

# Northern NY Agricultural Development Program 2006-2007 Project Report

## *Klebsiella* Mastitis in Dairy Cows: Sources, Transmission and Control Points

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### **Background:**

Mastitis is the most common and costly disease of dairy cattle. Losses due to mastitis have been estimated at \$200 per cow per year in the USA. The magnitude of losses depends in part on the organism causing mastitis. Judging by producer testimonials and culture results from our diagnostic laboratory, *Klebsiella* is an increasingly important cause of mastitis in New York. Its economic impact can be devastating because vaccines and antimicrobial treatment have limited impact on severity of mastitis, milk loss, death and culling due to *Klebsiella*. Prevention is the main control strategy for *Klebsiella* mastitis.

For many years, sawdust and shavings were considered the most important sources of *Klebsiella* and use of *Klebsiella*-free bedding was thought to be adequate for prevention of mastitis. In recent years, however, *Klebsiella* mastitis has become an issue in herds that use other bedding materials such as recycled manure or even sand bedding. In previous work, we showed that on average 80% of healthy adult dairy cattle shed *Klebsiella* in their feces during the summer. Fecal contamination of the farm environment, feed, water and animals is common, and it seems likely that exposure to *Klebsiella* could result from contact with sources other than bedding.

In this project, sources and transmission routes of *Klebsiella* mastitis on dairy farms were determined, so that critical control points for prevention could be identified. Initially, the presence of *Klebsiella* in feed and water, manure and bedding was evaluated. Early results suggested that animal hygiene may be an important indicator of the risk of exposure to *Klebsiella*. In the second half of the project, we specifically measured the association between hygiene scores and the presence of *Klebsiella* on the animals' legs and teats. We also evaluated the potential role of feed crops as source of *Klebsiella*. This work enabled us to pinpoint sources that harbor disease-causing *Klebsiella* species and that are critical points in the control of *Klebsiella* mastitis.

The second goal of this project was to educate dairy producers and farm advisors about sources, transmission and control of *Klebsiella* mastitis. Using the research results, we produced a fact sheet on *Klebsiella* mastitis control for distribution to dairy producers, veterinarians and other farm advisors in Northern New York. In addition, information was and will continue to be disseminated through ongoing QMPS education and extension activities, including presentations at the Miner Dairy Days, the National Mastitis Council Meeting, producer meetings, and continuing education meetings for veterinarians and publication in the dairy press.

This project benefits dairy farmers, the dairy industry and dairy cows. Herds that are involved in the project received herd-specific results and management advice. A large audience of dairy farmers, veterinarians and students is reached through direct contact with QMPS staff, through publications in professional and scientific journals and through teaching and continuing education courses. The W. H. Miner Agricultural Research Institute in Chazy, NY, is a partner in the project and will play an active role in dissemination of the results to producers and students.

The dairy industry as a whole and consumers of dairy products benefit from improved understanding of methods to control *Klebsiella* mastitis because they will contribute to improvement of production efficiency, milk quality, and shelf life of milk. Finally, dairy cows will benefit. *Klebsiella* mastitis is a painful and often fatal condition that has a strong negative impact on cow health and animal welfare. Every case that we can prevent may be a cow's life saved.

### **Methods:**

In June, July, August and September 2007, feed, water and environmental samples were collected from farms in Clinton, Franklin and St. Lawrence County. This included samples from bedding (unused bedding, used bedding, and spots that were heavily soiled with leaked milk) and from alleyways. At the Miner Institute, samples from soil, corn, alfalfa, rumen content and feces were collected. In the other two counties, lower legs and teat ends of cows were sampled. Per visit, legs of 50 animals and teats of 50 different animals were sampled. Legs were sampled in lactating cow pens, whereas teats were sampled in the milking parlor. Teat ends were swabbed before and after pre-milking udder preparation. The preparation routine included use of teat disinfectants based on peroxide or iodine. Animals that were sampled were also scored for cleanliness.

All samples were tested for presence of *Klebsiella* using culture media that we developed specifically for *Klebsiella* detection. The selective culture plates are not perfect, and other bacterial species may grow on them. Isolates that looked like *Klebsiella* were therefore tested with additional laboratory methods to determine whether they were really *Klebsiella*. For isolates from crops, soil, rumen content and feces, we used DNA sequencing and DNA fingerprinting to identify bacterial species and bacterial strains within species, respectively. This allowed us to determine which types of samples contain the type of *Klebsiella* that has the ability to cause mastitis in dairy cows.

