

Trends in udder health and emerging mastitogenic pathogens in South African dairy herds

I-M Petzer^{a*}, J Karzis^a, J C Watermeyer^a, T J van der Schans^a and R van Reenen^a

ABSTRACT

The aim of this study was to retrospectively analyse the results of milk samples obtained from South African dairy herds during the period 1996 to April 2007 in order to identify possible trends in isolates of microorganisms and their pathogenicity under field conditions. Milk samples were obtained from 7 of the 9 provinces in South Africa where there are low numbers of dairy cows. Although there is scientific limitation to a country wide survey, such as the variation in herd size, management skills, parity, milk yield, milking frequency and other parameters, the size of this database helps to give a fair indication of general udder health in South Africa. Cytology and routine bacteriology were performed on 379 000 milk samples of lactating cows and bacteriology on 11 946 samples from non-lactating cows. According to the results obtained, mastitis did not decrease in South Africa over the test period. The prevalence of mastitis and teat canal infection was lowest in 2002. Mastitis and teat canal infection increased from 2002 to 2006 from 8.1 % and 24.1 % to 15.4 and 30.0 % respectively. The percentage of mastitogenic pathogens isolated from cows over these years also varied. Previously unknown or almost eradicated mastitogenic pathogens such as $\alpha\beta$ haemolytic *Staphylococcus aureus* which is thought to be of human origin, *Streptococcus agalactiae* and *Enterococcus canis* were responsible for numerous mastitis outbreaks seen in the test samples. Coagulase-negative staphylococci were the most frequently isolated bacteria in milk samples from both lactating and dry cows, followed by *Staphylococcus aureus* and *Streptococcus agalactiae*. Although *Staphylococcus aureus* remained the principal mastitogenic pathogen in South Africa, owing to its chronic nature and resultant economic losses, most cases of mastitis were caused by coagulase-negative staphylococci. This finding increases the importance of coagulase-negative staphylococci (formerly described as a minor pathogen) significantly. Isolations of *Streptococcus agalactiae* peaked between 2000 and 2005 and decreased again by 2007. Coagulase-negative staphylococcal isolates increased from 2002 and were still on the increase in 2007. *Streptococcus agalactiae*, *Streptococcus uberis* and *Enterococcus canis* were isolated more frequently from milk samples of lactating cows compared with dry cows, while *Enterococcus faecalis* was isolated more frequently from dry cow samples.

Key words: emerging pathogens, mastitis prevalence, reverse zoonosis, South African dairy herds, udder health.

Petzer I-M, Karzis J, Watermeyer J C, van der Schans T J, van Reenen R **Present trends in udder health and emerging mastitogenic pathogens in South African dairy herds.** *Journal of the South African Veterinary Association* (2009) 80(1): 17–22 (En.). Department of Production Animal Studies, Section Udder Health, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, 0110 South Africa.

herds in South Africa decreased, while herd size increased. Consequently, the risks for the spread of udder pathogens increased due to amalgamation of herds and movement of cows. According to national statistics the prevalence of HIV of antenatal clinic attendees during 2006 was 29.1 % in South Africa²³. The possible effect of reverse zoonosis should not be ignored.

This retrospective study investigates trends in the bacteria isolated from milk samples as well as the prevalence of udder health from a national mastitis diagnostic service carried out over an 11-year period in South Africa.

MATERIALS AND METHODS

From 1996 to April 2007, 180 486 quarter milk samples and 198 514 composite cow milk samples of lactating cows, a total of 379 000, were submitted for diagnostic purposes to the milk laboratory of the Faculty of Veterinary Science, Onderstepoort. Milk samples were aseptically collected and submitted by producers, veterinarians and field workers within 48 hours after sample collection on ice to the laboratory. Somatic cell counting and bacteriological examination were done on all samples and testing for antimicrobial susceptibility took place. Dry cow udder secretion samples were also collected for bacteriological examination from 11 946 udder quarters and *in vitro* antimicrobial susceptibility of different mastitogenic pathogens was performed. Samples originated from herd examinations rather than individual mastitis cases. Results were analysed with the Milk Sample Diagnostic (MSD) software program developed during the same period by Abaci Systems (Onderstepoort) to assist veterinarians in analysing results for practical application. The MSD program integrates laboratory and clinical results by combining udder health parameters, clinical findings, milk production, reproduction and economics information. It provides present and historical udder health information at herd and individual cow level.

