

Regional Dairy Quality Management Alliance (RDQMA)

Introduction

We invite you to read through our 2009 annual report and learn about the results of our research project. We are pleased to share our findings and we are working hard to feed the information back to dairy farmers across the country. At the same time we are publishing our data in peer-reviewed scientific publications to advance the knowledge of animal health, farm profitability, and endemic infections on dairy farms.

One of the objectives of the project is to track endemic disease dynamics over time in Northeast dairy herds with well-characterized animals and herd management practices. The main pathogens of interest are four food-borne pathogens: *Salmonella* spp., shigatoxigenic *Escherichia coli*, *Listeria monocytogenes*, and *Campylobacter* spp. Besides these organisms we also study *Mycobacterium avium* subspecies *paratuberculosis* (MAP), the causative agent of Johne's disease in cattle, and other animal health pathogens.

The project

Each of three enrolled farms (Farms A, B, and C) receives quarterly farm visits from the project team in their state. At each visit, an online management survey is completed, environmental samples are collected, and blood samples are taken from all lactating cows. Individual fecal samples from lactating cows are taken every six months. Financial data and nutrient management data are gathered annually using the Dairy Farm Business Summary and a Mass Balance system, both Cornell University extension programs. Weekly bulk tank milk samples are analyzed for milk quality as well as for bacterial contamination. Data is stored in an Access Database and biological samples in a secure Biobank that will allow for future research endeavors. Additionally, cull cows are tracked from the farm to the slaughter house; tissues along with data are collected at slaughter and provided to us by our USDA-FSIS collaborators.



The mission of the RDQMA is to assure a healthy and safe food supply by advocating the adoption of Best Management Practices (BMPs) in Northeast dairies.

The RDQMA goals are to:

- promote animal health and welfare,
- improve productivity and profitability,
- encourage environmental stewardship among Northeast dairy farms.



Highlights from 2009

Some of the research highlights from the last year are presented in this annual report. A very short overview of the highlights includes the *Salmonella* strain competition that we are observing in one of the farms (page 2), *Listeria* persistence and evidence for biofilm formation on another farm (page 3), the presence of *E. coli* virulence factors on all three farms (page 4), the low level of antimicrobial resistance in major pathogens on dairy farms (page 5), a quantification of the production losses associated with different levels of shedding in Johne's disease (page 6) and then the incredibly complicated molecular epidemiology of MAP on our farms (page 7). Finally, the back page of the report provides an overview of activities that are going on in our group by listing recent publications and manuscripts that are currently in the process of being completed. Please take a look at these highlights and enjoy the discovery work on non-research, producer operated, commercial dairy farms.

A collaboration of:

Cornell University
The Pennsylvania State University
The University of Pennsylvania
The University of Vermont
USDA Agricultural Research Service
Food Safety and Inspection Service



-Results-

Salmonella

In addition to its impact on animal health, dairy farm-associated *Salmonella* represent a significant public health risk. Asymptomatic *Salmonella* shedding is common in dairy herds and prevalence estimates suggest that as many as 1/3rd of US dairy herds may be *Salmonella*-positive. Many serotypes have been identified in surveys of dairy cattle, dairy cattle facilities, and in raw milk, and more than half of the ‘top 20’ human clinical serotypes have also been isolated from dairy operations. Multiple serotypes are frequently identified in a single operation.

Each of the three study farms has provided us with opportunities to learn more about the huge variability in the dynamics of *Salmonella* within dairy herds. We have been testing samples from Farms A and B for *Salmonella* for over five years while those from Farm C have been tested for over four years. During this time, *Salmonella* has been isolated from all three herds. On Farm A, *Salmonella* was detected sporadically in fecal samples but with no apparent clinical consequences. *Salmonella* never established a ‘foothold’ in this herd. On Farm C, *Salmonella* serotype Uganda was isolated from environmental and several fecal samples during a full farm sampling. Testing of additional samples that were obtained immediately showed that *Salmonella* was not being shed in the milk from infected animals, that young calves on the farm were not infected, but that the organism could still be detected in composite manure samples obtained from shared alleys in the milking herd barns. Within two months however, no *Salmonella* could be detected on Farm C and it appeared that the outbreak had run its course. Several other serotypes have also been isolated from this herd but they have had minimal clinical impact and did not persist in the herd or in the environment.

