Farm-level Disease and Production Problem Investigations - Use of Molecular Tools in Outbreaks

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Abstract

Molecular tools are diagnostic and epidemiological tools that use DNA or RNA to characterize microbial pathogens at species or strain level, and to trace their sources and transmission patterns. Molecular tools are used to investigate problems in milk and cheese production and to elucidate the origin and epidemiology of disease outbreaks affecting single or multiple farms. Animal health products can be sources of pathogens that cause disease in dairy cattle, and dairy cattle can be a source of pathogens that cause disease in humans. In this paper, examples of a variety of molecular tools and their application in analysis of disease and production problems are presented. Bacterial and viral diseases are covered, including mastitis, respiratory disease, foot-and-mouth disease, and foodborne and zoonotic diseases.

Résumé

Les outils moléculaires sont des outils épidémiologiques qui font appel à l'ADN et à l'ARN pour caractériser des pathogènes microbiens à l'échelle de l'espèce et de la souche et pour retracer leur source et leur schéma de transmission. On utilise des outils moléculaires pour faire enquête sur des problèmes de production de lait ou de fromage et pour élucider l'origine et l'épidémiologie de flambées de certaines maladies touchant une ou plusieurs fermes. Les produits de santé animale peuvent être des sources de pathogènes à l'origine de maladies dans le bétail laitier, et le bétail laitier peut être une source de pathogènes à l'origine de maladies chez l'humain. Le présent exposé propose des exemples de différents outils moléculaires et leur application dans l'analyse des maladies et des problèmes de production. L'exposé traite de maladies bactériennes et virales, y compris la mammite, le complexe respiratoire bovin, la fièvre aphteuse de même que les maladies d'origine alimentaire et les zoonoses.

Introduction

Animal disease outbreaks occur at the global, national, regional and herd level. Production problems may occur on the dairy farm, or in the dairy processing plant. Finally, contact with animals on dairy farms or dairy products may result in disease in humans. When disease outbreaks or production problems occur, molecular tools are of great use. Traditional culture-based methods for identification of disease agents are often supplemented with or replaced by molecular methods that are more sensitive, more specific or faster than traditional methods. Once a disease agent is identified, molecular tools can be used to detect sources of the organism and to uncover its probable transmission routes. For treatment decisions, knowledge of the causative agent is often sufficient. For management decisions and prevention of new cases, knowledge of sources and transmission routes is essential. This presentation aims to summarize examples of use of molecular tools in outbreak investigations in dairy practice, drawing from the scientific literature and from our own experience with use of molecular tools to solve disease and production problems on dairy farms.

Molecular Tools

Molecules are everywhere, and everything on a dairy farm – be it animate or inanimate, protein, carbohydrate, lipid or DNA— is made of molecules. It is no surprise, then, that the word "molecular" has multiple meanings and its specific meaning depends on the context of its use. In the context of this paper, the term "molecular" is used to refer to diagnostic tools that rely on the use of DNA or RNA for identification of organisms at the genus, species or subspecies level. The term "genotyping" is also used for characterization of organisms based on their genetic material, and is contrasted to "phenotyping", i.e. characterization of organisms based on their phenotype, which results from the interaction between genotype and environment. Examples of phenotypic methods include serotyping, phage typing, and use of a series of biochemical tests such as the BBL Crystal, Vitek or API systems for bacterial species identification. Before the advent of the polymerase chain reaction (PCR) and other modern molecular methods, phenotyping methods were more easily accessible and cheaper than DNA-based methods. These days, DNA-based determination of the serotype of an organism through detection of the genes that encode the capsular

serotype, or species identification of unknown organism using DNA-sequencing of a housekeeping gene, can be cheaper than a phenotypic test. In addition, DNA-based methods tend to have a higher ability to characterize every test isolate, and to do so accurately. For example, we recently compared a number of methods for identification of coagulase-negative *Staphylococcus* species from dairy heifers. Almost all isolates could be identified using DNA-based methods, whereas approximately 20% of isolates could not be assigned an unambiguous species identity based on phenotypic methods. Those isolates that could not be identified by DNA-based methods belonged to as-yet unidentified bacterial species, or to species so closely related that it is dubious whether they are separate species at all.