Animal populations

Samples were received from dairy herds

INTRODUCTION

Bovine mastitis remains a major cause of economic loss in dairy herds and the industry, even though great technological advances have been made over the past decade. Knowledge of mastitogenic pathogens is important. Their categorisation reflects their basic epidemiology and can guide proactive management of dairy herds. Current information about pathogens is also needed due to their changing

trends over time. The prevalence of mastitis and its pathogens has been investigated in South Africa since the late 1970s. No official national survey has, however, been done to determine the mastitis prevalence, occurrence of udder pathogens and drug-resistant bacteria. Monitoring of udder health has been promoted in South Africa to enable the establishment of meaningful goals for effective udder health herd management. The National Milk Recording Scheme helped substantially by monitoring somatic cell count, protein, milk fat and milk urea nitrogen (MUN) on an individual cow basis. During this period the number of dairy

^aDepartment of Production Animal Studies, Udder Health, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

*Author for correspondence.
E-mail: inge-marie.petzer@up.ac.za

Received: June 2008. Accepted: January 2009.

in 7 of the 9 provinces of South Africa. These herds either had mastitis outbreaks, high bulk somatic cell counts, or were being monitored proactively for good udder health maintenance in herds. Stage of lactation, parity, milk yield and breed of dairy cows varied. Herd size varied from 11 to 2400 lactating cows.

Samples

Herd examinations were either done on quarter or cow milk samples, or a combination of both. All samples from dry cows were quarter samples. Dry cow samples were collected either from cows not treated with a dry cow remedy at drying off, or from cows in the close-up groups that received dry-cow intra-mammary treatment with a 3 to 4 week residual effect at drying-off. All samples were taken in an aseptic manner⁴ and transported on ice to reach the laboratory within 48 hours after sampling.

Laboratory examinations

The laboratory investigation took place in the milk laboratory at Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria. All milk samples were visually inspected in the laboratory and were then cultured on bovine blood tryptose agar (BTA) (Columbia Blood Agar Base, CM331 from Oxoid, plus 5 % defibrinated bovine blood), which supports the growth of most mastitogenic bacterial pathogens. Inoculated agar plates were incubated at $37 \pm 1^\circ\text{C}$, and evaluated for growth²¹. Somatic cell counting was performed with a Fossomatic 90 and Fossomatic 5000 (The Rhine Ruhr Group) according to the standard method for somatic cell counting²⁹. Phage typing of *Staphylococcus aureus* isolates was performed at the South African Institute for Medical Research (Johannesburg, South Africa).

Data management

Data were entered and stored in the MSD laboratory programme and Microsoft

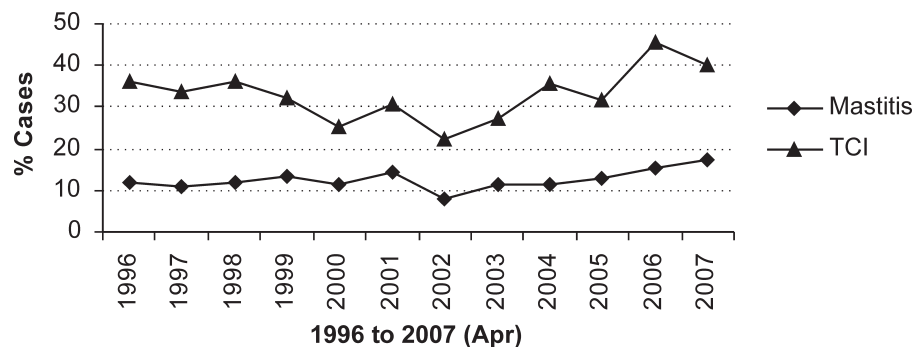


Fig. 1: Mastitis (M) and teat canal infection (TCI) in South African dairy herds.

Excel. Quarter milk samples from which pathogens were isolated and that had a somatic cell count (SCC) of equal to or above 400 000 cells/ml milk were considered positive for mastitis and those with a SCC of below 400 000 cells/ml as teat canal infections (TCI). Quarters in which no pathogens were isolated and which had a SCC of equal to or above 400 000 cells/ml were diagnosed as non-specific disturbance (NSD) and those with SCC below 400 000 cells/ml milk as normal.

RESULTS

Udder health in South African dairy herds (1996–April 2007)

Results from 180 486 quarter milk samples were investigated during this trial. The prevalence of both teat canal infection (TCI) and mastitis in South African dairy herds decreased from 1996 to 2002 (Fig. 1). The prevalence of mastitis during 2002 was 8.1 % and that of TCI 24.1 %. These percentages escalated to 15.4 % mastitis and 30 % TCI in 2006, respectively, increasing the overall infection rate in lactating cows from 32.2 % in 2002 to 45.4 % in 2006.

