of environmental IMI is influenced by two major climatic factors: temperature and humidity (Hogan & Smith 2012). With an increase in

8

temperature and humidity there will be an increase in the populations of pathogens in the environment the dairy cow finds itself in (Hogan & Smith 2012). Overcrowding in stalls increase temperature and humidity which in turn will increase the risk of cows having IMI due to environmental pathogens (Hogan & Smith 2012). Overcrowding will increase manure buildup, which in turn increases the contamination of bedding in stalls (Hogan & Smith 2012).

GRAZING SYSTEMS AND PASTURE MANAGEMENT

Pasture management play an important role in the incidence of IMI caused by environmental pathogens. The use of grazing systems reduces the exposure to environmental pathogens compared to total confinement systems (Hogan & Smith 2012). Pasture management practices such as the spreading of effluent manure over the pastures as means of manure disposal and also providing nitrogenous nutrients for plant production was investigated in New Zealand by Lopez-Benavides *et al.* (2007) as a risk factor for mastitis as a result of *Str. uberis*. It was concluded that *Str. uberis* was detectable for up to 2 weeks during the spring and winter, but was of very short duration detectable in autumn. The increase in stocking rate and the reduction in rotational grazing time between different pastures may lead to the increased shedding of faecal pathogens and the build-up of manure. This subsequently will increase the risk for IMI (Hogan & Smith 2012, Lopez-Benavides *et al.* 2007).

Streptococcus uberis and Klebsiella spp. strain typing

Although *Str. uberis* is classified as an environmental udder pathogen in dairies, there are studies that suggest that this pathogen has the epidemiological characteristics of the contagious pathogens regularly found in dairies (Abureema *et al.* 2014, Zadoks *et al.* 2003, Zadoks 2007). Epidemiological and molecular data from a study by Zadoks *et al.* (2003) suggested that cows are infected by *Str. uberis* from the environment and that within-cow and between-cow transmission can occur. The transfer of bacteria possibly occurs via the milking machine. Strain typing data from this study indicated that when multiple quarters of

9

a cow were infected by *Str. uberis* the infections were usually caused by the same strain indicating that within-cow transmission of bacteria was possible. This study also showed that a predominant random amplified polymorphic DNA fingerprinting (RAPD) strain of *Str. uberis* occurred that caused IMI within a group of cows. This possibly indicated that multiple cows became infected from a common environmental source or alternatively it could have been the result of between-cow transmission of the pathogen (Zadoks *et al.* 2003). Abureema *et al.* (2014), concluded that repeated occurrences of clinical mastitis, as a result of *Str. uberis*, were commonly caused by new strains, but that in a small number of cases the same strains were subsequently isolated.

Strain typing of *Klebsiella* spp., making use of RADP – PCR typing, in a study by Munoz *et al.* (2007), showed that *Klebsiella* spp. has a diversity of strains. This study describes two *Klebsiella* mastitis outbreaks on a single dairy farm. During the first outbreak a single strain of *Klebsiella pneumonia* caused the mastitis outbreak, indicating either contagious transmission or the exposure of multiple cows to a single environmental source for the pathogen. During the second outbreak multiple *Klebsiella* strains caused the mastitis outbreak, indicating opportunistic infections form multiple environmental sources.

Contagious pathogens

The source for contagious pathogens is usually infected mammary glands. Transmission from infected to non-infected glands predominantly occurs during the milking process.

Staphylococcus aureus is considered a major pathogen due to its widespread prevalence worldwide, causing contagious mastitis (Morin 2015).

In a review by Keefe (1997) *Str. agalactiae, as* a cause of subclinical mastitis worldwide, is described as an obligate intramammary and highly contagious pathogen with potential infected cattle acting as a source for these bacteria. Outbreaks as a result of *Str. agalactiae*

10

can be controlled effectively with whole herd antibiotic therapy and well implemented contagious pathogen control methods (Keefe 1997, Smith & Ward 1975).

Contagious pathogen control

Control measures for the spread of contagious pathogens include the implementation of the five-point-mastitis control programme (Morin 2015, Neave *et al.* 1969): Post milking teat disinfection; prophylactic dry cow antibiotic treatment; culling of all chronically infected cattle; treatment of clinical affected cattle and effective maintenance of milking machine. An expanded more proactive ten-point-plan for controlling contagious pathogens was proposed and includes the following established goals for the herd's udder health (Keefe 2012, NMC Online 2009, Ruegg 2012): maintain clean, dry and comfortable environment; proper milking procedures; maintenance and care of milking equipment; proper milking procedures; management of clinical mastitis during lactation; dry cow management; effective biosecurity and culling of chronically infected cows; monitoring the udder health status and review of the mastitis control programme to promote the pro-active approach.

Risk factors influencing prevalence of contagious IMI

MILKING MACHINE AND MILKING PROCESS

The milking machine (including factors such as age of the machine, machine cleaned after milking and machine test report present at farm) and the milking procedure (including procedures such as post-milking teat disinfection, udder preparation before milking and cleaning of unit after a mastitic cow was milked) were shown in a previous study as factors that are important in the spread of contagious bacteria (Schukken *et al.* 1991).

Pre-milking and post-milking teat disinfection with a chlorous acid-chlorine dioxide disinfectant significantly decreased the prevalence of new *S. aureus* IMI, compared to post-milking disinfecting alone (Oliver *et al.* 1993).

UDDER HEALTH STATUS

Although not proven to be a direct mastitogenic pathogen (Wellenberg *et al.* 2000), bovine herpes virus 4 seropositive lactating cows were associated with an increased susceptibility to mastitis as a result of *S. aureus* (Kálmán, Jánosi & Egyed 2004, Wellenberg, van der Poel & Van Oirschot 2002, Zadoks *et al.* 2001).

It was shown that quarters that recovered from previous *Str. uberis* and *S. aureus* infections had a higher rate of infection than quarters without any previous infection (Zadoks *et al.* 2001). In contrast to the aforementioned study, there is evidence to support the importance of bacterial interactions within the udder. Studies have shown that when *Str. agalactiae* is controlled through blitz therapy (whole herd intramammary antibiotic treatment to eliminate *Str. agalactiae*), regular *E. coli* mastitis is reported after the therapy (Bradley 2002)

TEAT-END CALLOSITY

In the study of Zadoks *et al.* (2001), it was shown that there is an association between teat-ends that has extreme thickened teat-end callus or roughness, and an increase risk to *S. aureus* infections. The pathogenesis behind this association is still unknown.

