

Québec/2004 Canada



23^e Congrès mondial de buiatrie • Québec, Canada, 11-16 juillet 2004
23 Congreso Mundial de Buiatria • Québec, Canada, 11-16 de Julio 2004

23rd World Buiatrics Congress • Québec, Canada, July 11-16, 2004
23. Welt-Kongress für Buiatrik • Québec, Canada, 11.-16. Juli 2004

Epidemiology of mastitis: paradigms, pattern and parables

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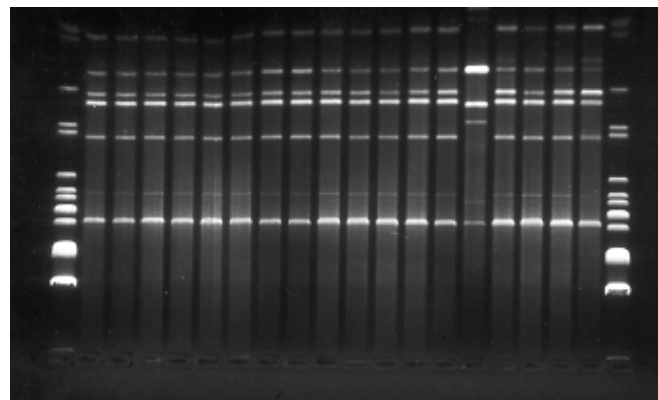
Introduction

Epidemiological research in bovine mastitis has been performed for many years with very early studies relying mostly on clinical observations and linking certain pathogenic bacteria to the clinical signs that were typically produced in the cow by these bacteria (e.g. *Streptococcus agalactiae* and *Arcanobacterium pyogenes*). In the last decade, molecular diagnostics have been added to the toolkit of the mastitis researcher community. These new tools have resulted in a better understanding of epidemiology and pathobiology of intramammary infection. In this paper we want to describe our recent research in the epidemiology of some major bacterial infections of the mammary gland using molecular diagnostic techniques.

E.coli

In recent publications, the occurrence of chronic *E. coli* intramammary infections was reported¹. Using DNA fingerprinting, the presence of indistinguishable isolates from repeated cases of clinical mastitis in the same quarter of the same cow was shown. An example of such an infection is shown in Figure 1. The isolates in lanes 2-14 were repeated cases of clinical coliform mastitis from the same quarter of the same cow and the isolates in lanes 16 to 19 came from a different quarter in the same cow. Because of the high number of *E. coli* strains in the dairy environment, it is unlikely that

recurrent isolation of one strain from the same quarter was the result of recurrent new infections. It is expected that recurrent coliform cases due to chronic infections will be observed more frequently in the years to come.



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Figure 1. Genetically indistinguishable isolates (evaluated by DNA fingerprinting using PCR-REP and ERIC primers) from recurrent clinical *E. coli* mastitis cases.

S. uberis

Typically, we classify *S. uberis* as an environmental organism, meaning that infection occurs due to organisms in the environment of cows. In the case of *S. uberis*, one would predict that most strains causing infections in a herd should be genotypically different since a enormous number of different genotypes of *S. uberis* exist in the environment of the cow. In some cases we have observed predominance of a single strain causing mastitis in multiple cows. This would indicate that transmission between animals occurs, and that the bacterium behaves more like a contagious organism. Data were from a dairy farm with 95 ± 5 lactating animals (mean \pm s.d.) where an outbreak of *S. uberis* infections was observed⁵. Data were collected during an 18-month observation period with 27 farm visits at 3-week intervals. Initially, a low prevalence and very low incidence of *S. uberis* infections was evident. At

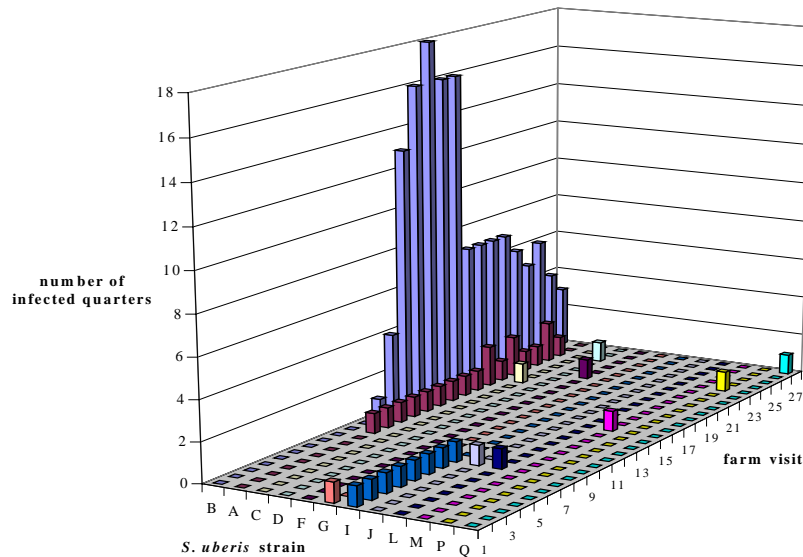


Figure 2. *S. uberis* strains on a farm with an outbreak of *S. uberis* mastitis.