Bacterial isolates from lactating and dry cows

Bacteria were cultured from 3118 out of a total of 11 946 dry cow udder quarter secretion samples and from 112 715 out of a total of 379 000 lactating cow samples. Identification of bacterial isolates from the dry cow secretions revealed that

coagulase-negative staphylococci were by far the most numerous at 61.71 %, followed by *Staphylococcus aureus* (17.28 %), $\alpha\beta$ haemolytic *Staphylococcus aureus* (7.81 %), *Enterococcus faecalis* (4.49 %), *Streptococcus dysgalactiae* (2.51 %), *Streptococcus uberis* (1.21 %), *Streptococcus agalactiae* (1.21 %) and other bacteria (3.69 %) (Table 1).

Similar percentages of coagulase-negative staphylococci, *Staphylococcus aureus*, $\alpha\beta$ haemolytic *Staphylococcus aureus* (STH) and *Streptococcus dysgalactiae* were isolated from dry and lactating milk samples. Higher percentages of *Streptococcus agalactiae* and *Streptococcus uberis* were isolated from lactating cows, while *Enterococcus faecalis* was more prevalent in dry cows.

Streptococci and enterococci isolated from lactating cows

Streptococci and enterococci isolated from milk samples (quarter and cow samples) differed from one year to the next (Fig. 2). The prevalence of *Streptococcus agalactiae* was high for the period 2000–2005 and has declined since. The prevalence of *Streptococcus dysgalactiae* isolates decreased after a major peak during 2002 (Fig. 2). The *Streptococcus uberis* isolates decreased during 2000 but have since increased. *Enterococcus faecalis* isolates decreased till 2004 only to start increasing again (Fig. 2). Most *Enterococcus faecalis* were isolated during 2001 and declined since then.

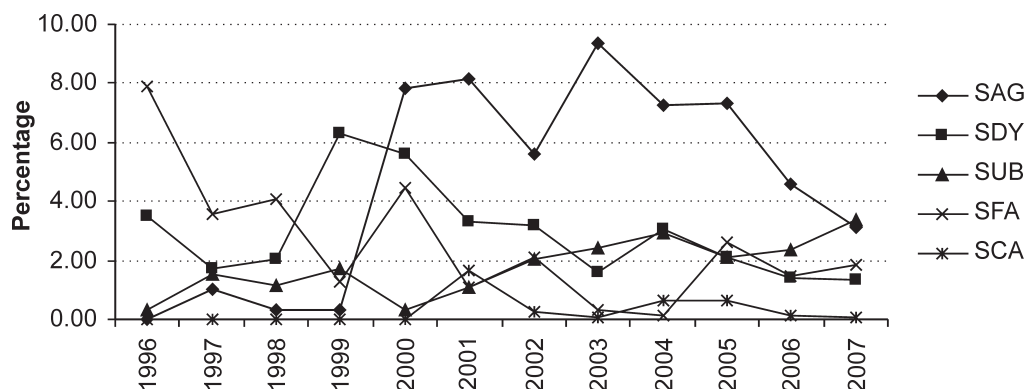


Fig. 2: Streptococci and enterococci isolated from milk samples in South African dairy herds from 1996 to April 2007. SAG: *Streptococcus agalactiae*; SDY: *Streptococcus dysgalactiae*; SUB: *Streptococcus uberis*; SFA: *Enterococcus faecalis*; SCA: *Enterococcus canis*.

Table 1: Bacterial isolates from lactating and dry cows in South African dairy herds (1996 to April 2007).

Bacterial isolates	Pathogens isolated from quarter samples of dry cows (%) (n = 3118)	Pathogens isolated from quarter and cow samples from lactating cows (%) (n = 112 715)	Difference in the bacterial isolations (%) between dry and lactating cow samples
<i>Staphylococcus aureus</i>	17.28	16.98	-0.44
<i>Staphylococcus aureus</i> ($\alpha\beta$ haemolytic)	7.81	8.12	1.01
<i>Streptococcus agalactiae</i>	1.21	5.92	33.03
Coagulase-negative staphylococci	61.71	60.96	-0.31
<i>Streptococcus dysgalactiae</i>	2.51	2.27	-2.51
<i>Streptococcus uberis</i>	1.21	2.25	15.03
<i>Enterococcus faecalis</i>	4.49	1.77	-21.73
Other bacteria	3.69	1.29	-24.10
<i>Enterococcus canis</i>	0.03	0.33	41.89
<i>Staphylococcus pyogenes</i>	0.06	0.11	12.50

In 73.9 % of dry cow samples and 70.3 % of lactating cow samples no growth was detected after culturing.

