

Epidemiology

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Epidemiology of bovine mastitis on the modern dairy farm - Introduction

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Objectives: Bovine mastitis remains one of the most important diseases on the modern dairy farm. Not only is mastitis associated with pain and decreased welfare of cows, but also with decreased quality of milk, with increased use of antibiotics, unwanted early removal of cows and ultimately with a negative impact on the economics of the farm. In the last decade much has been learned about the epidemiology of bovine mastitis, therefore the objective of this overview is to summarize these findings and to develop directions for further improvement of udder health on modern dairy farms.

Subclinical mastitis: The distinction between clinical and subclinical mastitis is one hand important and on the other hand also a gradual difference. Cows with very high somatic cell counts clearly have abnormal milk that in some cases looks like normal milk at the time of milking but starts flaking when standing in a collection vessel for only a few minutes. Still, the distinction is of value as subclinical mastitis primarily leads to increased somatic cell counts, delivery of milk into the bulk tank and therefore decreased milk quality. Whereas clinical mastitis leads to observed disease in cows and often to immediate treatment and milk withhold in the affected animals.

In recent years the improved diagnostic tools, particularly in the field of molecular diagnostics, has resulted in a much more precise diagnosis of bacterial organisms that are associated with intramammary infections. A much better insight into infections with staphylococci, streptococci and also gram-negative micro-organisms has improved our understanding of these infectious processes.

Improved molecular diagnostics has also resulted on important research with regard to the microbiome of the mammary gland. Current research would indicate that the mammary microbiome of health quarters is substantially different from quarters that are affected by either clinical or subclinical mastitis. Further research will be necessary to show whether the mammary microbiome plays a role in prevention of intramammary infections with major mastitis pathogens.

Clinical mastitis: Clinical mastitis has become increasingly important as subclinical mastitis may be controlled using the NMC 10-point plan. Increased on-farm hygiene, improved milking management and adequate treatment has on one hand reduced subclinical mastitis but at the same time put the spotlight on clinical mastitis.

In recent years much research has been done on the Immunology of the bovine mammary gland, including interventions on the immune system using diets, vaccinations or immune stimulations. These interventions have shown to be valuable in reducing the incidence of clinical mastitis, reducing

the length of clinical signs and the risk of culling of affected animals.

Societal pressure has resulted in a reduction of the use of antibiotics for the treatment and prevention of intramammary infections. This reduction of antibiotic use has ultimately lead to further improvements in managing risk factors for intramammary infections on farms but also on the increased importance of immune modulation.

Environmental versus contagious: In recent years, our understanding of the main infection routes that lead to intramammary infections has substantially changed. The main reasons for this were the increased use of molecular diagnostics that led to precise typing of bacterial strains and as a result a paradigm shift on the classification of mastitis causing micro-organisms. The classic approach was to divide mastitis causing microorganisms into environmental and contagious organisms. With streptococci and staphylococci being contagious and gram-negative being environmental. However, increased application of molecular typing schemes have shown that such a distinction does not hold up and that all bacteria that are able to cause persistent intramammary infections may lead to contagious behaviour on dairy farms. This had resulted in a portfolio of risk-factors, where transmission has become an important but not a singular part in the dynamics of intramammary infections.

Conclusions: Important changes in the biology, diagnostics, management and treatment of bovine mastitis in the recent decade has resulted in a vastly different understanding of intramammary infections and the treatment and prevention of these infections.

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Molecular epidemiology of mastitis: evidence versus paradigm

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The epidemiology of mastitis is generally described as contagious or environmental. Contagious pathogens have their reservoir in the mammary gland and are transmitted from animal to animal during milking. Environmental pathogens, by contrast, have their reservoir in the environment, and infections can occur at any time, even in non-lactating cattle. There are exceptions to this dichotomous classification. For example, insects may act as vectors, as shown for transmission of *Staphylococcus aureus* among heifers by flies, so contagious transmission can occur without involvement of the milking machine. Conversely, the infected mammary gland may serve as reservoir of infection, but with transmission via the environment, as shown for *Klebsiella* spp. in free-stall herds. Occasionally, we see iatrogenic point source transmission, as demonstrated for *Pseudomonas*-contaminated water or wipes, and for *Serratia*-contaminated teat dip.



