Understanding the Sources, Transmission Routes, and Prognoses for Mastitis Pathogens

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Take Home Messages

- Within animal species, we recognize different breeds. Within bacterial species, we recognize different strains. Strain typing may help to identify sources and transmission routes of mastitis-causing bacteria so that we can target interventions and choose appropriate treatment options.
- Many of the most common mastitis-causing bacteria, including *Staphylococcus aureus* and *Streptococcus uberis*, can originate from the environment and spread from cow to cow. It is wrong to consider a bacterial species as “contagious” or “environmental”. Transmission mechanisms are herd- and strain specific. Inspection of the farm, animals and records may be enough to identify the transmission route. Strain typing can help.
- Multi-herd outbreaks of unusual pathogens, e.g. *Pseudomonas* or *Serratia*, can have a point source that is common to all farms or farm-specific sources. Strain typing is needed to tell the difference.

Controlling Mastitis

In theory, controlling mastitis is easy. Prevent new infections and limit the duration of infections that occur if prevention fails. To some extent, we can achieve this through breeding and feeding. Breed cows with good udder confirmation and good udder health traits. Feed cows to meet their energy, vitamin and nutrient requirements. Both breeding and feeding will improve the cow’s ability to withstand the challenge posed by bacteria, which are inevitably present in and on the animals’ bodies and environment. A cow with a good immune system will also have a greater ability to limit the duration of infections. To some extent, this ability can be boosted by vaccination.

Unfortunately, past udder infection does not provide immunity to future udder infection. If anything, it is probably the other way around. Alternative methods to limit the duration of infection are treatment and culling. Generally speaking, “treatment” means the use of antimicrobials (commonly called “antibiotics” although technically there is a subtle difference, i.e. antibiotics are naturally occurring compounds whereas antimicrobials are man-made). Antimicrobial treatment, however, is not always successful. In addition, there is concern about the selection for antimicrobial resistance, which is a major issue in human health more than in udder health. If treatment fails, the only way to limit the duration of infection may be to “cull the quarter” or to cull the cow. Neither option is desirable. All in all, practical tools to improve the cow’s resistance to mastitis or to limit the duration of infection are limited in their effectiveness and desirability. Prevention is better than cure.

■ Mastitis Epidemiology

Epidemiology is the study of the spread of disease in populations. Even though the word may not trip off the tongue, the concept is extremely important. If we don’t know how a disease is spread, we also don’t know how to stop the spread. There are two main modes of transmission for mastitis: contagious and environmental. In the case of contagious transmission, also called cow-to-cow transmission, cows with mastitis are the main source of infection. Spread of the bacteria that cause the infection primarily happens during milking, e.g. via the milkers’ hands, udder cloths, or the milking machine. Use of milking gloves and individual towels will help to prevent this. Milking infected cows last or with a separate unit will also prevent spread of infection, provided that the unit is disinfected before the next milking. Environmental mastitis originates in the environment, e.g. in bedding, manure or water. Occasionally, bedding contains high numbers of bacteria even before it is used. Routinely, bedding is contaminated with manure, which contains everything bacteria need: moisture, warmth and nutrients. Water can be contaminated with bacteria from manure, or it may accumulate in a milking machine, particularly if hoses don’t have the correct slope and if the temperature of the cleaning water is too low. In addition to those common modes of transmission, there is also the possibility of transmission by people. This may happen via animal health products, such as teat wipes or teat dip. Alternatively, people may be a direct source of infection for animals because many mastitis-causing bacteria occur in people as well as in cattle. The human-to-animal route of transmission has various names, none of which are particularly attractive: humanosis, reverse zoonosis, or anthroponosis. An overview of transmission routes is shown in Figure 1.

Without knowledge of the most important sources or transmission routes of bacteria on a farm or during a multi-farm outbreak of mastitis, we cannot take adequate control measures. Strain typing can help us to identify sources and transmission routes so that we can target our control efforts.