In contrast to Farms A and C, Farm B experienced a sustained, subclinical outbreak of *Salmonella*. We previously reported that this dairy herd had an endemic infection of *Salmonella* Cerro. We report here on the gradual shift of the serotype being carried by this herd from Cerro to Kentucky (Figure 1). *Salmonella* Cerro was first detected on this farm in the summer of 2004 and 44% of the herd was infected in Sep. 2004. Symptoms of salmonellosis were not observed. Herd prevalence ranged from 8% to 88% in the following months leading up to Jan. 2006. In Jan. 2006, 87 of the positive cows were shedding Cerro but 2 of the animals were shedding Kentucky. Cerro was gradually supplanted as the predominant serotype and by the summer of 2007, Cerro represented less than 10% of the isolates and Kentucky was the dominant serotype.

Understanding the factors that enabled *S. Kentucky* to supplant *S. Cerro* in this herd may provide insights into the development of methods to control asymptomatic and perhaps clinical

infections. Although *S. Cerro* has caused disease in humans, it is an uncommon human isolate. *Salmonella* Kentucky is more frequently associated with human infections. Although asymptomatic carriage does not apparently impact animal production, it still represents a risk for public safety. Strain competition as we have now precisely documented (Figure 1) is a phenomenon that is common among bacterial and viral species. The quality of our data with very good longitudinal follow-up of individual animals over time will allow us to better understand this phenomenon.

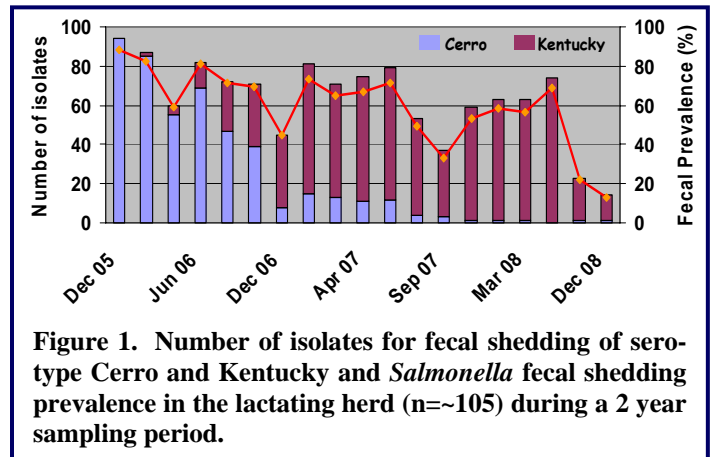
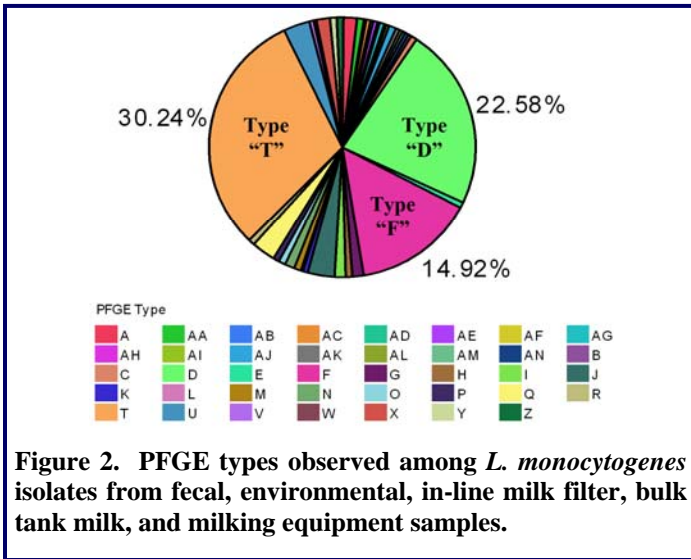


Figure 1. Number of isolates for fecal shedding of sero-type Cerro and Kentucky and *Salmonella* fecal shedding prevalence in the lactating herd (n~105) during a 2 year sampling period.

Listeria monocytogenes

Molecular typing can be used to determine the genetic relationship between bacterial isolates of the same species. Molecular typing using pulsed field gel electrophoresis (PFGE) of one isolate from each *L. monocytogenes*-positive sample collected from Farm A between Feb. 2004 and Jun. 2008, was recently completed. PFGE is a technique used to discriminate between bacterial isolates and is typically used in epidemiological studies of pathogenic bacteria to link clinical isolates with the outbreak source. Chromosomal DNA is extracted from the bacteria, enzymatically cut into relatively large pieces, and the resulting DNA ‘chunks’ are separated on a specialized agarose gel. The banding patterns are compared between isolates to determine the relatedness of the isolates – if the profiles are the same, the isolates are considered ‘clonal’. A total of 248 *L. monocytogenes* isolates from fecal, environmental, bulk tank milk, in-line milk filters, milking equipment, and parlor samples were typed by using *Ascl* and *Apal* restriction endonucleases. Combined restriction digest analysis yielded 39 PFGE types. The most predominant PFGE types from all categories of samples corresponded to PFGE type T (30.2%), D (22.5%), and F (14.95%) (Figure 2).