## **Results:**

### **Klebsiella in environmental samples**

Across herds and samplings, two-thirds of samples from alleyways, feces, and bedding tested positive for *Klebsiella*. Bedding drenched in milk due was *Klebsiella*-positive in 75% of cases. Most water samples, except those collected from faucets rather than drinking troughs, were *Klebsiella* positive, i.e. 83% of samples. Detection of *Klebsiella* was less common in TMR, which was mostly collected from feed alleys after cows had been eating. Only 30% of TMR samples tested positive. These results show that alleyways and soiled drinking water are important sources of *Klebsiella*.

### **Klebsiella on cows' legs and teats**

*Klebsiella* was very common on legs: on average, 59% of animals tested positive. In Herd 1, animal hygiene scores, environmental hygiene and detection of *Klebsiella* on legs were similar for August and September (56% vs. 60% of legs). In Herd 2, animal and environmental hygiene were much better in September than August and drastic reduction in the proportion of legs testing positive for *Klebsiella* was observed (98% vs. 22% for August and September, respectively). Results for teats showed a similar pattern: in Herd 1, 54% of teats tested positive for *Klebsiella* before udder prep, and 22% tested positive after udder prep, with little difference between months. In Herd 2, all animals had *Klebsiella* on their teats before udder prep, and 74% of animals still tested positive after udder prep in August. In September, only 34% of animals had *Klebsiella* on teats before prep, and there was almost no *Klebsiella* left (6%) after prep. Before udder prep, cows with clean udders were as likely to have *Klebsiella* on their teats as cows with dirty udders. After udder prep, however, most *Klebsiella* was removed from clean udders, whereas dirty udders still had *Klebsiella* on them in many cases. Bacterial isolates from swabs and from clinical mastitis cases were compared. The proportion of *K. pneumoniae* and *K. oxytoca* in clinical mastitis cases was farm-specific, and mirrored the proportion of *K. pneumoniae* and *K. oxytoca* on teat. This means that *Klebsiella* on teats is a very likely source of *Klebsiella* mastitis, that teat hygiene is very important in mastitis prevention, and that pre-dip is not sufficient to eliminate *Klebsiella* from dirty udder..

### **Klebsiella in soil, crops and the rumen**

Soil was collected in July, August and September. *Klebsiella* and *Klebsiella*-like organisms were detected 67% of corn plots and 57% of alfalfa plots that had received manure. Two of 3 alfalfa field plots that had not received manure for 50 years also tested positive. Based on DNA-sequencing, soil harbored four species of *Klebsiella* and *Klebsiella*-like organisms: *K. oxytoca*, *K. pneumoniae*, *K. variicola*, and *R. planticola*. Only the first two species are associated with mastitis. *Klebsiella* was isolated once from corn roots and once from alfalfa roots, but not from stems or leaves. In contrast, cultures from freshly cut corn, which was sampled just after harvest in September, showed heavy growth of *Klebsiella*. Of 10 corn varieties tested, 8 were positive for *Klebsiella* (80%). Most fecal samples (80%) and almost all rumen samples (95%) from cows at the Miner Institute contained *Klebsiella*. Almost all isolates from the rumen and feces were identified as *K. pneumoniae* based on DNA-sequencing results (93%), whereas almost all isolates from crops and soil were identified as one of the other species (87%). This implies that soil and crops are not important sources of mastitis-causing *Klebsiella*. Within many samples, multiple bacterial species could be found, and within a bacterial species, multiple strains could often be found within the sample.

### **Conclusions/Outcomes/Impacts:**

For years, wood-based bedding was seen as the most important cause of *Klebsiella* mastitis. Our earlier work on *Klebsiella* had shown that the bacteria are also very common in the feces of cows. This finding has been called a "paradigm shift" and raised two important questions: 1) does *Klebsiella* spread from the feces to the environment, so that the cows' udders are at risk of *Klebsiella* mastitis; and 2) does *Klebsiella* in the gut come from feed crops? The NNY project has enabled us to answer these questions with a resounding "Yes" for question 1, and an almost as resounding "No" for question 2.