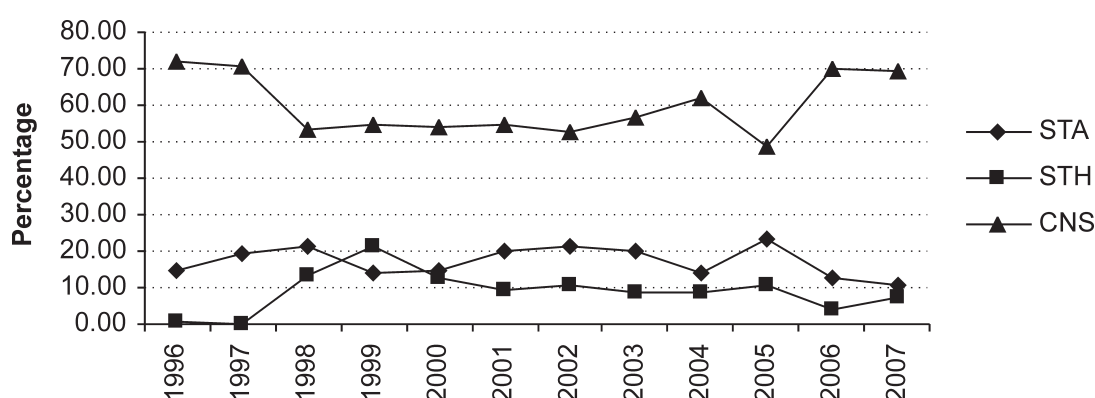


Fig. 3: Staphylococci isolated from milk samples in South African dairy herds from 1996 to April 2007. STA: *Staphylococcus aureus*; STH: *Staphylococcus aureus* (lytic type III); CNS: Coagulase-negative staphylococci.

Staphylococci isolated from lactating cows

The percentage of coagulase-negative staphylococci isolated from milk samples (quarter and cow) of lactating cows decreased in 1998, but has increased since 2003. A decline in *Staphylococcus aureus* isolates has been noticed since 2005 (Fig. 3).

High percentages of the *S. agalactiae* (76.4 %), *S. canis* (75.8 %), *S. dysgalactiae* (73.2 %) and STH (67.1 %) were isolated from quarters with mastitis (Table 2).

DISCUSSION

Mastitogenic pathogens isolated from cows' milk has changed over time, as less common mastitogenic pathogens filled niches that became vacant. Historically the development of new antibiotics, milking machines, housing systems and increased milk production are only some of the factors that created new opportunities for microorganisms to exploit. Prior to the use of penicillin G in 1943, *Streptococcus agalactiae* was the principle mastitogenic pathogen and the gap left was filled by *Staphylococcus aureus* when their numbers declined with the use of antibiotics. In European countries, dairy producers

have achieved success lately in reducing the incidence of contagious mastitis⁷. It has been estimated that the major contagious mastitis pathogens are now responsible for less than a third of all mastitis cases, compared with more than 75 % of all cases 20 years ago⁷.

Changes in udder pathogens isolated from milk samples in South African dairy herds were also noted in the 11-year period under investigation. Situations that created new opportunities for udder

pathogens under South African conditions include poor milking routine and milking machine maintenance, increase in herd size and introduction of many new cows into herds as well as increased milk yield. A matter of concern is a probable influence of reverse zoonosis that may have created a unique situation in South Africa due to many milkers that may suffer from compromised immunity²³. A gradual increase in udder infections occurred from 2002 until April 2007 (Table 1).

Table 2: Relationship between mastitis and teat canal infections (TCI) for the different bacteria isolated from quarter milk samples from 1996 to April 2007 (n = 19 311 isolates).

Bacterial isolates	n	Mastitis (%)	Teat canal infection (%)	Total mastitis cases (%)
<i>Staphylococcus aureus</i> (STA)	8477	52.4	47.6	23
<i>Staphylococcus aureus</i> (STH)	4713	67.1	32.9	16.4
STA and STH combined	13 190	57.7	42.4	39.4
<i>Streptococcus agalactiae</i>	2310	76.4	23.6	9.1
Coagulase-negative staphylococci	26762	28.5	71.5	39.5
<i>Streptococcus dysgalactiae</i>	1198	73.2	26.8	4.5
<i>Streptococcus uberis</i>	881	50.6	49.4	2.3
<i>Enterococcus faecalis</i>	877	51.7	48.3	2.4
<i>Enterobacteriaceae</i>	530	56.0	44.0	1.5
<i>Enterococcus canis</i>	293	75.8	24.2	1.2
<i>Streptococcus pyogenes</i>	46	58.7	41.3	0.1