All molecular tools rely on three basic activities: copying, cutting or sequencing of DNA or RNA. Copying generates high copy numbers of nucleic acid molecules, making it easy to visualize or measure them. This contributes greatly to the sensitivity of the methods. Cutting generates fragments of different sizes that can be separated by electrophoresis to generate banding patterns. In fact, copying can generate such fragments too. DNA-sequencing, which is offered for less than $5 per run at some commercial sequencing facilities, provides the exact genetic code. This can be used to detect and quantify identity and differences between species and strains of microorganisms. The exponentially increasing number and size of online DNA-databases allows for identification of an ever-larger number of bacteria, viruses and other pathogens. To copy, cut, bind or sequence specific targets, specific primers, restriction enzymes and probes are used. Combining the three basic activities of copying, cutting and sequencing with a wide variety of primers, probes and enzymes results in a sheer endless universe of molecular typing methods. When a sample is submitted to the laboratory with a request for "typing", it can take many phone calls to determine which method should be used, which target should be looked for, and estimated cost of the molecular typing. Rather than providing a theoretical overview of typing methods and possible applications, this paper will provide examples of use of molecular methods to solve disease outbreaks or production problems in dairy farms and plants. For a more comprehensive review of molecular terminology and molecular methods, the reader is referred to existing literature.[41]

Sour Milk and Exploding Cheese

One day, a bulk-tank milk sample arrived in our laboratory with the text, "Milk going sour. Please type." As stated before, typing can be done in a myriad of ways, and the method of choice depends on the specific question at hand. When there is no specific question, the best way to start is with a non-specific method. In this case, we started with culture of the sample on blood agar. The plate showed almost pure growth of *Staphylococcus* species and yeast. Staphylococci are not known to cause souring or fermentation, but yeasts are. Specific yeasts are used to produce kefir and other fermented dairy or grain products. A second bulk-tank sample, submitted several days later, was overgrown by gram-negative bacilli on blood agar. To see whether yeast was still present in the sample, we subsequently used MRS agar, a selective agar that supports growth of yeast, *Lactobacillus* and related bacterial genera. Using this semi-selective agar, yeast was also detected in the second sample of bulk-tank milk that was going sour in three days, despite refrigeration. To identify yeast species, phenotypic methods can be used. In our laboratory, which is specialized in mastitis bacteriology, identification of yeast by phenotypic methods is not done routinely. We routinely identify organisms based on DNA-sequence data. For bacteria, this is done by amplification of the 16S gene, which encodes a ribosomal subunit. Yeast does not have a 16S ribosomal subunit, but a 26S subunit. Primers for amplification of the 26S gene were ordered online (cost ca. US $5 per primer; two primers needed), the 26S gene was amplified, and the PCR product was purified and submitted for sequencing (cost ca. US $5 per sequencing reaction, two reactions run per PCR product). Two days later, sequence data were received by e-mail, checked for quality, and compared to one of the largest online DNA-sequence databases, GenBank, from the NCBI (http://www.ncbi.nlm.nih.gov/). The result? *Issatchenkia orientalis*. *Issatchenkia orientalis* has been found on grapes, citrus and in barley silage. It is used to make sourdough bread, to ferment coffee, and to prepare a local fermented food in Tanzania called togwa. It can decrease the pH of a sterile gruel solution to 3.6 in 24 hours.[25] Definitely a possible cause of milk going sour!

