

Shedding of *Mycobacterium avium* subsp. *paratuberculosis* into Milk and Colostrum of Naturally Infected Dairy Cows over Complete Lactation Cycles

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Summary and Implications

The primary mode of transmission of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is fecal-oral. However, MAP is also shed into the milk and colostrum of infected cows. The objective of this study was to identify if an association exists between stage of MAP infection and days in lactation with the amount of MAP present in milk and colostrum of naturally infected cows. Results indicated that MAP is primarily shed in early lactation and in cows with advanced infection. This experiment provides crucial information to dairy producers pertaining to the threat of MAP transmission via milk and colostrum. Producers now know that allowing a calf to suckle even once is exposing it to the highest concentrations of MAP and therefore possibly infecting the newest generation of animals.

Introduction

Mycobacterium avium subsp. *paratuberculosis* (MAP), the causative agent of Johne's Disease (JD), is an enteric pathogen that is primarily shed in the feces. JD is a slowly progressing wasting disease that does not manifest physical symptoms in ruminants until late in the disease, making early diagnosis difficult and costly. It is estimated that 91.1% of dairy herds are infected with this disease. The primary route of exposure for neonates is fecal-oral; however, MAP is also shed into the milk and calves can be exposed to this pathogen by suckling the dam or being fed colostrum or waste milk from infected cows. Despite this possibility, there is little information in the literature to document the shedding of MAP into the colostrum and milk of infected dams, particularly, the bacterial load and how this relates to the infection status of the dam and the stage of lactation. Yet, if producers could understand the association of disease with bacterial load in the milk, they might be more willing to make critical management decisions to further prevent dissemination of infection within the herd. The objective of this study was to determine if an association exists between disease status and days in milk with the amount of MAP shed into milk and colostrum.

Materials and Methods

Milk was collected from 42 Holstein cows well characterized for JD and housed at the National Animal Disease Center (NADC, Ames, IA) over a period of 7 years. All procedures performed on the dairy cows were approved by the Institutional Animal Care and Use Committee (NADC). Milk was collected into sterile containers after thorough cleaning of the teats on days 1, 3, 7, 14, 21, 28, 90, 180, and 305 after calving. Milk was concentrated and decontaminated with a solution containing *N*-acetyl-L-cysteine-1.5% sodium hydroxide (NALC-1.5% NaOH) and cultured in liquid BACTEC12B medium supplemented with mycobactin J, egg yolk, and the antibiotic mixture PANTA and solid Herrold's egg yolk medium, both incubated for 12 weeks. Direct real-time PCR was also performed targeting the species specific IS900 genetic element.

Results and Discussion

Results of culture and PCR on milk and colostrum samples collected from 50 lactation cycles from 42 cows are presented in Table 1. MAP was most frequently detected in samples that were obtained from cows in the clinical stage of disease compared to cows that were subclinically infected, regardless of detection method. Samples collected from clinical cows during the lactation cycle were 38.8% positive by PCR compared to 11.2% and 9.5% positive by culture on BACTEC 12B and HEY media, respectively. MAP was detected with less frequency in milk collected from subclinical cows regardless of detection method (Table 1). The highest level of MAP recovery was achieved by direct PCR with only 7.6% of samples positive. None of the samples from the control cows were positive by any of the detection methods. Over the course of the 305-day lactation cycle, samples within 14 of the 20 lactation cycles from clinical cows and 11 of the 24 lactations from subclinical cows were positive for MAP using PCR as the detection method. The incidence of detection was reduced to 7 positive lactations from clinical cows and 3 positive lactations from subclinical cows when using either method of culture to detect MAP in the milk samples.

Of the 20 clinical cows, 11 died from JD within one year of calving. Samples collected from these cows accounted for 12 of the 13 milk samples that were culture positive in BACTEC 12 B medium and all of the milk samples that were culture positive on HEY medium. In addition, 33 of the 45 milk samples that were positive for MAP via direct PCR were collected from this group of clinical cows. These data suggest that as JD progresses, the amount and frequency of MAP shed into milk and

colostrum continuously increases until the cow succumbs to JD.

Viable MAP was primarily shed into milk and colostrum in early lactation (DIM 0-60), and less frequently shed in mid (DIM 60-240) and late (DIM 240-305) lactation (Table 1). All of the MAP-positive milk and colostrum samples from cows with clinical JD were collected in early lactation. Likewise, 4 of 5 positive milk and colostrum samples from subclinical cows were collected in early lactation and only 1 positive sample was collected in mid-lactation. This trend persisted when the presence of MAP was detected by direct PCR. Of the direct PCR positive samples collected from clinical cows, 41 of 45 were detected in early lactation and 4 of 45 were detected in mid lactation. Similarly, 8 of the 14 positive samples were collected in early lactation, 3 in mid lactation, and 3 in late lactation.

This study definitively demonstrates that MAP is shed into milk and colostrum, preferentially by cows in advanced stages of JD and in early lactation. This study proves dam to

calf transmission of MAP by milk consumption is a viable route of transmission. To compound this issue, research has found that MAP that has been exposed to high-osmolarity environments like those found in milk and the mammary gland possess an invasive phenotype. In addition, calves less than 6 months of age are most susceptible to MAP infection. Therefore, if a newborn calf is allowed to suckle even once, it is experiencing the highest likelihood of infection by MAP. Additionally, if producers feed pooled colostrum, many calves could be at risk of infection by MAP. Veterinarians now have critical information to educate dairy producers on the risk of MAP transmission through milk and colostrum and the need to change calf rearing practices to accommodate these findings.

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Table 1. Isolation of *Mycobacterium avium* subsp. *paratuberculosis* from milk and colostrum collected from clinical, subclinical, and control cows cultured with BACTEC 12B and Herrold's egg yolk (HEY) media and detected by direct PCR targeting the IS900 gene. Data are organized in to early (days 0-60), mid (days 60-240), and late (days 240-305) lactation.

Disease Status	Days in milk	# Samples	Direct PCR	BACTEC 12B	HEY
Clinical	0-60	104	41	13	11
	60-240	10	4	0	0
	240-305	2	0	0	0
	Total	116	45	13	11
Subclinical	0-60	138	8	4	1
	60-240	35	3	1	1
	240-305	12	3	0	0
	Total	185	14	5	2
Control	0-60	32	0	0	0
	60-240	8	0	0	0
	240-305	3	0	0	0
	Total	43	0	0	0