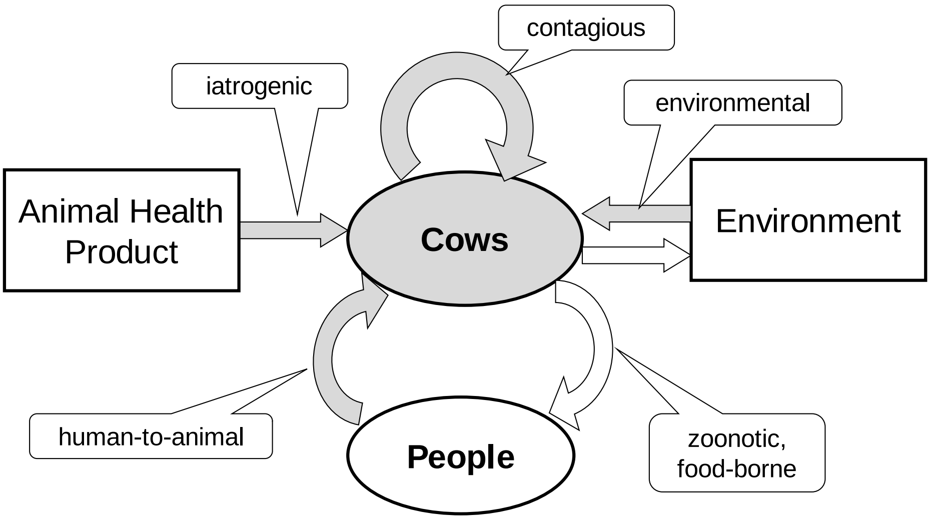

Figure 1. Possible sources and transmission routes for mastitis pathogens: from other cows, the environment, people or products. Mastitis control efforts must focus on the most important sources and routes.

▪ Strain Typing

Strain typing is often compared to DNA fingerprinting. A better analogy would be to compare strains of bacteria to breeds of animals. Not only is this a better comparison because farmers understand breeds but also because a DNA fingerprint is unique to an individual whereas a breed has generic traits that can be found in all individuals belonging to that breed. Similarly, a strain is a group of bacterial isolates that share a trait (Figure 2). The analogy is still not perfect. Breeds are described by breed standards, which clearly define the characteristics of the breed. A single cow, sheep, horse or dog can only belong to a single breed, even though that may be a "crossbreed" rather than a pure breed. A single bacterial isolate can belong to many "breeds" or, rather, strains, depending on the method that is used to define the strains.

Some strain typing methods are easy to understand and have immediate practical implications. For example, antimicrobial sensitivity testing can be used as a strain typing method. With this method, an isolate can be classified as penicillin susceptible or penicillin resistant. This information is helpful both in the choice of treatment and for the prognosis. Take *Staphylococcus aureus*, for example. Isolates (the live cultures we obtain from milk samples when processing them on farm or in the laboratory) can be penicillin susceptible or penicillin resistant. For penicillin-susceptible *Staph. aureus*, we can use a penicillin based treatment but it would be unwise to do this for a penicillin resistant strain. Thus, the susceptibility test informs our treatment choice. Even if we choose a different active compound, the penicillin resistant *Staph. aureus* may persist. Not only the treatment choice, but also the prognosis is different between the two types (Barkema et al., 2006). We can compensate for the poor prognosis of penicillin resistant *Staph. aureus* by extending the treatment duration or we can choose to cull rather than treat the affected cow.

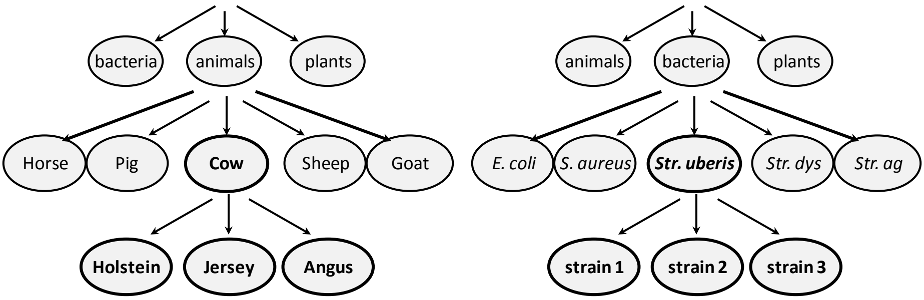

Figure 2. Analogy between breeds and strains. Animals from different breeds have enough in common to be considered the same animal species and enough different traits to be classified as separate breeds. Similarly, bacteria from different strains have enough in common to be considered the same bacterial species and enough different traits to be classified as separate strains.

There are many other strain typing methods, most of which have names that are abbreviated to “four letter words” such as PFGE, RAPD or MLST (Zadoks et al., 2011). For the person choosing, conducting and interpreting the typing assay, it is important to understand the differences between those methods. For the user, suffice it to say that they differ in cost, appropriate application, and turn-around time. To my knowledge, at the time of writing of this contribution, the only diagnostic laboratory to offer strain typing to farmers on a routine basis is Quality Milk Production Services at Cornell University in the USA. The insights we have gained from such testing, however, are available to all scientists and veterinarians. The aim of this contribution is to make them accessible to farmers too (Figure 3).