A lot of the milk our dairies produce is not consumed as fluid milk, but in processed form. Cottage cheese is a popular processed dairy product that is marketed in a range of flavors and packaging styles. One of its selling points is the convenience of a long shelf life. But what if the cottage cheese that is supposed to have a shelf life of 60 days starts popping its lid after 50 days? That is when the cottage cheese hits the fan. And that is when producers may be blamed for shipping milk that contains bacteria that cause product to go bad. Gas producing bacteria are not the organisms we normally look for in bulk-tank milk. Fortunately, we were given a lead in this case. The problem was thought to be caused by *Lactobacillus*. How do you recognize *Lactobacillus* on a blood agar plate? You don't. To tease out *Lactobacillus* from among the bulk- tank flora, semi-selective media, i.e. LBS (*Lactobacillus* Selection) agar were used. Con-

trol isolates of *Lactobacillus brevis* and *Lactobacillus plantarum* were included to ensure that the methods worked, and to help with phenotypic identification of putative *Lactobacillus* isolates from the test samples. Little is known about the prevalence of *Lactobacillus* in bulk-tank milk. If we only tested the milk from the herd that had been singled out as the "bad guy", we would not know whether its occurrence is common or exceptional. Therefore, bulk-tank milk from multiple herds was tested. Suspect colonies were identified from multiple samples and herds. For species identification, two methods were used: ribotyping and DNA sequencing. Ribotyping is a fully automated method with standardized reagents. Because of the automation and standardization, the banding patterns or DNA fingerprints that are generated can be compared to a library of fingerprints to allow for species or strain identification. Standardization and automation come at a cost: commercial fees for ribotyping can be as high as $170 per isolate. In our *Lactobacillus* investigation, ribotyping yielded clear banding patterns for every isolate, but only one isolate (*L. brevis*) was recognized at the species level through comparison with the database. This is an issue that can be encountered with a variety of molecular methods: the typing gives a result, but the result is not recognized. As the use of molecular methods grows, so will the reference databases, and their ability to identify organisms. Using DNA sequencing of the 16S gene, many more isolates could be identified at the species level: *L. brevis*, *L. plantarum*, *Pediococcus dextrinus*, *Weissella paramesenteroides*, and a variety of other *Lactobacillus* and *Leuconostoc* species. Many of these species are involved in fermentation, including fermentation of silage. In the laboratory, none of the species survived pasteurization, and with the exception of *L. brevis*, none of them produced gas. What caused the cottage cheese to explode remains an unanswered question. However, we know now that once you start looking with new methods, one can find all kinds of unexpected organisms in all kinds of places. Moreover, the herd that had been singled out as the culprit could resume shipping of milk to the processing plant.

How Environmental is Environmental Mastitis?

Enough about milk and cheese. Let's talk cows, dairy cows. The most common and costly disease of dairy cows is mastitis. The most common causes of mastitis are well known, and many of them can be identified using culture and phenotypic methods: *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae and Streptococcus uberis*. For other organisms, identification with phenotypic methods does not usually go beyond the genus level (e.g. *Enterobacter*, *Citrobacter*, *Corynebacterium*, *Bacillus*), or species level identification with phenotypic methods is not very reliable (*Enterococcus*, coagulase-negative *Staphylococcus* species).[37] For all these bacteria, molecular methods can be used to confirm their identity, usually by means of PCR with primers that are specific to a single bacterial species (e.g. *Staph. aureus, Strep. agalactiae, Strep. dysgalactiae, Strep. uberis*, *Strep. canis*), or to determine their species identity by means of PCR amplification and subsequent sequencing of housekeeping genes (e.g. *cpn*60, *rpo*B, and *sod*A genes, used for *Klebsiella*, *Serratia*, *Enterococcus*, *Streptococcus*, *Lactococcus* and *Staphylococcus* species).[10,22,28] In addition, molecular methods are used for differentiation of mastitis pathogens at the subspecies level, i.e. for strain typing. A strain is an isolate or a group of isolates exhibiting characteristics that set it apart from other isolates belonging to the same species. For example, isolates belonging to the species *Staph. aureus* can be subdivided into penicillin-sensitive and penicillin-resistant strains. Strain typing can help to differentiate between contagious and environmental mastitis, and to detect sources of mastitis-causing bacteria. The environment contains a large variety of bacterial species and strains. For example, one gram of soil can contain five or more strains of *Strep. uberis*[42] and one gram of feces can contain four or more strains of *K. pneumoniae*.[27] When mastitis originates from the environment, almost every cow and quarter will be infected with a different strain of the pathogen. When cows are infected by each other, multiple cows will have the same strain of the pathogen. Without knowledge of sources and transmission routes, targeted intervention is impossible, and mastitis outbreaks will be hard to control.