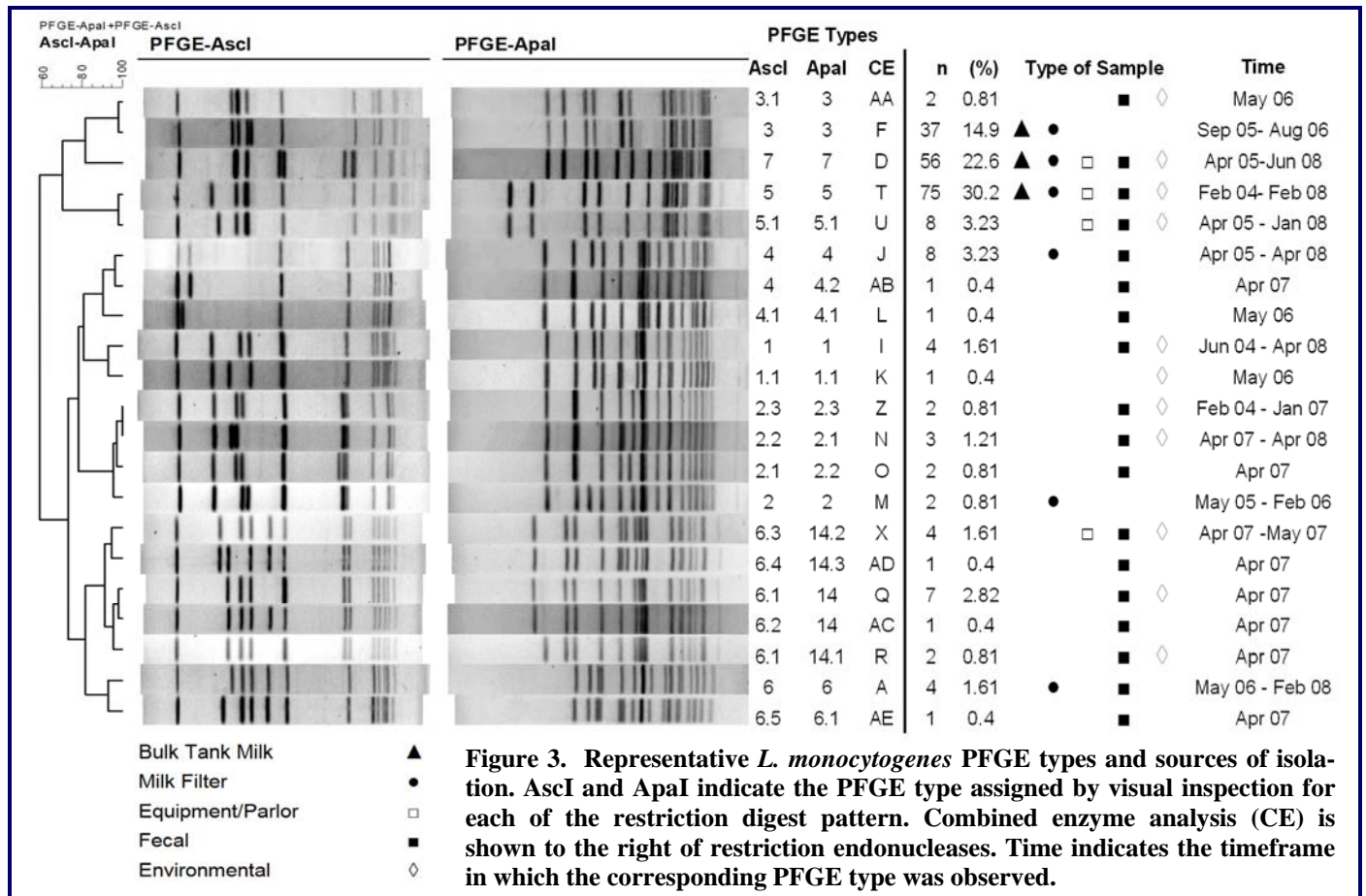
A large heterogeneity of PFGE types was found among isolates from fecal and environmental samples, while a large



their respective subtypes were only found in cow feces collected during the sampling conducted in Apr. 2007. Other PFGE types, such as PFGE types J and T (and their subtypes) persisted over time, although most of the PFGE types observed in *L. monocytogenes* isolates from feces were sporadically found. In 28 positive environmental samples, 19 different PFGE types were observed. Similar to fecal isolates, PFGE type T persisted over time in environmental samples, but in general a large diversity of sporadic PFGE types was observed.

