

# Northern NY Agricultural Development Program 2010 Project Report

## Klebsiella Mastitis Research in NNY Dairy Herds

### Project Leader(s):

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### Collaborator(s):

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### Cooperating Producers:

Fisher farms, St Lawrence County  
Miner Institute, Clinton County  
Bilow Farms: Franklin County

### Background:

Mastitis caused by gram-negative pathogens is a major concern on many well-managed dairy farms in North America. The main gram-negative pathogens that cause mastitis are *Escherichia coli* and *Klebsiella* spp. Cows with *Klebsiella* spp. Mastitis are more likely to die or to be culled than are cows with other types of mastitis. *Klebsiella* spp. mastitis causes a considerable and often sustained decrease in milk production, with average losses of 7.6 kg/d shortly after infection and 5 kg/d in subsequent months. *Klebsiella* spp. Prevention of exposure is the main strategy for control of *Klebsiella* spp. Mastitis. We have previously shown in our NNY research that antimicrobial treatment of *Klebsiella* cases is successful under precisely defined treatment conditions. In previous studies we have shown that *Klebsiella* mastitis is a large problem in NNY dairy farms. In this study our aim was to study the behavior of *Klebsiella* spp in the environment of the cow and farm and to better understand the genetic differences between clinical mastitis and environmental *K. pneumoniae* strains.

### Methods:

We collected a total of 305 samples from 3 dairy farms in Northern New York. Samples included clinical mastitis cases, soil, feed crops, feed, water, rumen content, feces, bedding, and manure from alleyways and holding pens. *Klebsiella* isolates from these samples were identified using *rpoB* sequencing methods and stored in freezer for future analysis. Suppressive subtractive hybridization was employed on three matched pairs of Clinical mastitis and environmental strains. This method is designed to identify the genetic differences between strains of bacteria.

### Results:

*Klebsiella* spp. were detected in 100% of rumen samples, 89% of water samples, and approximately 64% of soil, feces, bedding, alleyway, and holding pen samples. Detection of *Klebsiella* spp. in feed crops and feed was less common. Genotypic identification of species using *rpoB* sequence data showed that *Klebsiella pneumoniae* was the most common species in rumen content, feces, and alleyways, whereas *Klebsiella oxytoca*,

*Klebsiella variicola*, and *Raoultella planticola* were the most frequent species among isolates from soil and feed crops.

Random amplified polymorphic DNA-based strain typing showed heterogeneity of *Klebsiella* spp. in rumen content and feces, with a median of 4 strains per 5 isolates.

Observational and bacteriological data support the existence of an oro-fecal transmission cycle, which is primarily maintained through direct contact with fecal contamination or through ingestion of contaminated drinking water.

Fecal shedding of *Klebsiella* spp. contributes to pathogen loads in the environment, including bedding, alleyways, and holding pens. Hygiene of alleyways and holding pens is an important component of *Klebsiella* control on dairy farms.

We identified 13 genes uniquely present in the clinical strains. Sequences were tested for homology and screened against a larger pool of 18 clinical mastitis and 11 environmental strains collected from the same farms. Seven sequences were found in both clinical and environmental strains, while 6 were found solely amongst clinical strains. Sequences coding for parB, parA, arsD, and sulfate permease were found with similar frequency among clinical and environmental strains.

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

*Klebsiella* spp. are commonly present in soil, water troughs, rumen content, feces, bedding, alleyways, and holding pens on dairy farms. Predominant species in soil and plant samples differ from those in the gastrointestinal tract, water, bedding, alleyways, and holding pens, and the presence of *Klebsiella* spp. in plants appears to result from external contamination rather than endophytic colonization of feed crops. Oro-fecal transmission due to fecal contamination of animals, feed, and water appears more important for maintenance of *Klebsiella* spp. in the farm ecosystem than does introduction from external sources such as fresh bedding or feed. Fecal contamination of stalls, alleyways, and holding pens may result in exposure of teat ends to *Klebsiella*. Further studies would be needed to identify methods to reduce oral ingestion and fecal shedding of *Klebsiella* spp. Meanwhile, hygiene of alleyways and holding pens should be recognized as an important component of prevention of mastitis due to *Klebsiella* spp.

These results suggest that genes uniquely associated with *Klebsiella pneumoniae* clinical mastitis strains may contribute to adaptation to the bovine mammary gland and to virulence. These genes would be particularly useful as vaccine or treatment targets.

### **Outreach:**

Website materials:

<http://ahdc.vet.cornell.edu/sects/QMPS/QMPSNYKlebsiellamastitisbrochure.pdf>

Publications for the scientific community:

Zadoks RN, Griffiths HM, Munoz MA, Ahlstrom C, Bennett GJ, Thomas E, Schukken YH. Sources of *Klebsiella* and *Raoultella* species on dairy farms: be careful where you walk. J Dairy Sci. 2011 Feb;94(2):1045-51.