In recent years, the proportion of dairy herds with *K. pneumoniae* mastitis has increased significantly in New York State and other northern states. In addition, the proportion of *Klebsiella*-positive cows within positive herds has increased significantly,[40] and so has the number of reported *Klebsiella* mastitis outbreaks. *Klebsiella* spp occur in water, farm soil, bedding material,[33] living wood and bovine feces.[26] Based on its widespread occurrence in the environment and the heterogeneity of strains that can be found even in a single gram of feces, one would expect *Klebsiella* mastitis cases in a herd to be caused by a large variety of strains. Last year, we worked with a herd where two outbreaks of *Klebsiella* mastitis occurred. Isolates from both outbreaks, the milking machine, bedding, feed, feces and water from this herd were analyzed using random amplified polymorphic DNA (RAPD) typing. RAPD typing is a simple, cheap, PCR-based method that has limited standardization and reproducibility but is well suited for comparison of isolates from a single herd. In the first outbreak, nine of 10 *Klebsiella* isolates from milk

samples showed indistinguishable DNA banding patterns. The one cow that showed a different pattern was housed in a different management group. *Klebsiella* was not detected in the unused bedding material or in bedding from management groups other than the outbreak group. Based on the results, contagious transmission was suspected, with transmission via the milking machine or via the bedding material (Munoz *et al*, submitted). Intervention measures to prevent contagious transmission in the milking parlor and to improve environmental hygiene were proposed. After intervention measures were implemented, no new cases of *Klebsiella* mastitis were reported for several weeks. Then, a second wave of *Klebsiella* mastitis cases occurred, this time in multiple management groups. In this second outbreak, mastitis cases were caused by a wide variety of strains, showing that the second outbreak had a different origin than the first outbreak. The second outbreak was due to environmental mastitis, as most *Klebsiella* outbreaks are, and improvement of environmental hygiene was the most important management tool in this situation. Although predominance of a single strain in a herd with *Klebsiella* mastitis is an exception, it is not unique. We recently examined 45 isolates of *Klebsiella* from a second herd that suffered a major *Klebsiella* mastitis outbreak. Of 45 isolates from cows with clinical *Klebsiella* mastitis, 33% belonged to one predominant strain, 33% belonged to other strains that were found in more than two cows, and the remaining 34% belonged to strains that were identified only once. In this herd, fecal samples and bedding samples were not tested and mechanisms of transmission could not be identified with certainty.[40]

A less-common cause of mastitis is group G streptococcus, which is *Strep. canis*, at least when it is found in animals. Mastitis caused by *Strep. canis* is usually found in a single animal, and is considered to originate from the environment. Outbreaks of *Strep. canis* mastitis have been reported from a number of countries, including the USA.[5,14,32] To determine whether such outbreaks are of environmental origin or due to contagious transmission, strain typing is used. Methods used for strain typing included ribotyping,[32] and pulsed-field gel electrophoresis (PFGE).[14] PFGE is a highly discriminatory but labor intensive and hence, expensive method. In all *Strep. canis* outbreaks under analysis, whether in the USA, Germany or Italy, most or all cows in a herd were infected with the same strain of *Strep. canis*.[14,32,39] As for the *Klebsiella* outbreaks described before, this suggests that outbreaks due to *Strep. canis* are not environmental, but rather the result of contagious transmission. Management data support this.[5,32] As the name suggests, *Strep. canis* is primarily found in dogs, where it can be an innocent commensal or a life-threatening pathogen. In addition, it has been isolated from cats. In one case, a barn cat could be identified as the original source of the *Strep. canis* strain that caused a mastitis outbreak.[32] Infection of multiple cows from the same point source, i.e. the cat, cannot be ruled out completely, but management data make it far more likely that initial infection of one cow was followed by *Strep. agalactiae*-style contagious transmission. It is important to keep in mind that molecular data should not be considered in isolation, but always in combination with data on sample origin and dairy herd management.[41]

Wolves in Sheepskin

To protect our cows from disease, we use a range of management strategies and animal health products. Sometimes, the very measures we take to prevent disease result in disease outbreaks. Examples include outbreaks of bovine viral diarrhea (BVD) and mastitis.