In 101 *L. monocytogenes* isolates from in-line milk filter samples, 9 PFGE types were observed. The predominant PFGE types corresponded to PFGE type T (38.6 %), D (26.7 %), and F (26.7 %). Other PFGE types observed in in-line milk filter samples were PFGE types A, J, M (which matched fecal PFGE types), S, AJ, AC, and AO (unique types), which overall accounted for 7.9% of the PFGE types observed within this category of sample. In 45 *L. monocytogenes*-positive bulk tank milk samples, only 3 PFGE types were observed: PFGE type D (42.3%), T (38.4%), and F (19.2%). Likewise, PFGE types T and D were also found in milking equipment. PFGE type T was the most predominant type (66.6%) observed in *L. monocytogenes*-positive milking equipment samples. In milk meters, this PFGE type T persisted over a 9 months period.

homogeneity of PFGE types was observed among *L. monocytogenes* isolates from bulk tank milk and in-line milk filter samples (Figure 3). In 57 positive fecal samples, a total of 29 PFGE types were observed. Some PFGE types were found in specific sampling dates. For example, PFGE types Q, D, and



Undistinguishable or clonal PFGE types of *L. monocytogenes* isolates from fecal and environmental samples suggest that cows could be a likely source of *Listeria* for the farm environment. The large heterogeneity of PFGE types among *L. monocytogenes* isolates from fecal/environmental samples, contrast with a large homogeneity of PFGE types in milk filters, bulk tank milk, and milking equipment. A biofilm within the milk system could be a source of bulk tank milk contamination, explaining thus the homogeneity of PFGE types in these categories of samples. Bacterial biofilms are a common concern in food manufacturing. They are able to establish on many surfaces and can persist for long periods of time. If biofilms are established in the milking equipment, sloughing of cells from the biofilm can provide a continuous source of bacterial contamination of the milk. In our previous work (submitted for publication in the Journal of Dairy Science) we confirmed the presence of a biofilm in milk meters from Farm A. But the presence of a *L. monocytogenes*-containing biofilm could not be confirmed. However, our data suggest the presence of a persistent source of *L. monocytogenes*, consistent with the hypothesis of a biofilm. We are currently working in assessing the biofilm forming ability of representative *L. monocytogenes* strains from Farm A.

The next step in *L. monocytogenes* research will be the risk assessment of listeriosis caused by the consumption of raw milk contaminated with *L. monocytogenes*. The risk assessment model will include on-farm consumption of raw milk as well as the consumption of certified raw milk.

Escherichia coli

Food-borne diseases due to zoonotic bacterial pathogens are of increasing concern in the US and around the world. Enterohemolytic *Escherichia coli* (EHEC) are zoonotic pathogens that can cause severe disease in humans, causing a bloody diarrheal disease known as enterohemorrhagic fever which sometimes leads to the more serious hemolytic uremic syndrome (HUS). *E. coli* O157:H7 is the EHEC strain most commonly associated with enterohemorrhagic fever and HUS in the US but it is thought to cause only 50 to 60% of cases with other serotypes of EHEC responsible for the remainder. While the actual set of virulence genes required for a strain of *E. coli* to be considered an EHEC strain is not yet known, most strains carry genes for shiga-like toxins (*stx1* and/or *stx2*) and a hemolysin (*hlyA*) along with a pathogenicity island known as the locus of enterocyte effacement (LEE), which carries genes encoding a number of factors involved in the binding of the pathogen to intestinal cells. The genes encoding the bacterial surface protein intimin (*eaeA*) and the translocated intimin receptor (*tir*) are part of the LEE.

In this study, we used real-time polymerase chain reaction (PCR) assays to analyze fecal and environmental samples from

three study farms for the occurrence of virulence factors associated with EHEC within the coliform populations. The quantitative nature of the real-time PCR data is used to infer the likelihood of EHEC presence in samples. The real-time PCR detects the presence or absence of a target sequence in the populations of bacteria that grew in the EC enrichment and also provides a cycle threshold value (Ct, the number of PCR cycles at which fluorescence is detected) which is inversely proportional to the amount of target DNA in the sample. Thus, if PCR of a sample yields a relatively low Ct value, it is an indication that the target DNA sequence is present in a relatively high number of the bacterial genomes in that sample. The relative amount of each target in a sample is also indicative of the pathogen content of a sample.