Staphylococcus aureus

During 1989 the Faculty of Veterinary Science at Onderstepoort isolated a distinct $\alpha\beta$ haemolytic *Staphylococcus aureus* from milk samples from a then large commercial dairy herd for the first time. Phage typing identified it as belonging to lytic group III (with variation in its phage pattern) where all the other *Staphylococcus aureus* isolated from the milk of cattle belonged to lytic groups I and II. The owner, who suffered from chronic sinusitis, tested positive on a nasal swab for *Staphylococcus aureus* (lytic group III) (STH). Since then the STH has been isolated from large numbers of South African dairy herds, as well as from the people in close contact with those dairy cows (Tables 1 and 2). Since identifying the STH in herds and taking preventive measures, a decline in the percentage of isolates has been noticed. The presence of reverse zoonosis therefore warrants further investigation.

The percentage of STH isolated increased from low numbers during 1996 and 1997 to exceed the percentage of the other *Staphylococcus aureus* isolates for 1999 (Fig. 3). What is of concern is also the finding of a higher pathogenicity of the STH compared with the other *Staphylococcus aureus* bacteria as 52.4 % of *Staphylococcus aureus* isolated were from mastitic quarters, while 67.1 % of STH were mastitic (Table 2). Of the quarters infected with *Staphylococcus aureus* (including STH), 57.7 % had mastitis (Table 2). *Staphylococcus aureus* is still the principal mastitogenic pathogen in South Africa due to its chronic and destructive nature. Table 2, however, shows that most cases of mastitis were caused by coagulase-negative staphylococci and not *Staphylococcus aureus*.

A Norwegian survey ($n = 14\,152$ quarter milk samples) found *Staphylococcus aureus* to be the most prevalent pathogen isolated from 8.2 % of samples, while *Streptococcus dysgalactiae*, coagulase-negative staphylococci and *Streptococcus uberis* were isolated from 1.2 %, 3.3 % and 0.4 % of samples, respectively¹⁶. Results from the QMPS laboratory of milk samples in the Netherlands showed just over 12 % *Staphylococcus aureus* isolated in 1992, compared with 16 % in 2002³⁴. In this study, no growth was found in 70.3 % of samples cultured, compared with 76.6 % of samples in the Norwegian study¹⁶. Unlike the Norwegian study where *Staphylococcus aureus* was the most prevalent udder pathogen, the South African study found coagulase-negative staphylococci to be the most frequently isolated pathogen by far (60.96 % of isolates or 18.13 % of samples) (Table 1). *Staphylococcus aureus* (including STH) (25.10 % of iso-

lates or 7.47 % of samples) was the 2nd most abundant and *Streptococcus agalactiae* (5.92 % of isolates or 1.76 % of samples) the 3rd. These 3 organisms accounted for almost 92 % of isolates. *Streptococcus uberis* and *Streptococcus dysgalactiae* were both isolated from only 0.67 % of the samples and only a low percentage of Gram-negative organisms was cultured. In a study of smallholder dairy herds in Tanzania ($n = 213$ samples) the most prevalent mastitogenic pathogens were *Staphylococcus aureus* (25.7 %) and *Streptococcus agalactiae* (15.4 %) and there were high numbers of environmental pathogens: *Klebsiella pneumoniae* (14.3 %), *Escherichia coli* (14.1 %), *Pseudomonas aeruginosa* (7.5 %), *Streptococcus dysgalactiae* (5.2 %) and *Streptococcus uberis* (4.2 %)¹¹.

Streptococcus agalactiae

The *Streptococcus agalactiae* mastitis outbreaks may have influenced the number of isolates in this trial. These outbreaks were difficult to miss owing to the large number of clinical mastitis cases. During 2000 the 1st *Streptococcus agalactiae* mastitis outbreak in many years was confirmed in a South African dairy herd in the Western Cape region. This was followed by an increasing number of *Streptococcus agalactiae* outbreaks, peaking in 2003 until 2005, after which the percentage isolates became fewer (Fig. 2). The increase of movement of cows and lack of effective biosecurity in South Africa at the time are thought to be largely responsible for the increase in the *Streptococcus agalactiae* outbreaks. Individual cow SCC as high as 78 million cells/ml were recorded in South Africa. Infected quarters shed extremely high numbers of bacteria (500 million cfu/ml) and during an outbreak the bulk milk bacterial count can increase significantly⁹.