In 2006, *Serratia* mastitis outbreaks occurred in a number of dairy herds, mostly in the northeastern U.S. *Serratia*, outbreaks which have occurred before, have been associated with growth of the organism in bedding[16,19] and quarternary ammonium-based teat disinfectant.[35] In other cases, the source of the outbreak was not detected, despite testing of the environment, teat dips, or milking equipment.[29,38] Transmission via the milking machine is also thought to occur.[16] Strain typing can be used to determine whether *Serratia* isolates from a herd belong to a single or multiple strains. When multiple strains are detected, multiple sources must have been involved. This is typical for environmental mastitis. When a single strain predominates, infections may originate from a point source or contagious transmission. In the 2006 *Serratia* outbreaks, there was an extra twist to the story: most of the affected herds used the same chlorhexidine-based teat dip, and the question arose whether the teat dip could be the source of infection, like the quaternary ammonium teat dip had been before. To answer these questions, three kinds of comparisons where performed. First, within-herd comparison of isolates from multiple cows were done. Next, strains isolated from cows were compared to strains isolated from teat dip on the same farm. Finally, teat dip strains obtained from multiple farms were compared to each other. When comparing isolates from multiple cows within a farm, most cows within a farm were infected with the same strain of *Serratia*. In some herds, multiple strains, or even two species of *Serratia* were identified. This shows that transmission was predominantly due to contagious transmission or exposure to a point source, but there was some variability in sources of infection. When comparing isolates from cows to those from teat dip within a farm, the predominant strain from cows was also found in teat dip. This suggests that the teat dip acted as point source, or as the vehicle for conta-

gious transmission. When teat dip from multiple farms was compared, no two farms had the same strain of *Serratia* in teat dip. Even when multiple containers of teat dip originated from the same batch number, the contaminating strains of *Serratia* were unique to the farm.

One important lesson from this example is that strain typing allows for problem analysis in more depth than culture results and exposure data ever could. Another lesson is that teat dips contain disinfectants, but they are not kill-all sterilizing agents. Even in hospitals, contamination of disinfectants with *Serratia* and other pathogens occurs.[36] If teat dips are not handled properly, contamination with environmental bacteria may occur, some of which may be resistant to the active compound in the teat dip. Teat dip containers, be it dip cups or storage barrels, should not be left open or contamination with environmental bacteria may occur. Similarly, when pumps for spray units are placed on the floor while changing dip barrels, contamination may occur. Molecular methods can help to pinpoint where breakdowns in management occurred. People are the only ones that can fix such breakdowns.

Sometimes, animal health products arrive contaminated from the provider. Molecular analysis of *Pseudomonas aeruginosa* isolates from 11 mastitis outbreaks in Ireland showed that all outbreaks had been caused by the same strain. This strain was also isolated from a tub of teat wipes that was provided for free with dry cow therapy tubes. The purpose of the teat wipes, which were part of a sales promotion, was to clean and sterilize the teat ends of cows before infusion of dry cow product, so as to avoid intramammary infection.[7] Sadly, the good intentions of the pharmaceutical company backfired. Rather than preventing mastitis, as they were meant to do, these teat wipes caused mastitis. Fortunately, due in part to the cooperation of the company and the use of molecular methods, the source of the outbreak was identified, and additional cases could be prevented.