### **Next steps:**

This study utilized suppressive subtractive hybridization to isolate gene sequences unique to three clinical mastitis strains. In particular, one gene sequence, Ent638\_4324 was subsequently found to be present amongst a large set of clinical mastitis strains, and absent amongst a pool of environmental strains not associated with mastitis. Distribution and sequencing and homology analysis of this gene suggests that further investigation and characterization of its contribution to host adaptation and virulence is warranted, particularly with regards to the association of this gene with putative pathogenicity islands. Additionally, plasmid and antibiotic resistance profiles of clinical *Klebsiella* strains will provide further insight into *Klebsiella pneumoniae* persistence in the mammary gland and virulence during an IMI. The unique bacterial strain collection that we have developed from NNY dairy farms, will allow us to study the disease causing factors in more detail. It is expected that these studies will ultimately lead to the development of vaccines and science based preventative programs for NNY dairy herds

**Acknowledgments:**

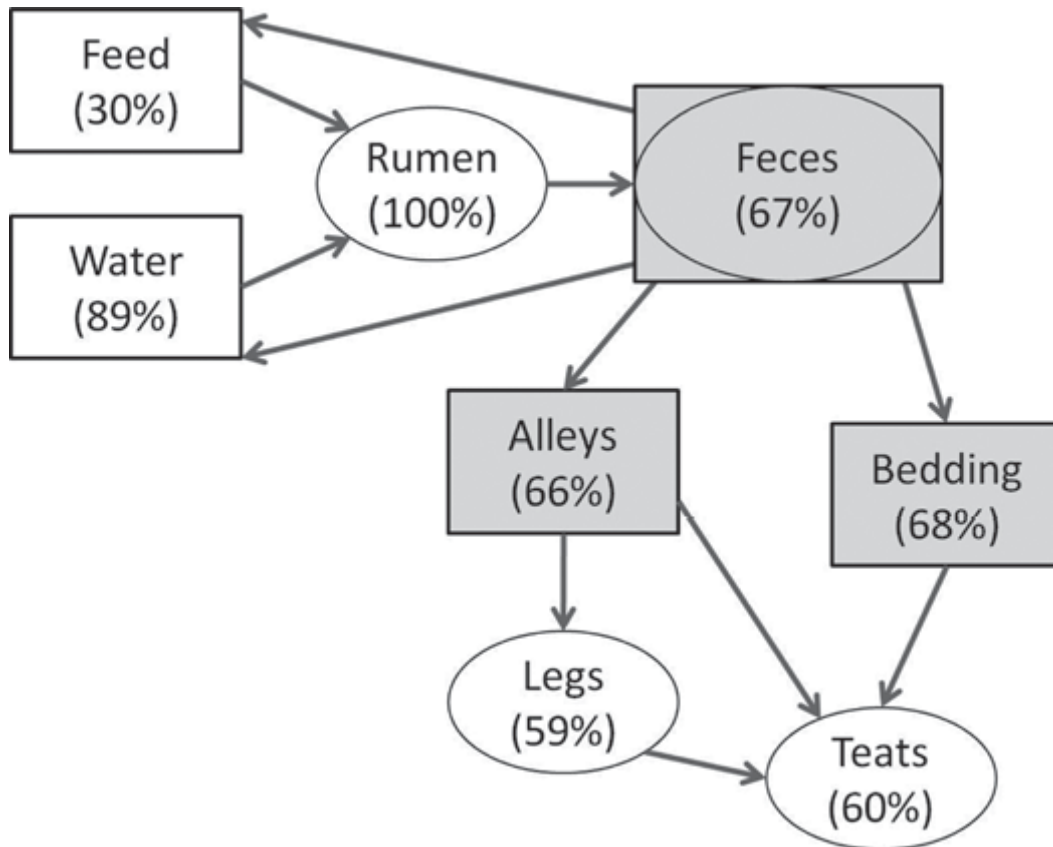
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**Reports and/or articles in which the results of this project have already been published:**

Zadoks RN, Griffiths HM, Munoz MA, Ahlstrom C, Bennett GJ, Thomas E, Schukken YH. Sources of *Klebsiella* and *Raoultella* species on dairy farms: be careful where you walk. J Dairy Sci. 2011 Feb;94(2):1045-51.

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Potential sources and routes of exposure of the mammary gland to *Klebsiella* spp. Ovals represent animals (rectum/feces, rumen, legs, and teats). Rectangles represent environmental sources. Gray rectangles are considered of primary importance for farm management and mastitis prevention. Proportion of samples testing positive based on 3 New York dairy farms described in the current study.