The *eaeA* gene encoding intimin was detected in the feces of 79% (range 54-97%), 73% (54-89%), and 92% (75-100%) of the cows on Farms A, B, and C, respectively. The incidence of the gamma allele of the translocated intimin receptor (γ -*tir*) that is associated with O157:H7 was generally low on all three farms but shows periodic spikes; it was detected in 28% (6-64%), 28% (2-58%), and 42% (3-90%) of the fecal samples from the respective farms. The *stx1* and *stx2* genes encoding shiga-like toxins were found less frequently in fecal samples from Farm A (*stx1* 47% [22-77%]; *stx2* 37% [25-57%]) than in those from Farm B (*stx1* 68% [53-84%]; *stx2* 69% [52-79%]) or Farm C (*stx1* 63% [39-88%]; *stx2* 62% [32-74%]). The Ct values for all 4 genes are clustered towards the high end (low gene concentration) on Farm A while the concentration of both *eaeA* and *stx 2* tend higher in Farm B samples (Figure 4 for Farm B). Except for *eaeA*, the concentration of virulence genes is generally low in enrichments of samples from Farm C.

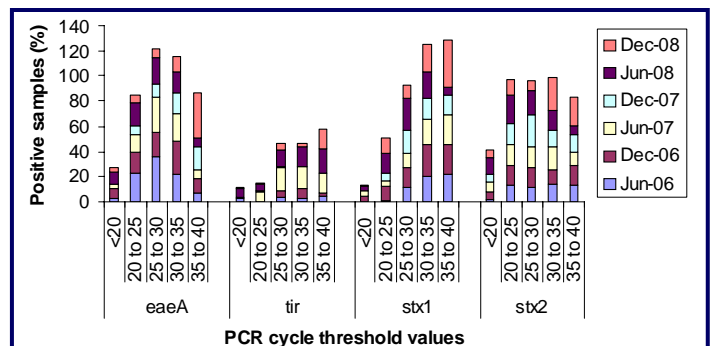


Figure 4. Distribution of cycle threshold (Ct) values among positive samples for Farm B *E. coli* virulence factors. [The Y-axis is the percent of positive samples (for any factor) that were positive for that particular one on a given sampling. They stack up to more than 100% because the axis values only pertain to one sampling (i.e., one color) at a time.]

Coliform bacteria containing one or several of the virulence factors associated with the ability to cause gastrointestinal disease seem to be ubiquitous on these three dairy farms

although the incidence of O157:H7 seems rather low. Other shiga-toxigenic forms probably are responsible for most of the *stx* PCR signals. In cows, carriage of O157:H7 does not seem to persist as, in the limited number tested so far, it was not detected in multiple samplings. In the environment, O157:H7 and shiga-toxin-containing strains in general seem to be more prevalent in areas housing younger animals. As part of this study, weekly milk and milk filter samples were tested by the multiplex PCR method used here and, although virulence factors were sometimes found in populations of coliform bacteria enriched from milk filters, they were rarely found in bulk tank milk.

Enterococcus, Escherichia coli, and Campylobacter

All environmental samples (collected quarterly) and approximately 25% of the fecal samples (collected biannually) from each farm were cultured for *Enterococcus*, *E. coli*, and *Campylobacter*. In 2008, 574 samples were cultured for *Enterococcus* and *E. coli*, and 505 samples were cultured for *Campylobacter*. The prevalence of positive samples across farms was 84.1% (n=483), 89.0% (n=511) and 34.3% (n=173) for *Enterococcus*, *E. coli* and *Campylobacter*, respectively. The three most prevalent species of *Enterococcus* (n=466) recovered were *E. hirae* (22.1%), *E. faecalis* (22.1%), and *E. faecium* (21.0%). *Campylobacter* isolates (n=173) were comprised of 98.8% *C. jejuni* (n=171) and 1.2% *C. coli* (n=2). The discrepancies between the number of isolates identified and those tested for susceptibility are due to the following: for each of *E. coli* and *Campylobacter*, one isolate died before being tested and for *Enterococcus*, 17 isolates did not grow in susceptibility testing panels.

Antimicrobial resistance

Antimicrobial susceptibility testing of the bacterial isolates collected in these studies were performed using antimicrobial panels similar to those used by the animal arm of the National Antimicrobial Resistance Monitoring System (NARMS; <http://www.ars.usda.gov/Main/docs.htm?docid=6750>).

In 2008, only 2 of 117 *Salmonella* isolates (1.7%) were resistant to any of the antimicrobials tested. Both isolates were identified as serotype Heidelberg and were resistant to kanamycin, streptomycin, sulfizoxazole and tetracycline. **Table 1** Shows the prevalence of antimicrobial resistance for *Salmonella* and generic *E. coli* isolates. Among the *E. coli* isolates (n=510) tested, 91.2% were susceptible to all antimicrobials tested, 4.9% were resistant to a single drug, and 3.9% were multidrug resistant. Among *Campylobacter* species (**Table 2**); 32.4% of the *C. jejuni* isolates (n=55) were susceptible to all antimicrobials tested while 67.6% (n=115) showed resistance to a single drug (tetracycline). Both *C. coli* isolates were resistant to only one drug (tetracycline). Among *Enterococcus* isolates