Molecular typing and unfortunate mishaps are not limited to the worlds of bacteria and mastitis. In 1999, outbreaks of bovine viral diarrhea (BVD) caused by BVD genotype 2 virus occurred in Italy and The Netherlands after use of a vaccine against infectious bovine rhinotracheitis that was contaminated with BVDV-2.[2,11] The original outbreaks in The Netherlands were diagnosed based on clinical investigations and use of monoclonal antibodies, rather than molecular methods.[2] In Italy, molecular methods (reverse transcriptase PCR) were used to confirm the genotype of the BVDV. To determine whether such outbreaks had occurred before, similar methods were used for a retrospective study of BVDV genotypes in Italy. All viruses in the retrospective study were classified as BVDV genotype I, highlighting that the use of contaminated vaccine could have resulted in the introduction of a new type of pathogen in Italy.[11] Outbreaks of viral disease may also result from the vaccine virus itself. Using molecular methods, most small scale outbreaks of foot-and-mouth disease (FMD) that occurred in Europe in the 1980s could be attributed to vaccine strains.[4] Finally, there are cases where viral disease was blamed on a vaccine, but use of molecular methods proved that suspicion false. This was the case in a Dutch herd where bovine herpesvirus 1 (BHV1) circulated. Because the herd was closed, and because a live-virus vaccine had been used in previous years, it seemed a foregone conclusion that the vaccine virus had caused the virus circulation. After viral shedding in vaccinated and unvaccinated carrier animals had been induced with corticosteroids, strain typing (using restriction enzyme analyses) showed that the virus circulation had not been due to the vaccine strain.[34] Despite efforts to maintain a closed herd, introduction of a different BHV1 strain must have taken place. Recurrence of viral disease in closed dairy herds has also been described for bovine respiratory syncytial virus (BRSV). When outbreaks occurred within a single dairy herd in different years, virus strains within years were shown to have a high level of genetic homogeneity, while virus strains from different years were genetically distinct. Recurrent introduction of BRSV into herds is the most likely explanation for this observation. As for the BHV1 outbreak, the route of introduction of BRSV into closed herds remained unknown.[21]

Dealing with Disaster

In a different presentation at this conference, FMD is discussed in detail. When dealing with FMD, molecular typing can be helpful in a variety of ways. FMD virus is an RNA-virus with a high mutation rate, resulting in multiple changes in the genetic code with each replication cycle.[20] To read the RNA-sequence, reverse transcription PCR is used, followed by sequencing of the amplified nucleic acid. Nucleotide sequencing is used to identify strains, and to track their sources and movement, including illegal trade.[3,20] When isolates are more than 85% identical at sequence level, they are placed in groups called "topotypes", a term that reflects limited geographical or topological distribution. For example, all recent FMD isolates from the Philippines belong to the Cathay topotype. This topotype was probably introduced from China or Hong Kong, the only other places where the topotype was known to exist.[20] Other FMD virus strains, specifically the PanAsia strain, do not have a limited geographical distribution at all. Between 1998 and 2001, the PanAsia strain caused a pandemic in Asia, with subsequent spread to Africa and Europe, resulting in FMD outbreaks in Korea, Japan, Russia, Mongolia, South Africa, the UK, France, Ireland and The Nether-

lands. The jump from Asia to Africa was made through feeding of pigs with uncooked swill from a ship that docked in Durban.[20] Its rapid spread throughout Eurasia is largely unexplained, and certainly unprecedented. The speed of spread is thought to be due to evolution of a new, highly transmissible strain that is not contained by control measures that effectively prevent the spread of other FMD virus strains. Establishment of international early-warning systems to monitor the development and spread of such strains may help to prevent future FMD pandemics.[20] So far, the PanAsian strain has not been detected in the Americas. Outbreaks with other FMD strains have been reported from South America, i.e., from previously FMD-free regions in Brazil and Argentina.[23] The outbreaks are attributed to FMD virus variants that are endogenous to South America, specifically those that also caused sporadic outbreaks in neighboring areas that are otherwise in advanced stages of FMD eradication. The strains from Brazil and Argentina were distinct from those used for vaccine production, from strains occurring in the Andean region and from strains occurring on other continents, implying that small pockets of FMD virus continue to exist but that re-introduction of virus from other parts of the world has not occurred.[23] The variability of FMD viruses has implications for vaccine development.[3] A vaccine that may be effective against one strain may fail to provide protection against other strains. In some countries, multiple serotypes and topotypes of the FMD virus occur, and a multivalent vaccine specific to the area is needed to control transmission of all circulating strains.[3] Updating of the antigenic composition of FMD vaccines in response to occurrence of new FMD strains during outbreaks may contribute to successful outbreak control. Molecular methods are indispensable for detection of new strains.[24]