■ Mastitis Control in Individual Herds

Most farmers and veterinarians are familiar with the concepts underlying mastitis control, such as detection and treatment or removal of existing cases and prevention of new cases by reduced exposure and improved host resistance. The weight that is given to each of those control measures may differ from farm to farm. If new infections are mostly due to cow-to-cow transmission, a reduction in the number of existing cases will inherently lead to a lower number of new cases. In such a situation, prevention of cow-to-cow transmission (parlor hygiene, teat dipping) and detection and removal of infected cows are crucial. If new infections mostly originate from the environment, removal of infected cows without a change in environmental exposure will not reduce the number of new cases. In that case, environmental hygiene or host resistance needs to be improved.

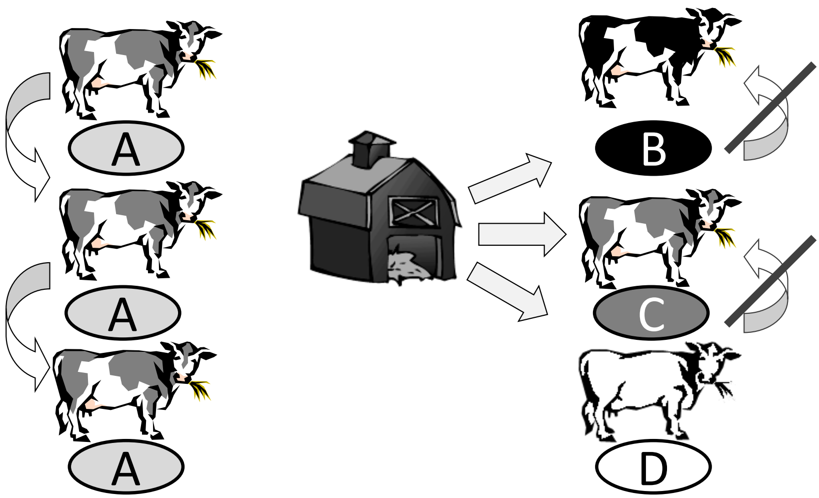

Figure 3. Contagious and environmental transmission as seen through strain typing: If cow-to-cow transmission occurs, all cows will be infected with the same strain (left). If cows are infected with different strains (right), they did not infect each other. Rather, they probably were infected from the environment, which contains many different strains of mastitis pathogens.

For decades, we have been told that some mastitis causing bacteria, e.g. *Streptococcus agalactiae* and *Staph. aureus* are “contagious” whereas others, e.g. *Streptococcus uberis*, *E. coli* and *Klebsiella* are “environmental”. When testing of milk samples yielded any one of those particular species, we thought we knew what the appropriate control strategy is. Things in nature, however, are rarely black-and-white, and failed mastitis control efforts provide many examples to illustrate this. Outbreaks of *Strep. uberis* or *Klebsiella* mastitis occurred when infected cows were not moved to the sick pen but left with the rest of the milking herd, even though those species are supposedly not contagious. *Staph. aureus* does not always respond to implementation of good milking parlor hygiene and a rigorous “search and destroy” programme (Sommerhauser et al., 2003) even though a well-behaved contagious pathogen should be controlled with that approach. *Strep. agalactiae* keeps popping up occasionally in previously free herds and is making a come-back in Northern Europe. Why does control fail?; partly because we don’t put in enough effort and partly because mastitis-causing bacteria don't follow the rules. For example, strain typing has shown that *Strep. uberis* and *Klebsiella* can spread cow-to-cow (Figure 4) and that *Staph. aureus* can originate from the environment.