At approximately sampling period 10, the start of an exponential growth of new infections could be seen. This outbreak halted around sampling 16. The prevalence remained high for a while, but then dropped to a much lower level. Towards the end of the observation period, the prevalence was still approximately three times as high as at the start of the study. Typing of the strains in the outbreak was performed using random amplified polymorphic DNA (RAPD) fingerprinting⁵. One large clonal outbreak with the strain named 'B' was observed (Figure 2). Additional arguments that favor cow-to-cow transmission and that indicated possible mechanisms of transmission were 1) finding identical strains of *S. uberis* in the milking liner up to 3 cow-milkings after milking of a *S. uberis* shedding cow, 2) finding only new infections with strain 'B' in lactation (in contrast to other strains that infect also during the dry period), and 3) the relative long duration of infection with strain 'B'. Survival analysis of observational data showed that infections with strain B lasted significantly longer than infections with other strains. Thus, infections with strain B would have a larger window of opportunity for spread, most likely occurring during the milking process, than other strains. Clearly, prevention programs for strain type B would be different compared to the other identified strain types.

S. aureus

In a recent study by Zadoks et al.⁴, they reported on *S. aureus* isolates that had been collected by Fox and coworkers during a cross-sectional study of dairy herds in Washington State in 1987. In this study, 225 viable *S. aureus* isolates were available from 43 herds, including isolates from bovine teat skin (n = 70), milkers' hands (n = 4), milking machine unit liners (n = 34), and bovine milk (n = 117). Teat skin isolates and milk isolates had been obtained from a random selection of cows in each herd. All milkers' hands had been sampled before milking of the cows, and all milking equipment had been sampled after the herd had been milked. Per herd, 1 to 20 isolates were available. We recently repeated

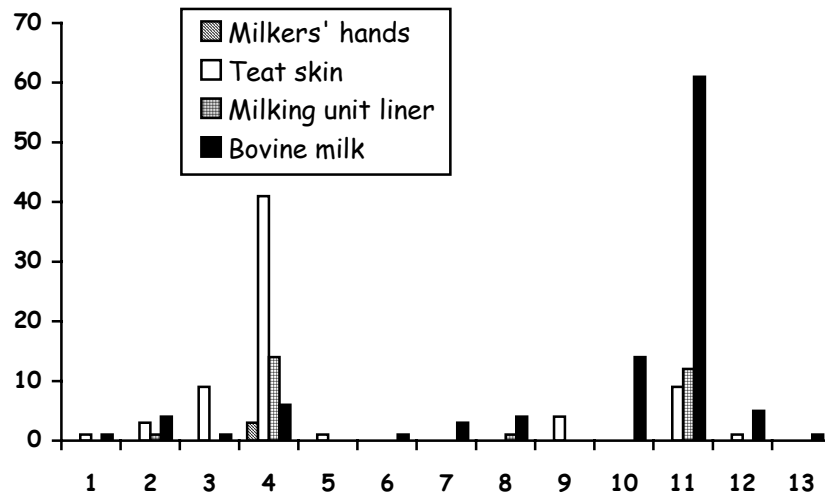


Figure 3. Distribution of Phage types over sources of isolation. Significant differences between teat skin and milk were observed for types 4, 10, 11.

differentiated between *S. aureus* strains that are predominantly isolated from healthy bovine teat skin and *S. aureus* strains that are predominantly isolated from milk. The association between site of isolation and strain is highly significant for the most frequently isolated phage types in this study. From these data, we conclude that *S. aureus* isolates are somewhat site specific, and that not all *S. aureus* isolates are equally likely to cause intramammary infections. The results are summarized in Figure 3.