FLIES AS VECTORS

It was shown that flies can act as potential vectors for the transmission of *S. aureus* in dairies. *Staphylococcus aureus* was acquired from flies that were caught in close proximity to the pre-weaning area, in a dairy with a high prevalence of *S. aureus* IMI. The calves were fed mastitic waste milk and it was suggested that this was the primary source of *S. aureus* causing IMI in the lactating cows (Roberson *et al.* 1994).

Changing trends in udder health and related pathogens

Over the last few decades there was a reduction in the incidence in IMI caused by contagious pathogens, mainly due to a better understanding of the epidemiology of these

12

pathogens and subsequent improved treatment and management protocols. Associated with this reduction is a noticeable tendency for an increased incidence of IMI caused by environmental pathogens (Bradley 2002, Leigh 1999, Shum *et al.* 2009, Smith 1983, Zadoks & Fitzpatrick 2009). In herds which controlled contagious mastitis, as evidenced by low SCC, environmental pathogens were generally responsible for high frequencies of IMI and clinical mastitis (Hogan *et al.* 1989). Herds with a high proportion of low individual SCC may have a higher risk for clinical mastitis (Bradley *et al.* 2007), probably due to the decreased immune stimulating effect of minor pathogens in the udder (Beaudeau *et al.* 2002).

A retrospective study done by Petzer *et al.* (2009a), evaluating the trends in udder health in South African dairies during the period 1996 to 2007, showed an increase in the incidence of IMI cases caused by environmental pathogens, particularly CNS. *Streptococcus uberis* showed an increase in frequency isolated and cultured over the eleven year study period. In this study it was noticed that the incidence of the major contagious bacteria was lower than that of the environmental bacteria. The cow milk samples were received from clients of the OML (Petzer *et al.* 2009a). A further increase in incidence of environmental IMI is evident in data collected from cow milk samples cultured between 2008 and 2012 (Petzer 2012, unpublished data).

Similar trends, with an increase in environmental mastitis, was noted in dairies in England with *E. coli* and *Str. uberis* being the major bacteria, New Zealand with *Str. uberis* the major contributing bacteria and New South Wales (Australia) with *E. coli* accounting for the major portion of mastitis cases (Bradley *et al.* 2007, Compton *et al.* 2007, Shum *et al.* 2009, Zadoks & Fitzpatrick 2009) .

Pasture-based dairies

Pasture-based dairies have only been in South Africa over the last twenty years. The impact of this system on udder IMI is not yet fully understood. It needs to be taken into account that

13

about 80% of all milk is currently produced in the coastal regions of South Africa with the majority of the dairies making use of pastures (Lactodata, 2015). It needs to be realised that dairies are increasingly under economic pressure, with farmers making use of slurry to fertilize their pastures. The potential impact of this practice on IMI has not been investigated in South Africa.

Total mixed ration based dairies

Housing of cows inside, all year round, in a zero grazing system was associated with an increased prevalence of *E. coli* compared to *S. aureus* in a study by Schukken *et al.* (1991), that identified and compared risk factors for *E. coli* and *S. aureus* in dairy herds with low SCC in the USA.

As a result of the accumulation of *Str. uberis* in straw bedding (Hogan & Smith 2012, Smith & Hogan 1993), it is not uncommon to have outbreaks due to these pathogens in cows housed in stalls or concentrated in small paddocks (Dodd 2003).

Conclusion

It is clear from the literature study that the prevalence of mastitogenic pathogens is not constant and it changes over time. Risk factors, including weather patterns and dairy management practices, have either an increasing or decreasing effect on the prevalence of mastitogenic pathogens. We realise that the prevalence of mastitogenic pathogens is changing in South Africa, as shown by Petzer *et al.* (2009a). There are gaps in the knowledge to what the prevalence of mastitogenic pathogens is in South Africa over the last 7 years. We are interested to know what the prevalence of *Str. uberis* is, since it is known that farmers spray slurry (liquid manure) to fertilize pasture and it was shown that *Str. uberis* is an environmental pathogen and found in manure. Although we will gain knowledge of whether there is a change in the prevalence of *Str. uberis* or not, it does not proof that the spraying of manure on pastures is a risk factor for the observed prevalence of these bacteria.

14

Having a better understanding of the bacterial isolation patterns and their prevalence in TMR and pasture-based dairy herds over an extended time period will aid to focus future research on environmental and contagious IMI in different dairy management systems.

15

OBJECTIVES OF THE STUDY

The objectives of the study are:

1.   To use retrospective culture results from composite cow milk samples, submitted to the OML during the years 2008 and 2013, to estimate the prevalence of IMI as a result of the following mastitogenic pathogens: *S. aureus, Str. uberis, Str. agalactiae,* CNS*, Str. dysgalactiae, Enterococcus faecalis,* other minor pathogens including Gram negative bacteria and pathogens contributing to contamination

2.   To compare the prevalence of the different mastitogenic pathogens isolated from both TMR and pasture-based dairies during the years 2008 and 2013.

3.   To estimate the within-herd prevalence of *Str. uberis* in TMR and pasture-based dairies during the years 2008 and 2013.

4.   To compare the within-herd prevalence of *Str. uberis* in *Str. uberis* positive TMR and pasture-based dairies during the years 2008 and 2013.

16

HYPOTHESES

H1$_0$:   The prevalence of specified mastitogenic pathogens isolated from composite cow milk samples did not differ:

    i.   Between TMR and pasture-based dairies within each of the two years,

    ii.   Between 2008 and 2013, both overall and within each of the two dairy systems.

H1$_A$:   The prevalence of specified mastitogenic pathogens isolated from composite cow milk samples will differ:

    i.   Between TMR and pasture-based dairies within each of the two years,

    ii.   Between 2008 and 2013, both overall and within each of the two dairy systems.

H2$_0$:   The within-herd prevalence of *Str. uberis* in *Str. uberis* positive herds will not differ:

    i.   Between TMR and pasture-based dairies within each of the two years,

    ii.   Between 2008 and 2013, both overall and within each of the two dairy systems

H2$_A$:   The within-herd prevalence of *Str. uberis* in *Str. uberis* positive herds will differ:

    i.   Between TMR and pasture-based dairies within each of the two years,

    ii.   Between 2008 and 2013, both overall and within each of the two dairy systems.