The main weakness of the contagious versus environmental paradigm, however, is how we as veterinarians interpret and implement it. Many veterinary curricula, textbooks, extension reports, and peer-reviewed publications classify bacterial species as contagious or environmental, regardless of the evidence. As a profession, we should have a more nuanced understanding of the sources and transmission routes of mastitis pathogens, because molecular epidemiology studies provide ample evidence that many bacteria can be spread in multiple ways. When we open our minds to this possibility, the distinction between modes of transmission can often be made even without using molecular typing. The tools that we normally use for mastitis investigations, such as inspection of the parlour, housing and animals for hygiene and management risks, and evaluation of data regarding parity, lactation stage and affected quarters, can largely be relied on to formulate a diagnosis and interventions. The success of those interventions will then inform on the accuracy of our diagnosis and the need for further investigation.

In genomic epidemiology, entire genome sequences are generated and compared as done, e.g., for SARS-CoV-2. This level of complexity is not needed for mastitis investigations, which can often be conducted using relatively simple molecular biology tools including polymerase chain reaction (PCR), or the more time-consuming former gold standard method of pulsed-field gel electrophoresis (PFGE). PCR amplifies bacterial DNA, PFGE cuts it. Either way, a bar code-like banding pattern is generated, revealing whether bacterial isolates from different sources have the same or different DNA fingerprint, e.g., across quarters, cows, and herds, or between animals and the milking machine, bedding, faeces, drinking water, teat dips and wipes, etc. Most practitioners and clinics would not have access to this technology within their laboratory but the scientific work to generate the evidence has already been done. It is largely the willingness to implement available knowledge that is the main hurdle.

Although a social science study into communication and uptake of evidence would be very valuable, the main focus of this presentation will be on the molecular evidence itself. It shows that major mastitis pathogens such as *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis* can all “behave” as contagious pathogens as well as environmental pathogens. The manifestation on a specific farm or even country will depend on the combination of pathogen strain and herd management. For example, *S. uberis* is almost exclusively environmental in New Zealand (different DNA fingerprints in different cows), whereas most clinical *S. uberis* mastitis in England is the result of contagious transmission (same DNA fingerprint in multiple cows). If we suggest to farms that milking time hygiene or teat dipping are not a priority when they have *S. uberis* mastitis in their herd, we may aggravate the problem. Even *Streptococcus agalactiae*, long described as an “obligate intramammary pathogen”, may have environmental sources, including people and bovine faeces. Several publications describe *S. agalactiae* problems that could only be controlled by approaching them as an environmental mastitis problem.

By presenting some of the examples described in this abstract, it is hoped that a growing number of cattle veterinarians will be willing to confront the contagious versus environmental paradigm with molecular epidemiology evidence and open

their mind to the fact that many bacterial species are more sophisticated than our dichotomous thinking.

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Epidemiology of infectious disease on dairy farms - Introduction

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Objectives: Infectious on dairy farms have gained in importance in recent years. Infectious diseases have resulted in subclinical and clinical disease in dairy cows, increased risk of zoonotic events whereby infections from cattle transmit to humans and also to increased societal concerns on welfare of calves and cows. In this presentation the recent experiences from the Netherlands will be shared. Lessons learned and important necessary developments in bovine health will be discussed.

Control of infectious diseases: In recent years control programs for infectious diseases have been developed for the Dutch dairy industry. This includes programs to reduce or eliminate infections with *Leptospira hardjo*, *Mycobacterium avium* subsp. *paratuberculosis*, Tuberculosis, *Brucella abortus*, Enzootic Bovine Leukosis virus (EBLV), *Salmonella* species, Bovine Virus Diarrhea Virus (BVDv), Infectious Bovine Rhinotracheitis virus (IBRv) and to some extent Blue Tongue Virus (BTV). On top of this, programs to diagnose and control list A diseases are present but have not been necessary in the last decade.

With the increased interest and concern about zoonotic pathogens, the control of *Leptospira hardjo*, *Mycobacterium avium* subsp. *Paratuberculosis* (MAP), Tuberculosis, *Brucella abortus*, and *Salmonella* species are important steps to decrease such concerns. The control of Enzootic Bovine Leukosis virus, Bovine Virus Diarrhea Virus and Infectious Bovine Rhinotracheitis are not indicated because of zoonotic concerns but because of animal health improvement in general or for trading reasons.