Farm soil and crops contain organisms that are closely related to *Klebsiella*, but rarely the type of *Klebsiella* that causes mastitis. *Klebsiella*-free crops or soils will not solve *Klebsiella* mastitis problems. The main source of *Klebsiella* in the cows' rumen and gut is the constant intake with feed and, more importantly, drinking water that are contaminated with manure. Manure also contaminates bedding. Most used bedding contains *Klebsiella*, even if the bedding was originally *Klebsiella*-free. The real solution to *Klebsiella* mastitis lies in hygiene, particularly hygiene of alleys and holding pens. Most alleys and holding pens contain manure and *Klebsiella*. As cows walk around, manure gets onto their legs. It may also splash onto their udders. When cows lie down, *Klebsiella* may be transferred "from feet to teat". *Klebsiella* on cows' teats is only partially removed by pre-milking udder preparation, even when teat disinfectants are used. This means that cows are at risk of new *Klebsiella* infections in the barn as well as in the milking parlor.

*Klebsiella* mastitis is difficult or impossible to control with vaccination, antibiotic treatment or use of sand bedding alone. Cows contaminate their environment with *Klebsiella*, putting themselves at risk of mastitis. By keeping the cows and their environment clean, the risk of *Klebsiella* mastitis can be reduced. Hygiene of pens and alleyway is a critical control point in prevention of *Klebsiella* mastitis.

### **Outreach:**

1. *Klebsiella* Mastitis. Presentation at Miner Dairy Days by G. Bennet, November 2007.
2. *Klebsiella* Mastitis Prevention and Control - QMPS tri-fold for dairy producers, distributed in Northern New York through QMPS, CCE offices and veterinarians.
3. See list of reports and articles (will be made available on-line once published)
4. Press release by Kara Dunn, NNYADP
5. QMPS veterinarians and technicians disseminate results through their on-farm consulting activities, and their education and extension activities for dairy producers, dairy veterinarians, and students of animal and veterinary science.

### **Next steps:**

Prevention is the most important component of mastitis control programs. The 2007 funding from the Northern New York Agricultural Development Program has enabled us to identify critical control points in prevention of *Klebsiella* mastitis (most importantly: pen, alley and animal hygiene). Despite producers' best efforts to prevent mastitis, some cases will occur. In those cases, producers will try to save the cow through treatment. For some mastitis pathogens, it is known that specific characteristics of the cow and the bacteria affect chances of cure. This

knowledge enables producers to select candidates for treatment as opposed to culling, and to choose an appropriate treatment duration. Knowledge of such risk factors is not available for *Klebsiella* or for its cousin, *E. coli*. The next step would therefore be to evaluate characteristics of cows and *Klebsiella* and *E. coli* strains that affect the chance of cure. Ideally, this is done in the context of a treatment trial. QMPS will conduct a coliform mastitis treatment trial in 2008, which provides a unique opportunity to evaluate factors affecting cure on New York dairies.

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### **Reports and/or articles in which the results of this project have already been published.**

1. *Klebsiella* mastitis - beyond bedding. Northeast Dairy Business, January 2008.
2. *Klebsiella* - Not By Bedding Alone. R. Zadoks, M. Munoz, H. Griffiths, G. Bennett and Y. Schukken. NMC 47<sup>th</sup> Annual Meeting Proceedings, January 2008.
3. *Klebsiella* In Feces Of Dairy Cattle - Where Does It Come From? R. Zadoks, H. Griffiths, M. Munoz, G. Bennett, E. Thomas, and Y. Schukken. NMC 47<sup>th</sup> Annual Meeting Proceedings, January 2008.
4. *Klebsiella* - From Feet to Teat. Submitted to Hoard's Dairyman.
5. Cleanliness Scores as Indicator of *Klebsiella* Exposure in Dairy Cows. M. A. Munoz, G. J. Bennett, C. Ahlström, H. M. Griffiths, Y. H. Schukken and R. N. Zadoks. Manuscript in preparation for Journal of Dairy Science.
6. Comparison of *Klebsiella* and *Raoultella* isolates from dairy farm soil, crops and animals. H. M. Griffiths, E. D. Thomas, M. A. Munoz, C. Ahlström, G. J. Bennett, and R. N. Zadoks. Short communication in preparation for Applied and Environmental Microbiology.

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