MATERIALS AND METHODS

Study design

This was a retrospective cross-sectional study on available composite cow milk sample bacterial culture results from dairy farms that were submitted during 2008 and 2013.

Study population

The study population was defined as all lactating dairy cows from the dairies that submitted composite cow milk samples to OML during 2008 and 2013.

Differentiation was made between TMR and pasture-based dairies. A TMR dairy was defined as a dairy where the main feeding system was based on a complete mixed ration fed to the cows. This system could be either free stall barns or open outside paddocks; there was no differentiation made between the two. A pasture-based dairy was defined as a dairy where the main feeding system was pasture-based. Within this system lactating cows might have received additional feed sources during the dry season, such as silage.

Composite cow milk samples from lactating cows were submitted for microbial culture and identification, as well as SSC determination. The microbial cultures were done for routine udder health monitoring within the herds. The samples were received from dairies situated across South Africa, with more than 50% from Kwa-Zulu Natal, Eastern Cape and Western Cape provinces. Approximately 81% of the total milk in South Africa is produced in these three provinces (Lactodata, 2015).

The composite cow milk samples were collected by trained animal technicians, referring veterinarians and farmers. Each sample was identified with the corresponding cow number. A composite cow milk sample is collected aseptically from foremilk of the four quarters of the same cow. This is done by collecting approximate equal volumes of milk from each of the

18

quarters into a single sterile sample tube (Lam *et al.* 1996). The samples were then transported on ice to reach the OML within 48 hours (Petzer *et al.* 2009a).

University of Pretoria, Faculty of Veterinary Science, Onderstepoort Milk Laboratory is situated north of Pretoria (S25°38'57.34"; E28°10'47.98", 1,230 m above sea level).

The OML has over 950 registered clients, which includes referring veterinarians. A total number of 46,067 and 130,870 cow milk samples (includes quarter - and composite cow milk samples) were received during 2008 and 2013 respectively. The OML received milk samples from 94 and 121 dairy producers during 2008 and 2013 respectively. There are 1,834 registered dairy producers in South Africa with an average number of 353 cows in milk per producer (Lactodata, 2015).

Study procedures

LABORATORY CULTURE OF COMPOSITE COW MILK SAMPLES

This part of the study was done in 2008 and 2013 by trained laboratory staff from the OML, a Department of Agriculture, Forestry and Fisheries approved laboratory.

Milk was plated out on bovine blood tryptose agar (BTA) (Oxoid, supplied by Quantum Biotechnologies (Pty) Ltd, Ferndale, South Africa). Inoculated agar plates were incubated at 37 $\pm$1 ^{o}C and plates were read after 18 to 24 hours and 48 hours. In addition all samples from clinical mastitis cases were enriched and re-cultured. Isolated bacteria were identified in accordance with standard laboratory milk culture methodology based on colony morphology as described by the IDF Document 132. Tests used included Staphylase, Strepkit, Catalase, DNase, KOH (Oxoid, supplied by Quantum Biotechnologies (Pty) Ltd, Ferndale, South Africa), Maltose (Merck NT Laboratory Supplies, Halfway House, South Africa) and API 20E and Staph API (Biomerieux South Africa (Pty) Ltd, Randburg, South Africa).

19

DATA TRANSFER

Milk recording data from 2008 and 2013 were transferred in a .csv file from the OML MSD®

program to a Microsoft Excel ® spreadsheet. Data consisted of the following variables:

    i.   Producer Code – specific numerical farm code for each producer.

    ii.   Date cultured.

    iii.   Bacteria cultured.

    iv.   Management system – TMR or pasture-based (This section of the data was not complete –See: *ADDITIONAL DATA COLLECTION*).

    v.   Cow number

DATA EXCLUSIONS

Data exclusion steps were included and the final dataset consisted of 16,415 and 45,815 composite cow milk sample data captured during 2008 and 2013 respectively from different milk producers across South Africa.

Dairies that were not known to be either TMR or pasture-based dairies and dairies that had both management systems were excluded from the study. However, a few farms were pasture-based with additional supplementation of silage only during the drier times of the year. These were included in the study as pasture-based dairies, as most of the cow's time was spent on pasture.

Composite cow milk samples from lactating cows, submitted from complete herds for udder health investigations and routine culture were used in the study. The average herd size for the dairies was not known, it was taken as the average of the consecutive number of milk samples submitted by a producer. Farms might have re-submitted a smaller number of milk samples (partial herd test) from the same cows to follow-up on a previous investigation of mastitis cases or mastitis outbreaks. These partial herd test results were excluded from the study to reduce the bias towards a specific bacteria cultured during a mastitis outbreak in a

herd. This was done by comparing the number of submitted samples on consecutive culture dates for the same producer. If a batch of samples was far less that the average herd size (i.e. 100 milk samples vs. 350 milk samples), it was taken that the batch with the lesser number of submitted samples was a possible partial herd test. If a herd was tested more than once during a year, one of the herd tests were selected randomly; the other samples were excluded from the dataset. Producers or veterinarians that submitted less than 50 cow milk samples were excluded, to reduce the potential risk of selecting a partial herd test, as these were usually to investigate specific mastitis outbreaks in herds. Partial herd tests were avoided as far as possible, but due to the data being retrospective and many farmers sold their dairies in the last few years, not all the farm information could be gathered.

ADDITIONAL DATA COLLECTION

Additional farm data, where it was not known whether a dairy uses a TMR or pasture-based dairy, was collected telephonically or via e-mail from the appropriate farm managers or technical assistants.

Data analysis

The prevalence of each specified bacteria was calculated, overall and by year and system, with exact 95% confidence intervals.

The specified bacteria that were evaluated were:

Staphylococcus aureus

Suspect human *Staphylococcus aureus*

Streptococcus uberis

Streptococcus agalactiae

Coagulase-negative *staphylococci*

Enterococcus faecalis

Streptococcus dysgalactiae

21

Two additional groups of bacteria were included:

OTHER: This included all the other minor bacteria and gram negative bacteria grouped together.

Contaminant bacteria (CU): This is regarded as samples where more than two different bacteria were cultured from the sample. It was included in the study as these contaminant bacteria have the potential to cause mastitis or might already be from a mastitic quarter. Knowledge of the prevalence of contamination is important for the veterinarian to realize potential problems during sample collection or break in the cold chain during sample transport. Contamination as a group was included in various other studies that evaluated the prevalence of bacteria in different dairies (Petrovski *et al.* 2009, Petrovski *et al.* 2011, Pitkala *et al.* 2004).