Disaster on a smaller scale, but more likely to occur in the USA or Canada, and no less disastrous when your clients or your family are involved, is disease in humans as a result of contact with dairy animals or dairy products. Many pathogens or commensals of dairy calves and cows can cause disease in humans, including *Cryptosporidium parvum*, *E. coli* O157:H7 and other shiga toxin-producing *E. coli* (STEC), *Salmonella enterica* serotype Typhimurium, and *Campylobacter jejuni*.[31] Most of these organisms are shed in feces. Exposure of humans may result from direct contact with animals or animal feces, from fecal contamination of raw milk or raw milk products, or from fecal contamination of the environment, including water sources. To trace the source of zoonotic or foodborne disease in humans, often children, molecular methods are used. Outbreaks of *Campylobacter jejuni* and STEC (*E. coli* O26:H-) in adults and children have been traced to raw milk consumption, and back to the farms from which the raw milk originated.[1,13] Infections with *E. coli* O157:H7 have been traced back to healthy goats, sheep and calves in petting zoos and dairy farms open to visitors.[6,8,15] Even when there is no direct contact between children and cows, calves or raw milk, *E. coli* O157:H7 may cause disease.[17] In one case, culture and molecular investigations revealed that fecal contamination of well water by healthy cattle resulted in bloody diarrhea and hospitalization of a child living on the farm. Without molecular tools, it would have been difficult to identify the source of infection and to prevent this from ever happening again.

In some cases, cows and people are infected with the same pathogen, and it is not clear who is the culprit and who is the victim. This is particularly true for *Staph. aureus*. Purulent dermatitis in a dairy farmer and multiple cases of mastitis in his cows has been described.[12] Similarly, occurrence of methicillin-resistant *Staph. aureus* (MRSA) in cattle and people has been described. In early reports, MRSA in dairy cattle was attributed to infection by milkers.[9] At the time, molecular methods to support the identity of human and bovine isolates were not available. More recent reports of MRSA in dairy cows and milkers do show that all isolates belonged to the same strain.[18] Based on PFGE typing, it is not possible to determine who infected whom. Based on multilocus sequence typing (MLST), a DNA-sequencing based method for which increasingly large reference databases exist, *Staph. aureus* isolates can be assigned to clonal complexes, some of which are host-species associated. Mastitis causing *Staph. aureus* mostly belongs to clonal complex 97 (CC97), while very few human infections are caused by members of that clonal complex.[30] MLST showed that the MRSA strains affecting humans and cattle belong to a clonal complex other than CC97.[18] Hence, infection of cattle by humans appears the most likely route of transmission. To prevent new cases, infected cows should be treated or culled, and the infected person should be treated or prevented from coming in contact with the cows.

Conclusion

Molecular methods are highly diverse, and can be used in a highly diverse range of applications. In this paper, examples were discussed for milk and dairy product quality, outbreaks of mastitis and viral diseases, contaminated animal health care products and vaccines, FMD and food borne disease. Many other examples exist, dealing with *Mycoplasma* in dairy herds and dairy calf operations; dairy products involved in food borne infections and food poisoning; zoonotic salmonellosis and cryptosporidiosis; detection of a wide range of pathogenic bacteria, viruses and protozoa using PCR-based methods; detection of virulence, toxin and antimicrobial

resistance genes etc. Molecular methods have contributed significantly to current insights into disease transmission mechanisms, and hence to our ability to control disease. Although not used routinely in dairy practice, the extra effort needed to get access to a laboratory that performs molecular typing may well pay off for investigation of disease outbreaks or production problems.

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