Table 1. Prevalence of antimicrobial resistance among Salmonella and E. coli isolates (in %)

Antimicrobial	<i>Salmonella</i>	<i>E. coli</i>
	n=117	n=510
Amikacin	0	0
Amoxicillin/Clavulanic Acid	0	0.6
Ampicillin	0	1.6
Cefoxitin	0	0.4
Ceftiofur	0	0
Ceftriaxone	0	0
Chloramphenicol	0	0.4
Ciprofloxacin	0	0
Gentamicin	0	0.2
Kanamycin	1.7	3.3
Nalidixic Acid	0	0
Streptomycin	1.7	2.8
Sulfizoxazole	1.7	2.8
Tetracycline	1.7	7.8
Trimethoprim/Sulfamethoxazole	0	1.2

Table 2. Prevalence of antimicrobial resistance among C. jejuni and C. coli isolates (in %)

Antimicrobial	<i>C. coli</i>	<i>C. jejuni</i>
	n = 2	n = 170
Azithromycin	0	0
Ciprofloxacin	0	0
Clindamycin	0	0
Erythromycin	0	0
Florfenicol	0	0
Gentamicin	0	0
Nalidixic Acid	0	0
Telithromycin	0	0
Tetracycline	100	67.6

Table 3. Prevalence of antimicrobial resistance among Enterococcus isolates (in %)

Antimicrobial	<i>Enterococcus</i> (n=466)
Chloramphenicol	1
Ciprofloxacin	9.4
Erythromycin	5.1
Flavomycin	67.6
Gentamicin	0
Kanamycin	7.5
Lincomycin	86.5
Linezolid	0
Nitrofurantoin	2.4
Penicillin	0.2
Synercid*	6.0
Streptomycin	3.2
Tetracycline	14.8
Tylosin	4.9
Vancomycin	0

**E. faecalis* excluded from Synercid % resistance calculation. Daptomycin and Tigecycline do not have CLSI approved breakpoints for resistance therefore resistance to these antimicrobials was not evaluated .

(n=466; **Table 3**) eight were susceptible to all antimicrobials tested, 13.5% were resistant to a single drug, and 84.8% were multi-drug resistant. These data continue to demonstrate considerable variability in antimicrobial susceptibility of zoonotic pathogens and commensal organisms isolated from healthy dairy cattle and their environment.

Johne’s disease: Impact on milk production and molecular approach to understand MAP dynamics

The RDQMA project has done much to advance our understanding of Johne’s disease (JD). JD is a chronic disease of ruminants caused by intestinal infection with the pathogen *Mycobacterium avium* subspecies *paratuberculosis* (MAP). The pathogen is pervasive on US dairy farms, with a herd prevalence of approximately 68%. In our previous reports we have described the identification of MAP super-shedders on these study farms. We have also recognized the role that super-shedders play in the creation of passive shedders. Over time, many passive shedders become infected with MAP in their intestinal tissues (adult infection). We recognized that virtually all active shedders have the potential to become super-shedders and super-spreaders of MAP. In this report, we are describing a few of the new findings of our research.

Johne’s disease: Impact on milk production in individual cows

JD has been estimated to cost the US dairy industry \$200 to \$250 million annually. The cost of JD is manifested in a variety of ways, but milk production losses are the most insidious. In this study, our objective was to estimate the effect of JD status on individual cow milk production using longitudinal data collected over a 4-yr period from 3 US dairy herds enrolled in the RDQMA project. JD status (uninfected, latent, low shedding, and high shedding), as indicated by MAP infection levels, was determined through quarterly ELISA serum testing, biannual fecal culture, and culture of tissues at slaughter. Milk production data were collected from the Dairy Herd Improvement Association. The effect of JD status on milk production was analyzed using a mixed linear model with an autocorrelation random effect structure.

JD status was found to have a significant effect on milk production, and this effect was not uniform across JD status categories. Our data indicate that cows that eventually will show low and high shedding of MAP are out-producing MAP-negative animals in the herd. Although latent animals produce more milk than uninfected animals, that difference decreases over time in the latent infection state. When an animal starts shedding low levels of MAP, the model predicts an initial milk production that is slightly higher than that of uninfected herdmates, but there is a greater rate of decrease in milk

production compared with the latently infected animals. Finally, animals in the high-shedding category have a meaningfully lower milk production than uninfected herdmates, with large decreases in production over time when remaining in the herd. **Figure 5** shows the predicted lactation curves for an average animal in herd A compared across JD status categories. Greater milk yield is evident during latency compared with uninfected herdmates, but the discrepancy in yield decreases as the disease progresses over time. This MAP-induced decrease in milk production is supported by the clinical progression of JD. As the organism invades the intestinal epithelium and begins to affect nutrient absorption, feed efficiency decreases and milk production is negatively affected.