The prevalence of each specified bacteria was compared using Fisher's exact test:

  i.   for TMR dairies between 2008 and 2013
  ii.   for pasture-based dairies between 2008 and 2013
  iii. between TMR and pasture-based dairies for 2008
  iv.   between TMR and pasture-based dairies for 2013

For each of the specified bacteria a multiple logistic regression model was used to estimate the association of year (2008 vs 2013) and system (TMR vs pasture-based) with the odds of culturing the organism, while adjusting for confounding. Herd was included as a random effect. The interaction between year and system was also assessed.

For *Str. uberis*-positive herds, the within-herd prevalence of *Str. uberis* was compared using a two sample *t*-test.

  i.   for TMR dairies between 2008 and 2013
  ii.   for pasture-based dairies between 2008 and 2013

22

iii.   between TMR and pasture-based dairies for 2008

iv.   between TMR and pasture-based dairies for 2013

The effect of year (2008 vs 2013) and system (TMR vs pasture-based) on the within-herd prevalence of *Str. uberis* was estimated using multivariable Poisson regression. The outcome variable was the number of positive cultures of *Str. uberis*, and the total number of samples tested was included as an exposure variable. Herd was included as a random effect. The interaction between year and system was also assessed.

Statistical significance was assessed at p<0.05. Statistical analyses were done using Stata 12.1 (StataCorp, College Station, TX).

Ethical approval

This study was approved by the University of Pretoria's Animal Ethics Committee (Protocol No: V015-15)

23

RESULTS

Prevalence of specified mastitogenic pathogens

Composite cow milk samples cultured for mastitogenic pathogens were used in the study. These samples were cultured during 2008 and 2013. Dairies were grouped into TMR and pasture-based dairies.

In total 62,230 (2008: 16,415 and 2013: 45,815) cultured cow milk samples (n) from 123 herds (N) were used in this study, 83 herds from pasture-based dairies and 40 herds from TMR dairies. Within each year-system group, TMR 2008 (n=12,269), pasture-based 2008 (n=4,146), TMR 2013 (n=7,752), pasture-based 2013 (n=38,063) the prevalence of each mastitogenic pathogen, with a 95% confidence interval was calculated (Table 1). Prevalences that were significantly different (p <0.05) are indicated in Table 1.

Streptococcus uberis showed a statistically significant increase in prevalence in both TMR (p=0.002) and pasture-based dairies (p=0.001) from 2008 to 2013. In 2013 the prevalence of *Str. uberis* was higher (p= 0.018) in pasture-based dairies compared to TMR dairies.

Staphylococcus aureus showed a statistically significant decrease (p =0.011) in prevalence in TMR dairies from 2008 to 2013. A statistically significant decrease (p<0.001) in pasture-based dairies from 2008 to 2013 was shown. In 2008 the prevalence of *S. aureus* was higher (p=0.023) in pasture-based dairies compared to TMR dairies, while the prevalence of *S. aureus* in 2013 was higher (p<0.001) in TMR dairies compared to pasture-based dairies.

Suspect human *Staphylococcus aureus* showed a decrease in prevalence in both TMR (p< 0.001) and pasture-based (p<0.001) dairies from 2008 to 2013. The prevalence for STH was higher in pasture-based dairies compared to TMR dairies in 2008 (p< 0.001).

24

Table 1: Prevalence of mastitogenic pathogens grouped by year (2008 and 2013) and system (Total mix ration (TMR) and pasture-based (PAS)).

Pathogen[**]	2008		2013	
	TMR	PAS[#]	TMR	PAS[#]
	Prevalence (%) (95% CI)	Prevalence (%) (95% CI)	Prevalence (%) (95% CI)	Prevalence (%) (95% CI)
	n=12269	n=4146	n=7752	n=38063
SDY	1.12 (0.94-1.32)	0.80 (0.55-1.12)	1.25 (1.02-1.52)[†]	0.64 (0.56-0.72)[†]
SUB	2.36 (2.10-2.65)[*]	2.63 (2.16-3.16)[*]	3.10 (2.72-3.51)[*†]	3.64 (3.45-3.83)[*†]
STA	4.71 (4.34-5.10)[*†]	5.62 (4.94-6.36)[*†]	3.95 (3.52-4.40)[*†]	1.71 (1.58-1.84)[*†]
STH	0.79 (0.64-0.96)[*†]	2.87 (2.38-3.42)[*†]	0.03 (0.00-0.09)[*]	0.09 (0.06-0.13)[*]
SAG	1.01 (0.84-1.20)[*†]	1.62 (1.25-2.05)[*†]	8.02 (7.43-8.65)[*†]	0.76 (0.67-0.85)[*†]
CNS	29.6 (28.8-30.4)[*†]	26.9 (25.5-28.3)[*†]	20.2 (19.3-21.1)[*†]	22.7 (22.2-23.1)[*†]
SFA	0.90 (0.74-1.08)[*]	0.75 (0.51-1.06)[*]	0.37 (0.25-0.54)[*†]	0.14 (0.10-0.18)[*†]
CU	13.7 (13.1-14.4)[*]	13.2 (12.1-14.2)[*]	16.5 (15.6-17.3)[*†]	4.90 (4.69-5.12)[*†]
OTHER	3.11 (2.81-3.43)[*]	3.59 (3.05-4.21)[*]	2.39 (2.06-2.75)[*†]	1.40 (1.28-1.52)[*†]
IMI	57.4 (56.5-58.2)[*]	57.9 (56.4-59.4)[*]	55.7 (54.6-56.8)[*†]	35.9 (35.4-36.4)[*†]

[*] Comparing similar systems in different years with $p < 0.05$.

[†] Comparing different systems in the same year with $p < 0.05$

[#] Pasture-based dairies

[**]Pathogens: SDY: *Streptococcus dysgalactiae*, SUB: *Streptococcus uberis*, STA: *Staphylococcus aureus*, STH: Suspect human *Staphylococcus aureus*, SAG: *Streptococcus agalactiae*, CNS: Coagulase-negative s*taphylococci*, SFA: *Enterococcus faecalis*, CU: Contamination, OTHER: Minor pathogens including gram negative bacteria, IMI: Intramammary infection.