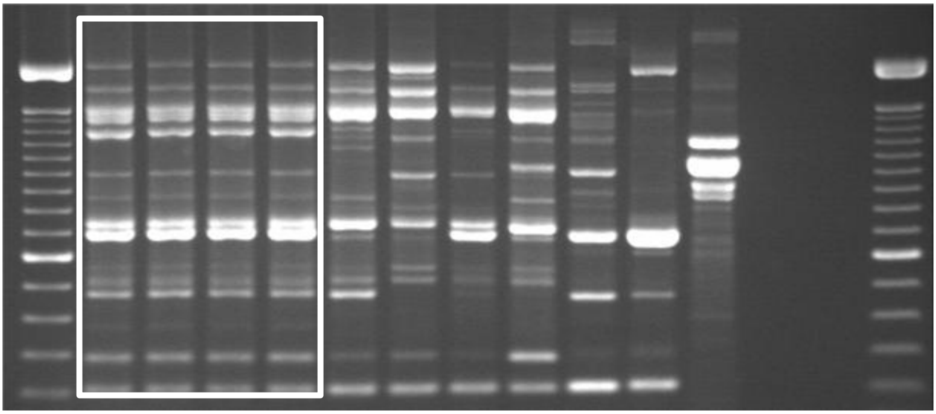

Figure 4. Example of strain typing results for mastitis-causing bacteria. Each "DNA fingerprint" or barcode-like typing result runs from top to bottom and represents a single cow with mastitis. The box encloses 4 identical results showing that 4 cows shared the same strain of *Klebsiella*. Other cows from this herd were infected with different strains of *Klebsiella*. The regular patterns on the left and right are DNA-ladders, which are included for quality control reasons, as are the empty lanes.

There are many examples now of mastitis problems in individual herds that were caused by bacteria that "didn't follow the textbook". *Staph. aureus* can behave as a contagious pathogen, but it can also behave as an environmental pathogen (Gurjar et al., 2012). *Strep. uberis* can behave as an environmental pathogen, but it can also behave as a contagious pathogen (Zadoks et al., 2003; Gurjar et al., 2012). Even *Klebsiella*, which is generally associated with bedding or manure, can spread in a contagious manner when a single infected cow leaks a lot of *Klebsiella* positive milk (Munoz et al., 2007). *E. coli* is one of the few mastitis pathogens without documented cow-to-cow transmission.

If strain typing is not routinely available to farmers and veterinarians, what good is this knowledge? A lot of good, potentially. By recognizing that almost any pathogen can follow either mode of transmission, the mastitis control programme can be tailored to the farm-specific situation rather than the textbook. Many *Staph. aureus* problems are due to cow-to-cow transmission, but in herds with low somatic cell count and excellent parlor hygiene and teat dipping, the focus may need to shift to environmental *Staph. aureus*. Many *Strep. uberis* problems are due to environmental contamination, particularly if they occur around calving, but if environmental hygiene is good and *Strep. uberis* cases continue to occur, please check the parlor routine, teat dipping, and segregation of infected cows. It doesn't take rocket science or even strain typing to implement this knowledge, just an open mind.

■ Multi-herd Outbreaks

There are only a few documented examples of multi-herd outbreaks of mastitis caused by unusual mastitis pathogens. One was an international outbreak of *Pseudomonas* mastitis in dry cows, with cases in The Netherlands and Ireland. The other one was a multistate outbreak of *Serratia* mastitis in lactating cows, with cases in New York, Wisconsin and Vermont. In both outbreaks, there was a suspicion that this was a iatrogenic problem due to a contaminated animal health product (Figure 1) and in both cases strain typing was used to investigate that suggestion (Daly et al., 1999; Muellner et al., 2011). To cut a long story short: in the *Pseudomonas* outbreak, contaminated teat wipes were the suspected source and the presence of a single strain of *Pseudomonas* in all investigated herds was confirmed, supporting the notion of a common source. An unopened tub of teat wipes also contained this strain, confirming that the contamination had happened during production, prior to distribution of the product to the affected farms. At first glance, the *Serratia* outbreak scenario appeared quite similar: all affected herds used a newly introduced teat dip. But in contrast to the *Pseudomonas* outbreak, each herd was affected by a different strain of *Serratia*. The herd-specific strains could be found in the teat dip, but only once teat dip containers were opened and used on the farm. Even when farms used dip from the same batch, the *Serratia* strain was farm-specific. In this situation, the introduction of bacteria had not happened during production but the individual farms. Let's hope such multi-herd outbreaks continue to be rare. If they do occur, strain typing is a quick and very useful tool to differentiate between different scenarios and to address both the mastitis problem and potential legal implications.

■ People, Cattle and Other Animals

Cows, the environment and contaminated products are not the only potential sources of mastitis pathogens. In rare cases, dogs or cats may be implicated, such as in the case of *Streptococcus canis* outbreaks (Tikofsky and Zadoks, 2005) but a more common occurrence is probably the introduction of mastitis pathogens by people. People and cattle share many bacteria: *Strep. agalactiae*, *Staph. aureus*, *Staphylococcus epidermidis*, *E. coli* and *Klebsiella*.