Streptococcus agalactiae is found both in humans and in animals, but zoonotic transmission is thought to be non-existent or insignificant. The strains that cause disease in humans are usually biochemically, metabolically or serologically different from the strains that cause disease in animals¹². *S. agalactiae* is the leading cause of neonatal meningitis, pneumonia and sepsis in humans^{12,22} and is also an important cause of morbidity in *peri-partum* women, non-pregnant adults with chronic medical conditions and immuno-compromised people. Its primary human habitat is the colon, but it can cause infections in the respiratory, urinary and reproductive tracts in 10–40 % of women¹⁰. A study done by Schukken²² in the Netherlands, in which a combination of ribotyping and serotyping was used, showed

2 bovine isolates to be indistinguishable from the human strains. The possibility of zoonosis and reverse zoonosis, although slim, could therefore not be completely ruled out.

Coagulase-negative staphylococci

The coagulase-negative group of staphylococci is most frequently isolated from milk samples in herds that have controlled major pathogens⁶. Coagulase-negative staphylococci constituted the most prevalent isolate in this study for the period 1996 to 2007 in both lactating and dry cows (Table 1). They were isolated from 22.6 % of quarter milk and 23.1 % of cow milk samples and were the most frequently isolated microorganism at 61.0 %. Our data are in agreement with studies in Finland, Ontario, Wisconsin, New York and Pennsylvania and in Germany where coagulase-negative staphylococci were isolated from 50 %, 51 %, 24 %, 23 % and 35 % of positive cultures, respectively^{13,14,17,26}. Surveys carried out in Norway and the Netherlands found coagulase-negative staphylococci to be less common. In Norway they were isolated from 14 % and in the Netherlands from 16 % of positive cultures^{17,18}.

The coagulase-negative staphylococci were considered minor pathogens with a low pathogenicity and somatic cell counts of infected quarters were found to increase 2- to 3-fold above that of the uninfected glands, causing the impact on SCC of composite to be minor²⁰. However, Table 2 shows that 28.5 % of coagulase-negative staphylococcal udder infections caused mastitis (both clinical and sub-clinical) with a somatic cell count of above 400 000 cells/ml milk. This value is much higher than 3 to 10 % reported by Ruegg²⁰. A further alarming finding was that the coagulase-negative staphylococci were responsible for most mastitis cases in South Africa (Table 2). This places coagulase-negative staphylococci as a mastitis pathogen in South Africa in a different perspective.

In human medicine, coagulase-negative staphylococci are described as common pathogens which cause infections in immuno-compromised patients³³. It is, however, difficult for clinicians to decide whether an isolate of coagulase-negative staphylococci represents the causative agent or is a non-specific contamination of another infection. Isolates from milkers' skin showed an identical antibiotic resistance pattern to that in milk isolates on the same farm²⁷. The 1st national symposium on 'Risk Management for the Limitation of Antibiotic Resistance' reported that genes responsible for resistance towards certain antibiotics can be

shared by a number of different bacteria via horizontal gene transfer of whole clusters. The latter may explain multi-resistance and cross-species spread of resistance⁵. Poutrel¹⁹ already indicated in 1988 that competition or antagonism between bacteria in mammary infections was a general phenomenon.

Enterococcus canis

Enterococcus canis isolates in this trial almost all originate from 1 outbreak in 1 dairy herd and no milk samples collected from dry cows in this herd. *Enterococcus canis* is commonly found in dogs and cats, where it causes infections of the skin, reproductive and respiratory tracts and the udder. *Enterococcus canis* has occasionally been isolated from milk of dairy cows, but was unknown as a mastitogenic pathogen in dairy cows. An *Enterococcus canis* mastitis outbreak occurred in a well-managed, high-producing dairy herd in South Africa during 2001 (IMP, pers. obs.). The SCC of infected quarters varied between 9000 and 47 328 million cells/ml milk with severe parenchymal damage. Treatment achieved little success (IMP, pers. obs.) in contrast to other described outbreaks^{1,28}. Of the 293 *E. canis*-infected quarters studied, 75.8 % had mastitis (Table 2). An *Enterococcus canis* mastitis outbreak in dairy cows in New York was reported on in 2005²⁸. Based on results from bacterial cultures and ribotyping, a cat with chronic sinusitis was the most likely source of this outbreak. Of the lactating cows, 22 % were infected with *Enterococcus canis* and the bulk SCC varied from 750 000 cells/ml to 1.8 million cells per/ml milk. Infected cows had macroscopically normal udders and treatment success was good (87.5 % cure in the dry period and 67 % during lactation). Chaffer¹ reported in 2005 on an *Enterococcus canis* mastitis outbreak in Israel with a 38 % infection rate and a bulk milk SCC of above 1 million cells per/ml milk.

It has been reported that the incidence of Lancefield group G streptococci (GGS) infections in humans is increasing in many parts of the world^{1,2,8,12,24,25,30}. Both human and animal GGS can cause chronic pharyngitis and sinusitis in their hosts⁸. However, Lancefield group G, beta-haemolytic streptococci in dairy cows usually belongs to the species *E. canis*, whereas those in humans rarely do^{3,8,32}.