Streptococcus agalactiae showed an increase ($p < 0.001$) in prevalence in TMR dairies from 2008 to 2013, with a decrease ($p < 0.001$) in pasture-based dairies during the same period. The prevalence of *Str. agalactiae* in TMR and pasture-based dairies differed from each other during 2008 and 2013 ($p < 0.001$) with a higher prevalence in pasture-based dairies in 2008 and higher ($p = 0.002$) prevalence in TMR dairies in 2013.

Coagulase-negative s*taphylococci* showed the highest prevalence of all the pathogens, in all four the year-system groups. There was a decrease ($p < 0.001$) in the prevalence in both TMR and pasture-based dairies from 2008 to 2013. The prevalence in TMR and pasture-

based dairies differed from each other in both 2008 and 2013; the prevalence in TMR dairies was higher (p=0.001) in 2008 and lower (p<0.001) in 2013.

The CU group cultured the second highest in all the year-system groups, with the lowest prevalence of this group in 2013 in pasture-based dairies.

The IMI prevalence (which includes CU and OTHER) was also calculated for each year-system group (Table 1). The prevalence of IMI decreased significantly in both TMR (p=0.023) and pasture-based (p<0.001) dairies from 2008 to 2013. The prevalence of IMI was significantly lower (p <0.001) in pasture-based dairies compared to TMR dairies in 2013.

The prevalence of each pathogen was compared between years using multiple logistic regression models to estimate the odds ratio (OR) for 2013 compared to 2008, adjusting for system, with herd as a random effect (Table 2). *Streptococcus uberis*, *Str. agalactiae* and CU showed an increase in prevalence in 2013 compared to 2008 (OR>1). On the other hand, *S. aureus*, suspect human *S. aureus,* suspect human *S. aureus*, CNS, *Enterococcus faecalis,* OTHER and the prevalence of IMI were significantly lower in 2013 (OR<1).

**Table 2: Comparing prevalence for 2008 and 2013 after adjusting for system using multiple logistic regression.**

Pathogen[**]	OR[*]	95% CI	*P*-value
SDY	1.08	0.79-1.48	0.610
SUB	1.79	1.38-2.33	<0.001
STA	0.52	0.46-0.59	<0.001
STH	0.03	0.01-0.08	<0.001
SAG	5.55	3.70-8.31	<0.001
CNS	0.68	0.62-0.75	<0.001
SFA	0.41	0.25-0.69	0.001
CU	1.24	1.09-1.41	0.001
OTHER	0.82	0.66-1.03	0.091
IMI	0.71	0.65-0.78	<0.001

* Odds ratio for 2013 vs. 2008

[**]Pathogens: SDY: *Streptococcus dysgalactiae*, SUB: *Streptococcus uberis*, STA: *Staphylococcus aureus*, STH: Human *Staphylococcus aureus,* SAG: *Streptococcus agalactiae*, CNS: Coagulase-negative *Staphylococcus* spp., SFA: *Enterococcus faecalis*, CU: Contamination, OTHER: Minor pathogens including gram negative bacteria, IMI: Intramammary infection.

Within herd prevalence of *Streptococcus uberis*

A total of 43/48 herds in 2008 (89.6%; 95% CI: 77.3-96.5%) and 83/87 herds in 2013 (95.4%; 95% CI: 88.6-98.7%) contained at least one cow that cultured positive for *Str. uberis*. Twelve herds submitted samples in both 2008 and 2013 and of these 10 herds tested positive for *Str. uberis* in both years. For *Str. uberis*-positive herds, the distribution of the within-herd prevalence for each year is shown in the histograms (Figure 1).
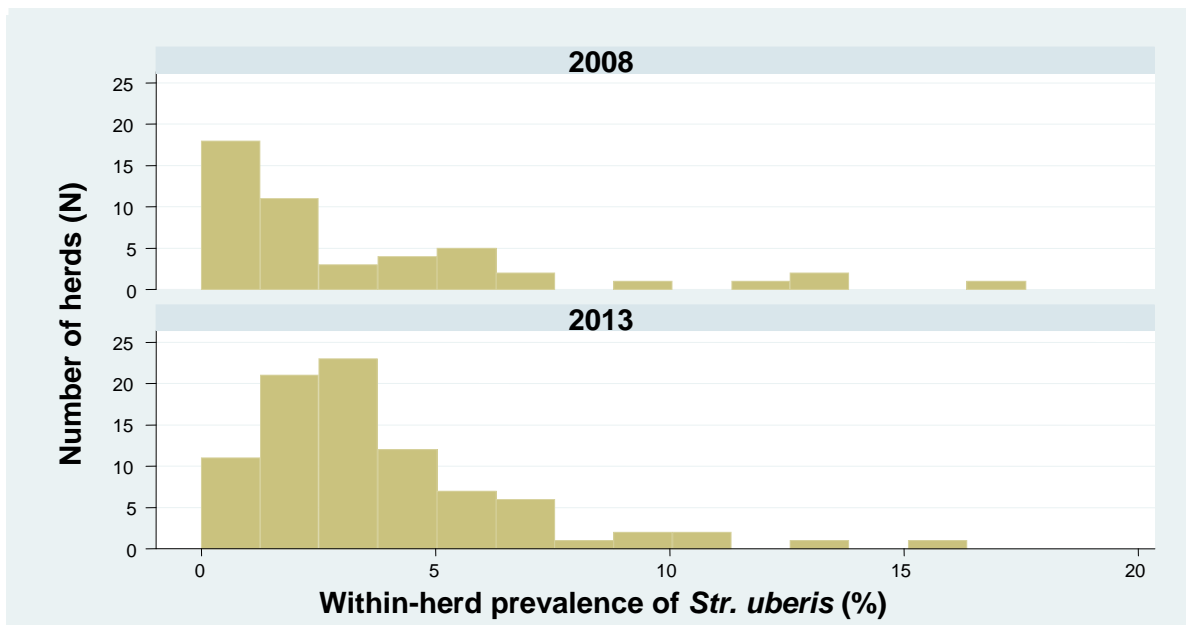
Figure 1: Distribution of the within-herd prevalence (%) of *Str. uberis* in 2008 and 2013.

The median within-herd prevalence of *Str. uberis* for the combined dairy systems in 2008 and 2013 was 1.72% and 3.10%, respectively (Table 3). The count ratio (CR) for 2013 relative to 2008, obtained from the Poisson regression model, adjusted for system, was 1.75 (95% CI=1.35-2.26), indicating a significant difference between the two years (*p*<0.001). The interaction between year and system was not statistically significant (*p*=0.181), and was therefore not included in the model.