S. agalactiae

S. agalactiae is the leading cause of human neonatal sepsis, pneumonia and meningitis and is also a major cause of bovine mastitis. In a recent study we compared phenotypic and genotypic characteristics of 52 human and 52 bovine *S. agalactiae* isolates to provide some insight into the zoonotic transmission potential of this organism. Serotypes and ribotypes of the isolates were compared and antibiotic resistance profiles and the presence of genetic determinants encoding resistances to tetracycline and erythromycin were determined. Combination of ribotyping and serotyping showed only two bovine isolates as indistinguishable from human isolates. Ribotyping results are shown in Table 1. Resistances to tetracycline and erythromycin were higher among human isolates (85% and 27%, respectively) than among bovine isolates (15% and 4%, respectively).

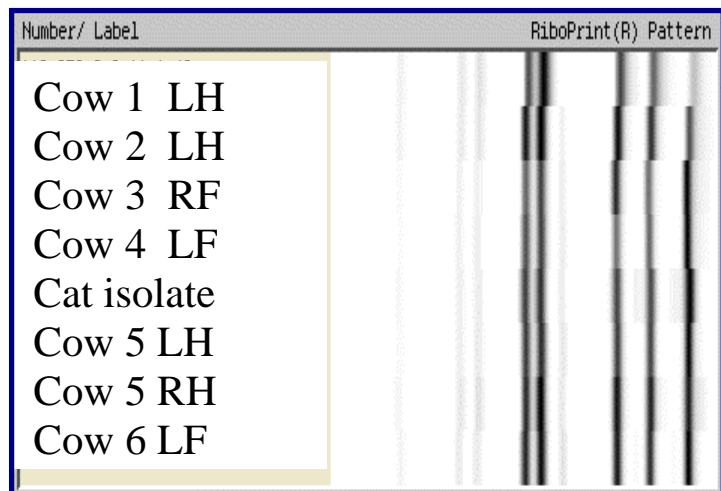
Table I. Ribotype distribution of human and bovine *S. agalactiae* isolates from New York State.

Ribotype	Human		Bovine		H vs. B
106-1501-S-8	39	75.0%	1	1.9%	<0.01
116-549-S-1	7	13.5%	3	5.8%	0.50
106-1501-S-5	4	7.7%	12	23.1%	0.01
116-611-S-7	2	3.8%	3	5.8%	0.65
106-1502-S-6	0	-	9	17.3%	<0.01
106-1502-S-2	0	-	6	11.5%	0.01
106-1502-S-3	0	-	4	7.7%	0.03
106-1504-S-1	0	-	3	5.8%	0.08
Other (9types)	0	-	11	3.8%	-

The *tetM* resistance determinant was found in all tetracycline resistant human isolates, while *tetO* was the predominant resistance gene among bovine isolates. Only the two bovine isolates that were indistinguishable from the human isolates had the *tetM* resistance determinant. Our results suggest that the presence of mostly distinct *S. agalactiae* populations in bovine and human hosts. Gene transfer of antimicrobial resistance genes between host-specific populations was not evident, and if any exists it would appear to flow from human to bovine isolates.

S. canis

In a herd with 90 lactating cows Tikofsky et al.³ observed 46 cows (51%) culture positive Group G, 16 cows (18%) *Staphylococcus aureus*, 2 cows (2%) *Streptococcus* spp., 18 cows (20%) *Staphylococcus* spp., 17 cows (19%) *C. bovis*, and 1 cow (1%) *A. pyogenes*. The isolation of Group G streptococcus (*S. canis*) is somewhat rare and unexpected in dairy cows. It usually is an infection that occurs in dogs, cats or people. In this herd we also sampled the cat and the owner. Both of these had a chronic sinusitis. The cultures showed no growth in the material from the owner, but did result in *S. canis* from the cat's nostrils. Ribotyping results are shown in Figure 4.



and an isolate from cat's nostrils.

Discussion

Classical classifications of bacterial species can now be challenged using very precise molecular and epidemiological data. In itself it is of no surprise that substantial variation in

epidemiology exists within one bacterial species. There is a large variation in the genome of individual strains within species, and very different clinical patterns emerge from these strains. It is likely that we will continue to develop a better understanding of strain-specific epidemiology within bacterial species causing bovine mastitis. This will have a major impact on the specific control strategies that are successful to prevent and or reduce intramammary infections in herds.

Abstract

Dans cet article, nous présentons des données scientifiques qui indiquent que la classification épidémiologique classique doit être révisée. Nous proposons une approche basée sur une caractérisation moléculaire des souches bactériennes. Cette approche permettra d'élaborer des stratégies de contrôle spécifique à chaque souche bactérienne/ferme.

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