The development of these control programs is particularly dependent on the ability to control transmission between animals within a farm and transmission between farms.

Within farm transmission: Understanding within farm transmission has been particularly important for infectious diseases such as MAP, salmonellosis, BVD and IBR. Elimination of MAP from farms has been difficult, but long term control programs have certainly resulted in important progress as indicated by a reduced incidence, less severe shedding and disease and a delay in the time to shedding. Carefully designed diagnostic programs for BVD and IBR have turned out to be essential to eliminate within farm transmission and certification of farms as infection free. *Salmonella* control programs are based on long term control of the key risk factors and on bulk milk monitoring, subsequent individual animal diagnostics and culling of persistently infected animals. Once a farm has been

certified as infection free, prevention of between farm transmission becomes the focus of the national control program.

Between farm transmission: A number of the indicated infectious diseases are controlled based on National legislation and with the aid of National enforcement of diagnostic programs and subsequent control procedures. This includes to control programs for Tuberculosis, *Brucella abortus*, Enzootic Bovine Leukosis virus (EBLV) and to some extent Blue Tongue Virus (BTV). As a consequence all cattle farms in the country are included in monitoring and control and the risk of between farm transmission is minimal. In contrast, control programs for *Leptospira hardjo*, *Mycobacterium avium* subsp. *paratuberculosis*, *Salmonella* species, Bovine Virus Diarrhea Virus (BVDV) and Infectious Bovine Rhinotracheitis virus (IBRV) are implemented by the animal industries and without legislation or enforcement support by the government. Hence, the success of these programs fully depend on the ability of the industry partners to work together and decided on control programs that are implemented across different players in the industry. It has been remarkable that this appears to be successful. Important components of the control programs are cattle registration, documentation of animal movements and dedicated participation of farmers in the developed control programs.

However, the control of these infectious diseases in the dairy industry alone will not lead to elimination of the infectious agents from the country as beef cattle farms, calf raising farms and veal calf operations are also potential sources of these infectious organisms. Further cooperation between these industries will be essential for a further reduction in between farm transmission.

Conclusions: Control of infectious diseases on dairy farms has gained in importance in recent years. Concerns with regard to zoonotic diseases, animal welfare and reduced use of antimicrobials all result in an increased need for continuous improvement of animal health and reduction or elimination of infectious diseases on dairy farms.

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Beyond mastitis: molecular epidemiology insights into transmission and control of bacterial, parasitic and viral diseases of cattle

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Introduction: As sequencing of DNA and RNA has become increasingly accessible and affordable, molecular methods have added significantly to our understanding of the transmission and control of bacterial, parasitic and viral disease of cattle. They have provided insight into past movement of pathogens and their current distribution, and inform rational design of future eradication strategies, diagnostic tests, and vaccines. They also help us understand how the pathophysiology of disease and the transmission of organisms and, hence,

control strategies may differ, or need to differ, between production systems, countries, or continents. Parasites are comparatively large infectious agents. They are eukaryotic, like cattle and humans, and have large genomes spread across multiple chromosomes, which means that sequencing of entire genomes is still very challenging. Bacteria are prokaryotes, with smaller and simpler genomes than parasites. Less than 15 years ago, sequencing of the first bovine *Streptococcus agalactiae* genome cost more than the annual salary of the postdoctoral researcher analysing it. Now, it can be done within days or weeks for less than 100 Euro. Viruses are much smaller yet, and sequencing of entire genomes is cheaper and faster than for bacteria, although it can be tricky for segmented genomes such as those of influenza D, which was first described in cattle in 2011.

Parasites: In parasites, sequencing of housekeeping genes is a useful tool for accurate species identification. This approach led to the realization that most rumen fluke in the Republic of Ireland and the United Kingdom did not belong to the species *Paraphistomum cervi*, as long assumed, but to the species *Calicophoron daubneyi*. The two species have different life cycles and intermediate host snails. Control of rumen fluke, which has emerged as a common cattle parasite across much of western Europe, requires knowledge of how to manage the environment to limit the snail population and cattle exposure to infectious cysts on pasture. Where the culprit was thought to be a freshwater snail, it is actually a mud snail, completely changing our options for environmental control. Other desirable tools for diagnostics and management of parasitic diseases would be molecular markers of anthelmintic resistance. Although initial results for, e.g., the liver fluke *Fasciola hepatica*, were promising, they could not be confirmed in other studies, countries, or continents.