Table 3: Comparison of median within-herd prevalence in *Streptococcus uberis* positive herds in 2008 (*n*=48) and 2013 (*n*=87).

2008	2013			
Median % (IQR*)	Median % (IQR*)	CR**	95 % CI	*P-value*
1.72 (0.88-5.00)	3.10 (1.72-4.70)	1.75	1.35-2.26	<0.001

* Interquartile range

** Count ratio

28

Figure 2 illustrates the distribution of within-herd prevalences of *Str. uberis* in the four different year-system groups. It is noted that the distribution of all four the graphs is right skewed, with pasture-based 2013 having the highest concentration of herds ($n \approx 20$) around 3% within-herd prevalence for *Str. uberis*.
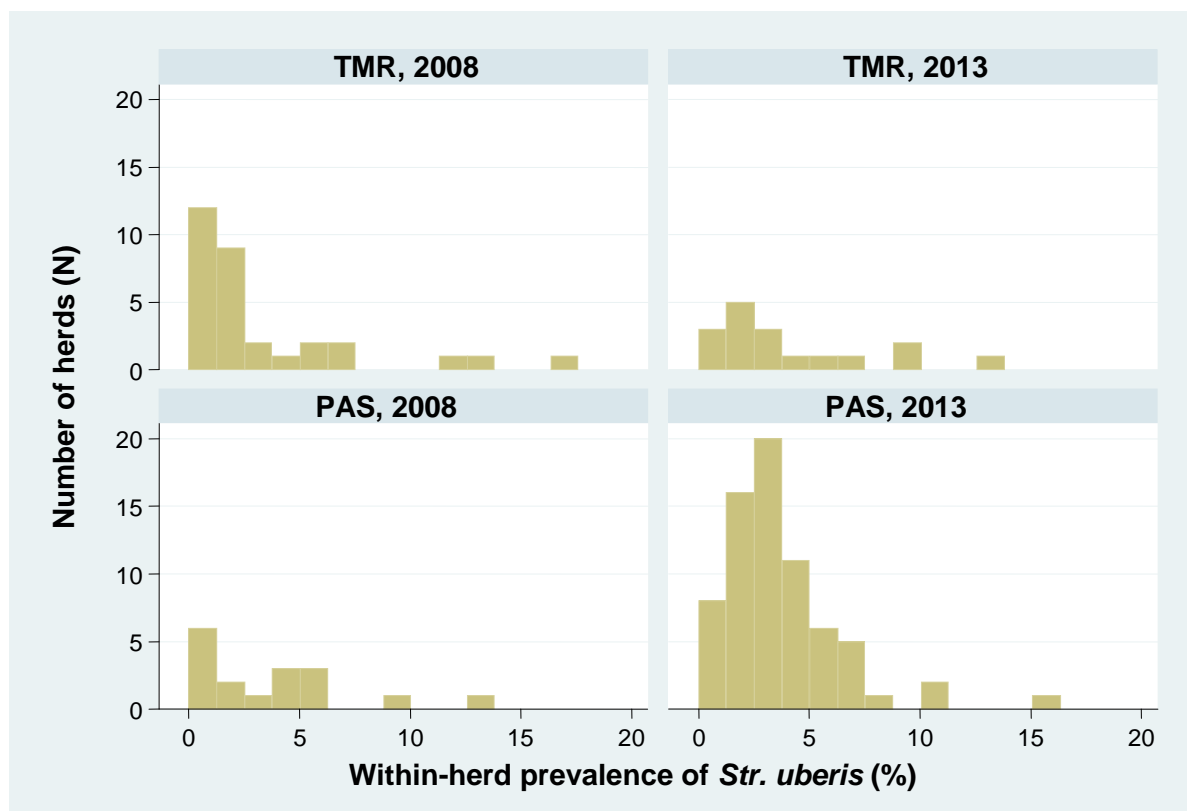
Figure 2: Distribution of the within-herd prevalence of *Str. uberis* (%) for TMR 2008, TMR 2013, pasture-based 2008 and pasture-based 2013.

Despite the lack of statistical evidence for interaction between system and year, in order to further investigate differences, separate Poisson regression models were used within each year to compare the within-herd prevalence of *Str. uberis* between pasture-based and TMR dairies (Table 4). The prevalence was slightly higher in the pasture-based dairies than the TMR dairies during both years; however, the difference was statistically significant only in 2013, likely partly due to the larger sample size.

29

Table 4: Comparison of the median within-herd prevalence in *Streptococcus uberis* positive herds in different systems within the same year.

Year	PAS[***] Median% (IQR[*])	N[#]	TMR Median% (IQR[*])	N	CR[**]	95 % CI	*P-value*
2008	2.57 (0.99, 5.31)	17	1.55 (0.69, 4.17)	31	1.11	0.89-1.39	0.34
2013	3.35 (1.78, 4.59)	70	2.59 (1.37, 6.02)	17	1.17	1.02-1.35	0.02

[*] Interquartile range

[**] Count Ratio for PAS vs TMR, estimated using Poisson regression

[***] Pasture-based dairies

[#] Number of herds

Similarly, separate Poisson regression models were used within each dairy system to compare the within-herd prevalence of *Str. uberis* between years (Table 5). Although there was an increase in prevalence from 2008 to 2013 in both dairy systems, it was larger, and statistically significant, in the TMR dairies.

Table 5: Comparison of the median within-herd prevalence of *Streptococcus uberis* positive herds in similar dairy systems within different years

System	2008 Median% (IQR[*])	N[#]	2013 Median%(IQR[*])	N	CR**	95 % CI	*P*
TMR	1.55 (0.69, 4.17)	31	2.59 (1.37, 6.02)	17	2.13	1.50-3.03	<0.001
PAS[***]	2.57 (0.99, 5.31)	17	3.35 (1.78, 4.59)	70	1.38	0.94-2.01	0.10

* Interquartile range

** Count Ratio for PAS vs. TMR, estimated using Poisson regression

*** Pasture-based dairies

[#] Number of herds

DISCUSSION

Prevalence of mastitogenic pathogens

Changing trends in the prevalence of contagious and environmental pathogens, as a cause of mastitis in dairy herds, have been noticed over the last few decades worldwide (Bradley *et al.* 2007, Leigh 1999, Shum *et al.* 2009, Smith 1983).   In countries worldwide there are various mastitogenic pathogens that are recognised as being the most prevalent within that country: England, *E. coli* and *Str. uberis*, New Zealand, *Str. uberis* and New South Wales (Australia), *E. coli* (Bradley *et al.* 2007, Compton *et al.* 2007, Shum *et al.* 2009, Zadoks & Fitzpatrick 2009). According to Petzer *et al.* (2009a), CNS is the most prevalent bacteria in South Africa. Coagulase-negative *staphylococci* were isolated from 60.96% of 112,715 milk samples.   *Streptococcus uberis* emerged as a relative important environmental pathogen during this period, isolated from 2.25% of these samples. The contagious bacteria group (*S. aureus*, *Str. agalactiae* and *Str. dysgalactiae*) was isolated from 25.17% of these samples.