Streptococcus pyogenes

A small number of *Streptococcus pyogenes* have been isolated from milk samples in South African herds (Table 1) since 2004 and 58.7 % of these 46 isolations cause mastitis (Table 2).

Streptococcus pyogenes is adapted to hu-

mans and has no natural reservoirs in animals, but is known to transmit via reverse zoonosis¹⁵. Humans can be asymptomatic carriers of *Streptococcus pyogenes*¹⁵, which has also been reported to infect the bovine udder. Contaminated milk can re-infect humans drinking raw milk¹⁵.

CONCLUSION

We acknowledge limitations in this study. However, in spite of the limitations of descriptive studies for research purposes, they have an important practical application for the evaluation of the efficacy of udder health management programmes on dairy farms. Changes in udder pathogens that occurred over the 11-year period were pronounced. Of particular importance was that the number and type of microorganisms isolated from milk samples and information on the field pathogenicity of the isolates could be determined. It also became evident that contagious mastitis is still responsible for 49 % of mastitis cases. Commercial dairies in South Africa must remain alert to the ever-increasing risk of reverse zoonosis due to our unique situation²³.

ACKNOWLEDGEMENTS

The authors acknowledge the volume of work and dedication of all field workers who assisted with sampling and especially personnel of Ecolab, as well as private veterinarians and dairy producers in South Africa who made use of our laboratory and enabled us to obtain this volume of information.

REFERENCES

1. Chaffer M, Friedman S, Saran A, Young A 2005 An outbreak of *Streptococcus canis* mastitis in a dairy herd in Israel. *New Zealand Veterinary Journal* 53: 261–264
2. Bert F, Brangger C, Poutrel B, Lambert-Zechovsky 1997 Differentiation of human and animal strains of *Streptococcus dysgalactiae* by pulsed-field gel electrophoresis. *FEMS Microbiology Letters* 150: 107–112
3. DeVriese L A, Hommez J, Kilpper-Balz R, Schleifer K 1986 *Streptococcus canis* form sp. nov. A species of group G streptococci from animals. *International Journal of Systematic Bacteriology* 36:422–425
4. Giesecke W H, du Preez J H, Petzer I M 1994. *Practical mastitis control in dairy herds*. Butterworths, Durban
5. Grugel C 2006 First national scientific symposium *Risk Management for the Limitation of Antibiotic Resistance*. *International Journal of Medical Microbiology* 296, Suppl. 2: 1–3
6. Harmon R J, Langlois B E 1995 Mastitis due to coagulase-negative *Staphylococcus* species. *Proceedings of the National Mastitis Council*, Vol 34. National Mastitis Council, Madison WI: 56–64
7. Hillerton J E, Bramley R T, Staker R T, McKinnon 1995 Patterns of intramammary infection and clinical mastitis over a 5-year period in a closely monitored herd apply-