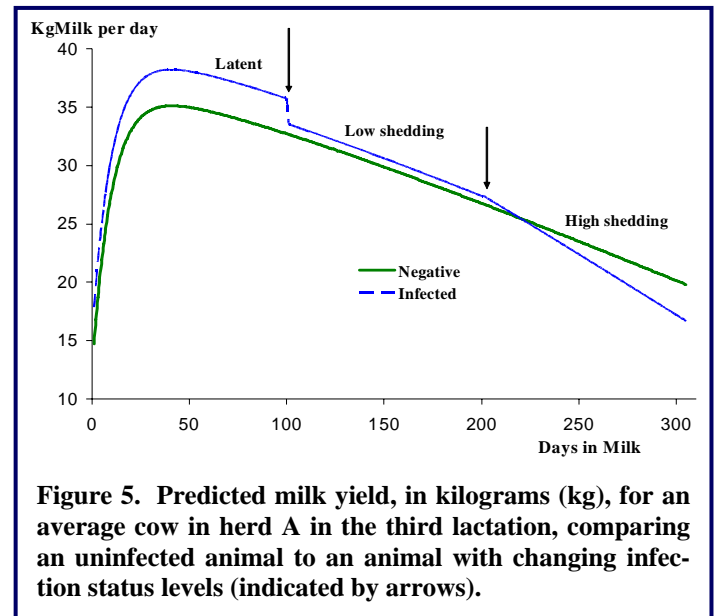


Figure 5. Predicted milk yield, in kilograms (kg), for an average cow in herd A in the third lactation, comparing an uninfected animal to an animal with changing infection status levels (indicated by arrows).

This analysis provides strong support that JD status affects milk production in all infected animals, with increasing losses in milk production as disease progresses. The above-average milk production of animals later affected by JD further highlights the important loss of milk production in high potential animals. These results will be useful in making culling decisions on an individual-animal, economic level, especially as animals shedding MAP also spread the infection through environmental contamination.

Johne’s disease: DNA-based sequencing technique to understand MAP dynamics

Based on the new dimension of super-shedders, researchers suggested that significant proportion of low shedders (culture-positive fecal samples) are likely to represent passive shedding (or false positive fecal samples), i.e., by excreting the bacteria as pass-through infectious material and not true infections.

In this study, we used a DNA-based molecular subtyping technique to better understand the epidemiology of JD and MAP dynamics in dairy cattle. Short sequence repeats (SSR) refers to repeats of nucleotides, which can be identified as variable number tandem repeats (VNTR) in the genome of the organism and can be used as markers for strain typing. By using a multi locus short sequence repeat (MLSSR) technique in combination with the observed MAP shedding level, we aimed to evaluate: (i) whether low shedders of MAP were passive shedding (pass-through) animals or whether they were truly infected, and (ii) whether these animals were possibly infected by the super-shedders. For MLSSR strain typing, we have used four loci (locus 1, 2, 8, and 9) with the highest genetic diversity values (D-values).

For three farms, from a total of 142 isolates (from fecal and tissue samples) analyzed, we found 15 different strains (i.e., genotypes or MLSSR types); 9 types on Farm A, 7 types on Farm B, and 6 types on Farm C (Figure 6). The results indicated herd-specific infections; a clonal infection in herd C with 89% of animals sharing the same strain (type 2), different strains in herds A and B. On Farm C, almost all isolates from super-shedders were type 2. Type 4 was the most predominant one on Farm A (59%) and most super-shedder isolates belonged to this type. On Farm B, we found a variety of strains from a limited number of isolates and animals from which these samples were collected were purchased from different sources.

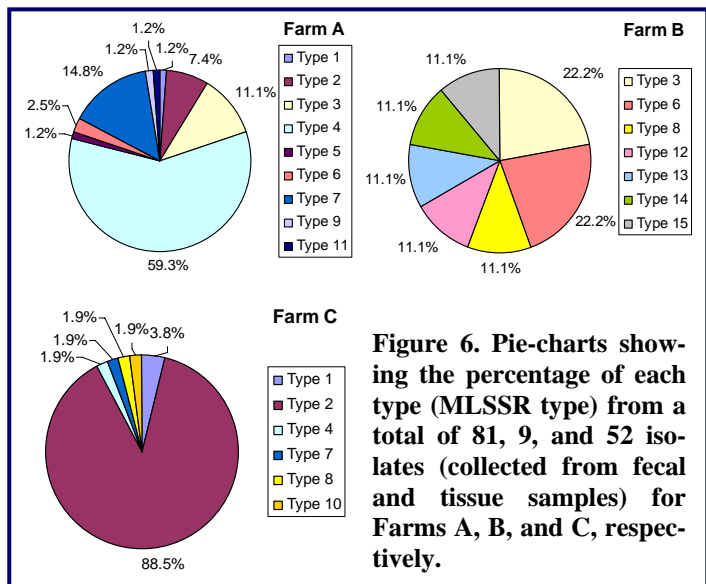


Figure 6. Pie-charts showing the percentage of each type (MLSSR type) from a total of 81, 9, and 52 isolates (collected from fecal and tissue samples) for Farms A, B, and C, respectively.