In the current study the prevalence of mastitogenic pathogens in composite cow milk samples during 2008 and 2013 in TMR and pasture-based dairies was investigated. This was the first study in Southern Africa that compared the prevalence of mastitogenic bacteria, grouped into four different year-system groups. In the study done by Petzer *et al.* (2009a) the prevalence of mastitogenic pathogens was determined from all quarter and composite cow milk samples submitted for identification of mastitogenic pathogens (including partial herd tests). In the current study the prevalence of the pathogens was determined from single routine complete herd batches of composite cow milk samples submitted by dairies.

As a result of the unknown herd sizes, it was not possible in all cases to identify whether a dairy submitted samples from a complete herd or not. Combining prevalence results from the study done by Petzer *et al.* (2009a) with the current study, to evaluate a larger timeframe of bacterial trends in the South African dairies, should be done with caution. It should be

31

noted that partial herd tests and quarter milk samples were excluded from the current study, whereas the study of Petzer *et al.* (2009a) included all samples (including partial herd test). As mentioned before partial herd tests, might include the investigation of a specific outbreak of mastitis and this might over represent the prevalence of that specific pathogen.

Environmental pathogens

The prevalence of *Str. uberis* is increasing as noticed during 2008 and 2013. This is similar to the findings of Petzer *et al.* (2009a) that concluded that *Str. uberis* increased in prevalence from 1996 to 2007. From our current study it was noticed that the major increase in *Str. uberis* prevalence occurred in pasture-based herds in 2013. Although no specific risk factors have been studied, it would be worthwhile investigating what the effect of increased spreading of manure (slurry) on pastures has on the prevalence of *Str. uberis,* since this practice has been favored in the last few years in pasture-based dairies. In a study done by Petrovski *et al.* (2009) in New Zealand, mostly pasture-based dairies, *S. aureus* was isolated from 23.7% and *Str. uberis* was isolated from 23.3% of all clinical mastitis cases (data July 2005 – May 2006, Northland region). Petrovski *et al.* (2011) did a similar study comparing culture results from all milk samples submitted to five laboratories in New Zealand from August 2003 to December 2006 and the findings were concurrent with the previous study, with *Str. uberis* (23,6%) and S. *aureus* (23,5%) the two bacteria most commonly isolated. These findings from the aforementioned studies by Petrovski *et al.* indicated the importance of *Str. uberis* in pasture-based dairies in New Zealand, as a result of an increasing prevalence of *Str. uberis* in pasture-based dairies; this should be taken note of in South African pasture-based dairies in future, because of the different control measures that need to be implemented for environmental pathogens (Smith 1983, Smith & Hogan 1993).

Coagulase-negative s*taphylococci* are the most prevalent group of bacteria in the current study. This is in agreement with the data published by Petzer *et al.* (2009a). Work that was done in Finland showed similar results, with CNS having the highest prevalence (Pitkala *et*

32

al. 2004, Pyorala &Taponen 2009). In a Norwegian study, CNS (3.3%) and *S. aureus* (8.2%) were the two major bacteria cultured (Østerås, Sølverød & Reksen 2006). A recent review by Vanderhaeghen *et al.* (2015), discussed the different CNS species commonly causing IMI. They indicated that molecular identification methods are very important to understand the epidemiology of CNS, since *S. epidermides* and *S. haemolyticus* are common human adapted pathogens. The other species have different environmental sources. In none of the studies done in South Africa (current and Petzer *et al.* 2009a) was any molecular identification methods used to differentiate between different CNS species. As one of the most prevalent bacteria in South African dairies the next step would be to utilise molecular identification methods to identify the most common CNS species and furthermore study its associated epidemiology and potential risk factors, to understand why it is such a prevalent pathogen in South African dairies.

Contagious pathogens

Noted by Petzer *et al.* (2009a), *S. aureus* is still one of the major concerning pathogens in South Africa when referring to mastitis, due to its chronicity as mastitogenic pathogen. In the current study *S. aureus* showed slight decline in prevalence during 2008 and 2013. Specific reasons for this decline could not be identified. Bradley (2002) concluded that a decline in the prevalence of contagious pathogens, in the UK, was seen over a forty year period. A possible reason for this decrease in prevalence of contagious pathogens can be ascribed to improved control methods specifically targeting the contagious pathogens. In contrast to the studies by Petzer *et al.* (2009a), Bradley (2002) and the current study, *S. aureus* is still the major mastitogenic pathogen found in other countries. In a Norwegian study *S. aureus* was the most prevalent pathogen, cultured from 8.2% of all samples (Østerås, Sølverød & Reksen 2006).

Streptococcus agalactiae showed a marked increase in prevalence in TMR dairies in 2013. This is in agreement with Petzer *et al.* (2009a) that also showed marked increases in

33

prevalence in 2001 and 2003. These peaks were ascribed to large outbreaks in *Str. agalactiae* mastitis cases. Although this is difficult to prove on the current data, it is also suggested that the peak seen in 2013 in TMR dairies can be due to several outbreaks of *Str. agalactiae* in South African dairies (Petzer 2015, personal communication). *Streptococcus agalactiae* is a highly contagious obligate intramammary pathogen and is one of the major causes of subclinical mastitis with a potential high cure rate after antimicrobial therapy and well managed sanitary procedures (Keefe 2012, Smith & Ward 1975). As a result farms become free from *Str. agalactiae* (Andersen *et al.* 2003, Barkema *et al.* 2009) with a subsequent decrease in prevalence of *Str. agalactiae*. However, if the on-farm biosecurity is lacking on a farm and newly purchased infected cows are introduced onto the farm, rapid spread of IMI due to *Str. agalactiae* is possible (Barkema *et al.* 2009).

