

be housed and milked together with cows suffering from *Mycoplasma mastitis*,<sup>5</sup> it is of special epidemiological interest that cows that were milked last although sharing the same barn (Dairies A, B, C) or cows milked with separate milking units (Dairy D) seemed to have posed no risk for the remaining cows in the herd. This observation confirmed previous published reports that infection is mainly spread from infected to uninfected cows by milker's hands and milking machines.<sup>2,3,5,13</sup> However, mycoplasmal mastitis outbreaks in New York State have been frequently associated with respiratory problems<sup>1,6</sup> and airborne transmission suspected among animals housed in poorly ventilated barns.<sup>1,6,14</sup>

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## Oxytetracycline Residues in Milk After Intrauterine Infusion of Dairy Cows With Retained Fetal Membranes

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#### Abstract

**Our objectives were to establish the duration, by high performance liquid chromatography, of milk residues of oxytetracycline (OTC) after intrauterine infusion of 5 g OT of cows with retained fetal membranes. We also determined the predictive values positive (PVPT) and negative (PVNT) of the Delvo-P (Gist Brocades), Cite Probe (Idexx), Charm Farm, and Charm II (Charm Sciences) tests for OT residues above 30 ppb, and above the stated minimum detection level of each test. Cows with mastitis were not included. Milk samples were collected from 50 cows at 24 hour intervals during treatment, and at 12 hour intervals after the cessation of treatment. The results of this work plus important implications for dairy practitioners will be discussed in this presentation.**

Oxytetracycline (OT) is widely used as an intrauterine infusion for the treatment of retained fetal membranes (RFM) in dairy cattle. It has a broad spectrum of

antimicrobial activity and remains active in the presence of organic debris (Olson et al 1984). However, it is not approved by the Food and Drug Administration (FDA) for any use in lactating dairy cattle. OT is used as an intrauterine infusion in lactating cattle under the Extra Label Use Provisions set forth by the FDA (FDA Compliance Policy Guide 7125.06). One of the stipulations of these provisions is that the prescribing veterinarian inform the producer how long after the cessation of treatment the milk must be withheld from sale to ensure that treatment residues are not present in the milk. Because OT is not approved for lactating cattle, there are no milk withholding times listed on the label. Veterinary practitioners have commonly recommended that milk be withheld only during treatment.

Recent public concern about residues in milk and meat in general, and OT in particular, has prompted the

FDA to set a "safe level" of OT in milk at 30 ppb, and to screen bulk milk for OT at this lower level. Many tests are now available that can detect as few as 30 ppb of OT (Sundlof 1990). As a result, tanker loads of milk have been found to be contaminated with OT, and in some instances, the cause of contamination has been found to be the inadequate withholding of milk from cows that have received intrauterine OT infusions.

The analysis of OT residues in milk of individual cows after IU infusion of cows with RFM, using modern analytical techniques, has not yet been reported. Our objectives were 1) to establish the duration of milk residues of oxytetracycline after intrauterine infusion of cows with retained fetal membranes, using high performance liquid chromatography (HPLC); and 2) to determine the positive and negative predictive values of several commercial on-farm antimicrobial residue tests for the presence of oxytetracycline in milk.

Holstein cattle on a single 1400-cow commercial dairy were enrolled if they had retained their fetal membranes at least 12 hours, and did not have evidence of mastitis. Cows were treated with 5 grams of OT (50 ml of 100 mg/ml solution in a povidone base) by intrauterine infusion. Treatment was initiated at the time of enrollment and was administered once a day for at least 2 days, or until the membranes were released. Cows that became febrile (rectal temperature > 103.5°) were also given 10,000 IU per pound of procaine penicillin G intramuscularly for 2-4 days. Milk from cows treated with OT alone was discarded for 4 days after the last treatment. Milk from cows treated with penicillin was discarded for 5 days after the last treatment.

Samples of milk were collected from each quarter of each cow before milking. Equal amounts of milk from each quarter were pooled for OT analysis. Milk samples were collected from cows at 24 hour intervals during treatment, and 12 hour intervals after the last treatment. Sampling was halted when 4 consecutive samples were negative using the Cite Probe<sup>1</sup> commercial ELISA test for tetracyclines.

On farm tests included the Delvotest-P<sup>2</sup>, the Cite Probe, and the Charm Farm Test<sup>3</sup>. Samples were tested by the Charm II<sup>3</sup> test by laboratory technicians at the Western Dairymen's Cooperative Inc (WDCI) of Thornton, Colorado. HPLC was performed at the University of Florida: Samples were frozen and shipped every 2 weeks.

Samples were obtained from 54 cows with RFM. Three cows had no OT residues in any milk sample. The duration of OT residues ranged from 0 to 144 hours after the last infusion, with a mean of 52.3 hours. Neither the

number of infusions received, nor the presence of rectal temperature above 103.5°, affected the mean duration of OT residues in milk.

The predictive value of a positive test (PVPT) indicates the likelihood that OT residues were present in milk samples which were test positive, while the negative predictive value (PVNT) indicates the likelihood that there were less than 30 ppb, (or less than the manufacturer's level of detection) of OT in samples which were test negative. At the 30 ppb "Safe Level" (FDA), the positive predictive value ranged between .57 (Charm Farm) and .74 (Cite Probe), while the negative predictive value was between .69 (Charm Farm) and .82 (Charm II and Cite Probe). At the manufacturer's reported minimum detection level for the Delvotest (200 ppb) and the Charm Farm Test (80 ppb), the positive predictive values increased dramatically (fewer false positives), but the negative predictive values declined.

The following conclusions were made:

1. Significant residues of OT appeared in milk during intrauterine infusion of cows with retained fetal membranes.
2. Levels of OT in milk remain above the FDA "Safe Level" for more than 2 days after the last treatment, on average. However, 10 cows had more than 30 ppb OT in milk for 96 hours or more.
3. All of the commercial tests evaluated were plagued by false negative and false positive results. The results provide further evidence that there is a serious lack of milk antibiotic residue tests that are reliable when used on samples from an individual cow.

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<sup>1</sup> Idexx Corporation, Portland, ME

<sup>2</sup> Gist-Brocades, Inc., King Of Prussia, PA

<sup>3</sup> Charm Sciences, Inc., Malden, MA