On Farm C, 100% and on Farm A, 17 to 70% of cows shed the same strain as that of contemporary super-shedders. For available tissue data, tissues from about 82% of cows other than super-shedders were culture-positive for MAP, indicating a true infection. Based on the results of MAP strain-typing and shedding levels, at least 50% of low shedders have the same strain as that of a contemporary super-shedder. The results of

this study indicate that very few cows had characteristics of a possible pass-through animal; many more cows were truly infected. Sharing of the same strain of low shedders with the contemporary super-shedders suggests that low shedders may be infected by the super-shedders. Successful control strategies for JD require a good understanding of the epidemiology of a disease. The use of SSR strain-typing combined with observed shedding levels provided a unique opportunity to get a better insight into herd infection and dynamics of MAP.

RDQMA Project direction for 2009 and 2010

For the coming year the basic features of the project will stay on course by continued intensive longitudinal surveillance of the farms that are currently enrolled. While collecting all these data, our research team is also developing new methods and techniques. A series of recent papers from both the ARS scientific teams and the university scientists on this project have testified to this (see the list of publications in this news bulletin). On top of generating these enormously valuable baseline data and research methods there are a number of additional projects that the team will work on:

- We continue to implement the intervention strategy (a vaccine) to reduce the prevalence of *Salmonella* spp. on the *Salmonella* infected farm. This farm provides a unique opportunity to estimate vaccine efficacy under field conditions.
- We plan to implement a major effort on sequencing the *Salmonella* isolates from our farms. The objective is to better understand the biology behind persistent *Salmonella* infections in dairy cows.
- Further studies into the mathematical modeling of Johne’s disease on dairies. We plan to model the efficacy of vaccination against Johne’s disease using stochastic modeling.
- We continue our efforts in *Listeria* infection ecology on dairy farms. We will study the formation of biofilms in milking equipment and methods to eliminate or prevent these biofilms.
- A risk assessment component will be added to the project. With the data that we have collected on bulk tank milk infections and slaughter house data, we will be able to develop risk assessment models to quantify the safety of the raw food supply.

If you or your staff would be interested in the result of our annual RDQMA meeting in November 2009, in State College, PA, please contact Abani Pradhan (contact information in the last page) and we will be sure to provide you with the meeting summary.

Thank you for your interest!
The RDQMA project team.



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**Manuscripts under review/in preparation/
work in progress:**

- Fedorka-Cray, P. J., et al. 2009. Antimicrobial resistance patterns of *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterococcus* spp. isolated from dairy cattle in the northeastern United States.
- Karns, J. S., et al. 2009. PCR analysis of pathogenic *E. coli* on three dairy farms in the northeastern US.
- Latorre, A. A., et al. 2009. Biofilm in milking equipment on a dairy farm as a potential source of bulk tank milk contamination with *L. monocytogenes*.
- Latorre, A. A., et al. 2009. Molecular epidemiology of *Listeria monocytogenes* on a New York state dairy farm: Heterogeneity among fecal and environmental isolates and homogeneity in bulk tank milk and in-line milk filter isolates.
- Mitchell, R. M., et al. 2009. A meta-analysis of the effect of dose and age-at-exposure on shedding of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in experimentally infected calves and cows.
- Pradhan, A. K., et al. 2009. Multilocus short sequence repeat analysis of *Mycobacterium avium* subsp. *paratuberculosis* isolates from dairy herds in northeastern United States of a longitudinal study indicates low shedders are truly infected.
- Smith, J. M., et al. 2009. Johne's "supershedder" results in extensive environmental contamination with *Mycobacterium* subsp. *avium paratuberculosis* organisms.
- Smith, R. L. et al. 2009. The effect of Johne's disease status on reproduction and culling in dairy cattle.
- Van Kessel, J. S., et al. 2009. *Salmonella* serotype shift during an endemic dairy infection.
- Zanella, R., et al. 2009. Identification of loci associated with tolerance to Johne's disease in Holstein cattle.

Peer-reviewed publications:

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