Changing trends in mastitis and pathogen prevalence

In the current study and that of Petzer *et al.* (2009a) it is noted that there is a difference in the prevalence of environmental and contagious pathogens. In both these studies the prevalence of the environmental pathogens are higher than that of the contagious pathogens. In pasture-based dairies during 2013, CNS and *Str. uberis*, both environmental pathogens had the highest and second highest prevalence respectively. Literature earlier that 1996, comparing the difference in prevalence between contagious and environmental pathogens is not readily available for South Africa. The changing trends resulting in a decrease in contagious mastitogenic pathogens and increase in environmental pathogens have been noted in other studies and reviews (Bradley 2002, Smith 1983). Contagious IMI are usually recognised by high SCC in the milk (Keefe 2012). Improved control of contagious pathogens, potentially leave a gap to be filled by the previously minor groups of environmental pathogens, as seen by the increased prevalence of environmental mastitogenic pathogens in low SCC herds (Bradley 2002, Hogan *et al.* 1989). Whether the minor pathogens play a role in a protective competitive manner with the major pathogens still needs to be investigated. Future challenges will be to continue with contagious pathogen

34

control strategies, but also to focus on the management and control of emerging environmental pathogens (Bradley 2002, Smith 1983, Zadoks & Fitzpatrick 2009).

Within-herd prevalence of *Streptococcus uberis*

No studies have been done in South Africa to investigate the within-herd prevalence of *Str. uberis.* The study done by Petzer *et al.* (2009a) indicated that the prevalence of *Str. uberis* over an eleven year period increased. The results from the current study have shown a similar outcome. Firstly the prevalence of *Str. uberis* is higher for 2013 compared to 2008, as previously discussed. Secondly the median within-herd prevalence of *Str. uberis* is higher for 2013 compared to 2008 ($p<0.001$). From the aforementioned observations it can be deduced that *Str. uberis* increased in prevalence, which is in agreement with the study done by Petzer *et al.* (2009a). Care should be taken when interpreting the within-herd prevalence results, as it should be noted that the lower number of *Str. uberis* positive farms (dairy which cultured at least one positive *Str. uberis* sample) for pasture-based dairies in 2008 and TMR dairies in 2013 might be a limitation in the current study. Therefore it is not recommended to use any inferences, made from the within-herd prevalence of *Str. uberis* in the current study, on a national scale.

Limitations of the study

This study was conducted using samples submitted to the OML, and was therefore not based on random sampling from the entire dairy population. This could have resulted in several sources of selection bias which may have influenced the prevalence estimates.

Although routine whole herd sampling (lactating group) was done, the possibility exists that dairy farms that had previous outbreaks of mastitis, as a result of a particular pathogen, subsequently decided to regularly submit routine whole herd milk samples to monitor udder health in the herd. These samples would not have been excluded as a partial herd test as the whole lactating group was tested. Not all dairies were involved in routine udder health management programs and therefore did not submit routine samples on a regular basis;

these dairies would then have been excluded from the study, subsequently resulting in selection of dairies that were interested in routine udder health programs (improved udder health status). This may have excluded dairies that had mastitis problems at that stage.

Although the methods for culturing and identifying the bacteria in the laboratory stayed relatively constant from 2008 to 2013, other procedures such as collection methods, personnel collecting the samples and travel distances (time) differed during the two years. This may have resulted in slightly different sensitivities for detecting pathogens between the two years, although this is unlikely to have significant biased the results.

The small sample sizes in some comparison groups likely resulted in reduced statistical power to detect differences. This might have been the case when the within-herd prevalence of *Str. uberis* was compared between TMR and pasture-based dairies during 2008 and 2013. It was noted that there were only 17 pasture-based dairies in 2008 and 17 TMR dairies in 2013 used in the study. This may have resulted in decreased power of the statistical testing in the study; taking into consideration that there are over 1800 registered dairy farmers in South Africa. In the case of only 17 pasture-based and TMR dairies, it may be that farms with extremely high or low within-herd prevalence of *Str. uberis* were over represented, resulting in biased results.

The total population of TMR and pasture-based dairies in 2008 and 2013 is not known, therefore it is not known whether they were proportionally represented in the samples used; if not, this could have resulted in bias in the estimate of the overall prevalence.

CONCLUSION

One of the objectives of the study was to determine the prevalence of specified mastitogenic pathogens in TMR and pasture-based dairies during 2008 and 2013. Although statistically significant differences in prevalence of the pathogens were shown between the different management systems and years, it should be appreciated, that as a result of large data sets, even small practically insignificant differences might have shown to be statistically significant. Therefore discussions were focussed on pathogens that have shown to be of major economic importance in the dairy industry worldwide.

Staphylococcus aureus and *Str. agalactiae* are considered major contagious pathogens worldwide. *Staphylococcus aureus* showed an overall decrease in prevalence during 2008 and 2013. Although no specific reason could be found for this decrease, the possibility of improved contagious bacteria mastitis control was discussed. *Streptococcus agalactiae* however showed a steep increase in prevalence during the same period and this increase can potentially be ascribed to localised outbreaks of *Str. agalactiae* mastitis nationally. Continued contagious pathogen control, management and on-farm biosecurity is important for the future reduction in the prevalence of contagious pathogens in South African dairies.

Coagulase-negative *staphylococci, Str. uberis* and *Str. dysgalactiae* are traditionally regarded as environmental pathogens. Although CNS is the most prevalent mastitogenic pathogen in both TMR and pasture-based dairies, it showed a statistically significant decrease in prevalence for both TMR and pasture-based dairies during 2008 and 2013. Similar to the study of Petzer *et al.* (2009a), *Str. uberis* showed a statistically significant increase in prevalence in the current study, with the highest prevalence of *Str. uberis* in pasture-based dairies during 2013. *Streptococcus dysgalactiae* did not show a statistically significant increase during 2008 and 2013. The only significant difference in prevalence for

37

Str. dysgalactiae was between TMR 2013 and pasture-based 2013, with TMR 2013 having the highest prevalence.

Taking the overall increase and the increased within-herd prevalence of *Str. uberis* during 2008 and 2013 into consideration it can be deduced that the prevalence of *Str. uberis* is higher than more than a decade ago. The highest overall and within-herd prevalence for *Str. uberis* was shown to be in pasture-based dairies during 2013. As discussed, various risk factors influence the prevalence of environmental pathogens in dairies and it would be worthwhile to investigate the influence that slurry spreading on pastures has on the prevalence of intramammary infections as a result of *Str. uberis* and other environmental pathogens.

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41

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