Bacteria: Differences in pathogen characteristics between continents can be considerable, and evidence-based control strategies may differ between countries. In our contribution on molecular epidemiology of mastitis, the difference between New Zealand and the UK is used as an example, whereby *Streptococcus uberis* mastitis is almost exclusively environmental in New Zealand but commonly contagious in the UK, as it is in mainland Europe or the USA. Another striking geographical difference is observed for *Coxiella*, the causative agent of Q-fever in humans and coxiellosis in ruminants. In the USA, up to 95% of bulk tank milk may test positive for *Coxiella* but reports of Q-fever are unusual. In Europe, Q-fever is primarily associated with small ruminants. By contrast, cattle are considered the main source of Q-fever in Australia. The difference may be due, at least in part, to differences in *Coxiella* strains, which may also impact on the efficacy of diagnostic assays coated with antigens from different parts of the world.

Viruses: The most detailed molecular epidemiology information is available for viruses. Strain typing is essential to inform selection of vaccines for a high-impact pathogen like Food and Mouth Disease (FMD) because dominant serotypes may differ between countries or change over time. It may also inform on the role of animal movements and non-bovine host species as reservoirs, both for epidemic viral diseases like FMD and for endemic viral diseases like bovine viral diarrhoea virus disease (BVDV). Viral sequence analysis has shown that sheep, humans, or inanimate fomites may act as source of BVDV. Insight into occurrence and prevention of such excep-



tions will become increasingly important as BVDV programs progress, which they are likely to do now that it has been added to the OIE list of notifiable diseases.

Although it is impossible to provide a comprehensive overview of the molecular epidemiology of bovine infectious diseases, selected examples will be presented to illustrate their contribution to our ability to understand and manage the health of cattle populations.

Public Health, Food Security and Antimicrobial Resistance

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Vulnerability of rural wells and organic produce to multiple drug resistant (MDR) bacteria from dairy cattle manure

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Objectives: Assess the public health risk of microbial contamination of raw produce such as leafy green vegetables and rural groundwater wells used for drinking water from AMR-MDR enteric bacteria in dairy manure and slurry.

Material and methods: A. We collected feces or manure from live animals, pen soil, and manure storage ponds on California commercial dairies in the San Joaquin Valley to quantify the occurrence of AMR-MDR commensal and pathogenic bacteria. AMR-MDR determination included phenotypic methods (disk diffusion, MIC) and/or whole genome assessments of the resistome using the Comprehensive Antibiotic Resistance or ResFinder database.

B. In parallel, we determined if rural municipal wells either on the dairy or in proximity of a dairy exhibited MDR bacteria and whether field irrigation of forages with effluent from manure storage ponds created a subsurface plume of bacteria. Groundwater samples were obtained using domestic wells or by drilling monitoring wells on the dairy and in nearby rural communities. Bacteria were concentrated using filtration methods, followed by primary bacterial culture and biochemical characterization of isolates.

C. Lastly, a series of randomized controlled trials were conducted in experimental Romaine lettuce production fields whereby furrows were spiked with animal feces that contained specific concentrations of marked *E. coli*. These inoculated lettuce fields were then overhead irrigated and heads of lettuce hand harvested, rinsed, and the concentration of *E. coli* determined for in order to calculate the percentage of bacteria transferring from fecal deposits in the furrows onto the heads of lettuce.

Results: A. AMR-MDR commensal bacteria were most prevalent in young calves on California commercial dairies compared to other age classes and production stages. Over 50% of hutch calves were colonized by MDR *E. coli* within 6 weeks of age, while cull dairy cattle exhibited prevalences of 30-40% of MDR *Enterococcus*. Use of genomic tools to characterize the larger resistome compared to phenotypic AMR methods found that hutch calves were more likely to harbor MDR bacteria compared to other age classes of dairy cattle, but numerous resistance genes were also found throughout all age classes of dairy cattle.

B. AMR genes were widely dispersed in manure matrices throughout the dairy environment and cattle loafing areas, manure lagoon storage system, surface pen soil, and in dairy manure solids used for soil amendments for forage crops and in limited cases produce farming. For example, concentrations of AMR *E. coli* ranged from 6×10^4 cfu/100 ml of manure slurry