People are the natural host for two important staphylococcal species: *Staph. aureus* and *Staphylococcus epidermidis*. Both species can be transmitted from people to cows with subsequent cow-to-cow transmission (Zadoks et al., 2011). In recent years, methicillin-resistant *Staph. aureus* (MRSA) has emerged as a cause of mastitis in dairy herds. The first case was reported in the 1970s and was probably due to infection of cows by a human strain of MRSA. The next case was reported in 2007 and was probably also due to infection of cows by a human strain of MRSA. In countries with a high pig density, such as Belgium or The Netherlands, MRSA mastitis has been linked to strain ST398 from pigs. In the USA, MRSA mastitis continues to be linked to human sources based on strain typing (Haran et al., 2012). Fortunately, MRSA is extremely rare in Canadian dairy cattle.

Another mastitis pathogen that is very rare in Canada is *Strep. agalactiae.* The organism has almost been eradicated. The same was true in Northern Europe, until the turn of the millennium. Since 2000, there has been a re-emergence of *Strep. agalactiae* in Denmark and, more recently, Finland and Norway. There are many theories about contributing factors, including changes in the use of milking machines, teat dip and dry cow treatments. The only thing that is certain is that many of the infections are caused by strains of *Strep. agalactiae* that also occur in people. A considerable proportion of healthy adults, some 20 to 40%, carry *Strep. agalactiae* in their gut or their urinary tract, mostly without symptoms. In infants and elderly people, *Strep. agalactiae* may cause disease. It was thought that people and cattle were mostly affected by different strains of *Strep. agalactiae* but ongoing work in Denmark, Finland and Sweden shows that the majority of mastitis cases are caused by strains of *Strep. agalactiae* that may also affect people (Figure 5). Or maybe they originate from people. That would provide an explanation for the introduction of *Strep. agalactiae* in closed herds and its occasional occurrence as cause of clinical mastitis in low somatic cell count herds (Zadoks et al., 2011). Like MRSA, *Strep. agalactiae* is not a current problem in Canada but if it should emerge, strain typing will be a useful tool to track its movements.

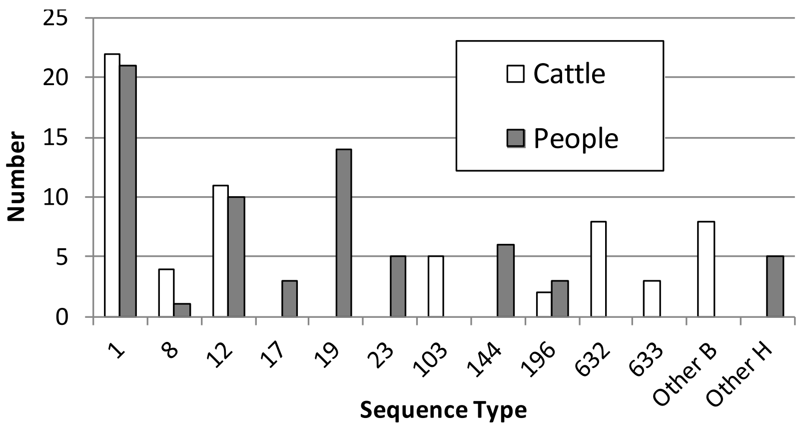

Figure 5. Distribution of 63 *Streptococcus agalactiae* strains from cattle and 69 from people from Finland, characterized based on Sequence Type. Some strains are limited to people, e.g. ST19, or cattle, e.g. ST632, but some of the most common strains, e.g ST1 and ST12, occur in both host species.

■ Conclusion

Strain typing, the differentiation of mastitis-causing bacteria at subspecies level, has taught us a lot about sources and transmission routes of mastitis pathogens. Most bacterial species can spread via more than one route, including from cow to cow (one strain detected in multiple animals) or from the environment to the cow (different strains detected in different cows). In some cases, an environmental point source such as a treatment product or even a dog, cat or person acts as the source of infection and in those situations, initial introduction from the environment may be followed by cow-to-cow transmission. Both removal of the original source and prevention of further within-herd spread are needed to control such outbreaks. Even without strain typing data, information on affected animals (e.g. lactating or non-lactating) and current mastitis control strategies (e.g. use and concentration of teat dip) may provide information about likely transmission routes. For *Staph. aureus*, strain typing based on antimicrobial sensitivity testing may inform treatment decisions.

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