- ing mastitis control measures. *Journal of Dairy Research* 62: 39–50
8. Ikebe T, Murayama S, Saitoh K, Yamai S, Suzuki R, Isobe J, Tanaka D, Katsukawa C, Tamara A, Katayama A, Fujinaga Y, Hoashi K, Watanabe H 2004 Surveillance of severe invasive group-G streptococcal infections and molecular typing of the isolates in Japan. *Epidemiology and Infection* 132: 145–149
9. Keefe G P 1997 *Streptococcus agalactiae* mastitis: a review. *Canadian Veterinary Journal* 38: 429–437
10. Kilian M 1998 Bacterial pathogens and associated disease: *Streptococcus* and *Enterococcus*. Online at: www.cdc.gov/ncidod/dbmd/diseaseinfo/ (accessed December 2007)
11. Kivaria F M, Noordhuizen J P T M 2007 A retrospective study on the aetiology and temporal distribution of bovine clinical mastitis in smallholder dairy herds in Dar es Salaam Region, Tanzania. *The Veterinary Journal* 173: 617–622
12. Lewthwaite P, Parsons H K, Bates C J, Mckendrick M W, Dockrell D H 2002 Group G streptococcal bacteria: an opportunistic infection associated with immune senescence. *Scandinavian Journal of Infectious Diseases* 34: 83–87
13. Lim G H, Leslie K E, Duffield T F, Timms L L, Dingwell R T 2007. Adherence and efficacy of an external sealant to prevent new intramammary infections in the dry period *Journal of Dairy Science* 90: 1289–1300
14. Makovec J A, Ruegg P L 2003 Antimicrobial resistance of bacteria isolated from dairy cow milk samples submitted for bacterial culture: 8905 samples. *Journal of the American Veterinary Association* 222: 1582–1589
15. Institute for International Cooperation in Animal Biologics. Iowa State University, College of Veterinary Medicine 2005 *Streptococcus*. Online at: <http://www.cfsph.iastate.edu/Factsheets/pdfs/streptococcus.pdf> (p. 11)
16. Østerås O, Sølvørød L, Reksen, O 2006. Milk culture results in a large Norwegian survey – Effects of season, parity, days in milk, resistance, and clustering. *Journal of Dairy Science* 89:101–1023
17. Pitkälä A, Haveri M, Pyörälä S, Myllys V, Honkanen-Buzalski T 2004 Bovine mastitis in Finland 2001 – Prevalence, distribution of bacteria, and antimicrobial resistance. *Journal of Dairy Science* 87: 2433–2441
18. Poelarends J J, Hogeveen H, Sampimon O C, Sol J 2001 Monitoring subclinical mastitis in Dutch dairy herds. *Proceedings 2nd International Symposium on Mastitis and Milk Quality, Vancouver, BC, Canada, 13–15 September 2001*: 145–149.
19. Poutrel B 1988 Effect of naturally occurring intramammary infections by minor pathogens on new infections by major pathogens in cattle. *American Journal of Veterinary Research* 49: 327–329
20. Ruegg P L, Dohoo I R 1997 A benefit to cost analysis of the effect of premilking teat hygiene on somatic cell count and intramammary infections in a commercial dairy herd. *Canadian Veterinary Journal* 38: 632–636
21. Sandholm M M 1995. *The bovine udder and mastitis*. Praesidium Books, Northlands
22. Schukken Y H, Zadoks R N, Tikofsky L, Dogan B, Klaessig S, Simpson K, Wiedmann M, Boor K 2004 Epidemiology of mastitis: paradigms, patterns and parables. *Médecin Vétérinaire du Québec* 34: 48–50

23. South Africa HIV & AIDS statistics 2006. Online at: <http://www.avert.org/safrica/stats.htm>
24. Sylvestsky N, Raveh D, Schlesinger Y, Rudensky B, Yinnon A M 2002 Bacteremia due to beta-hemolytic *Streptococcus* group G: Increasing incidence and clinical characteristics of patients. *American Journal of Medicine* 112: 622–626
25. Tadeka N, Kikuchi K, Asano R, Harada T, Totsuka K, Sumiyoshi T, Uchiyama T, Hosoda S 2001 Recurrent septicemia caused by *Streptococcus canis* after a dog bite. *Scandinavian Journal of Infectious Diseases* 44: 927–928
26. Tenhagen B-A, Köster G, Wallmann J, Heuwieser W 2006 Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. *Journal of Dairy Science* 89: 2542–2551
27. Thorberg B-M, Kühn I, Aarestrup F M, Brändström B, Jonsson P, Danielsson-Tham M-L 2006 Pheno- and genotyping of *Staphylococcus epidermidis* isolated from bovine milk and human skin. *Veterinary Microbiology* 10: 1016
28. Tisofsky L L, Zadoks R N 2005 Cross-infection between cats and cows: origin and control of *Streptococcus canis* in a dairy herd. *Journal of Dairy Science* 88: 2707–2713
29. Van den Heever L W, Katz K W, Prinsloo J D, Giesecke W H, Rawlins G, Jones A 1983 Standard methods for counting somatic cells in bovine milk in the Republic of South Africa. *Department of Agriculture Technical Communication* 190: 6–8
30. Whatmore A M, Engler K H, Gudmundsdottir G, Efstriou A 2001 Identification of isolates of *Streptococcus canis* infecting humans. *Journal of Clinical Microbiology* 39: 4196–4199
31. Wilson D J, Gonzalez R N, Das H H 1997 Bovine mastitis pathogens in New York and Pennsylvania: prevalence and effects on somatic cell count and milk production. *Journal of Dairy Science* 80: 2592–2598
32. Woo P C, To A P, Tse H, Lau S K, Yuen K Y 2003 Clinical and molecular epidemiology of erythromycin-resistant beta-hemolytic Lancefield group C streptococci causing bactericidal bacteremia. *Journal of Clinical Microbiology* 41: 5188–5191
33. Ziebuhr W, Hennig S, Eckart M, Kränzler H, Batzill C, Kozitskaya S 2006 Nosocomial infections by *Staphylococcus epidermidis*: how a commensal bacterium turns into a pathogen. *International Journal of Antimicrobial Agents* 28: s14–s20