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***Mycobacterium avium paratuberculosis
(MAP): Single Tests Can Fool You.***

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Testing cattle for Johne's disease is challenging because of the low responsiveness of the cow's immune system to the infection and the intracellular location of MAP. It is informative to do repeated testing on cows suspected to be infected, to see what the probability of positive results is over time and across different types of tests.

Recently, four cows from herds where Johne's Disease was known to be present, were subjected to repeated sampling and testing over five consecutive days. One milk sample and one manure sample were collected daily. The daily manure sample was split and cultured three times at the Animal Health

Table 1. Milk ELISA and Fecal Culture Results on Four Cows Tested on Five Consecutive Days

Day	Test	Cow 1	Cow 2	Cow 3	Cow 4	
Day 1	Milk ELISA	0.02	0.02	1.48 (P)	0.08 (Susp)	
Day 2		0.01	0.06	1.37 (P)	0.05	
Day 3		0.01	0.02	1.64 (P)	0.04	
Day 4		0.01	0.02	1.46 (P)	0.03	
Day 5		0.01	0.02	1.49 (P)	0.02	
Separator						
Day 1	A	Fecal culture	N	N	P	P
	B		N	N	P	N
	C		N	N	P	N
Day 2	A		N	N	N	P
	B		N	N	N	P
	C		N	N	N	P
Day 3	A		N	N	P	P
	B		N	N	P	N
	C		N	N	P	N
Day 4	A		N	N	P	N
	B		N	N	P	N
	C		N	N	P	N
Day 5	A		N	N	P	N
	B		N	N	P	N
	C		N	N	P	N

Laboratory, University of Guelph. The milk samples were tested at CanWest DHI. Cows with milk ELISA S/p ratios of less than 0.07 were classified as negative; those with results from 0.07 to 0.10 as suspicious; and those greater than 0.1 as positive. The results are shown in Table 1. Cells with results classified as positive on either test are shaded.

This is a very small sample size so conclusions are limited. Cows 1 and 2 were both negative on the milk ELISA and both were fecal culture negative on ALL manure samples. Cows 3 and 4 had evidence of infection. Cow 3 was classified as a HIGH positive on all 5 days of milk ELISA testing and on 4 of 5 days based on fecal culture. Cow 4 had a suspect ELISA value on one day, but negative ELISA results on the next 4 days. On fecal culture, she was negative on 3 of 3 cultures on 2 days, positive on 3 of 3 on another day and positive on only 1 of 3 cultures on the remaining 2 days. Ultimately she was positive on 5 of 15 cultures (33%).

There is seldom complete agreement between tests. Care should be taken if one test type is to be used to confirm the results of another. Fecal culture “missed” cow 3 on one day compared to milk ELISA; milk ELISA “missed” cow 4 on one day compared to fecal culture. If the goal of a testing program is to identify the cows most at risk of shedding MAP, then, in this case, there is a high probability that on any given day cow 3 would have been identified by either test as being a cow at high risk of shedding MAP to young calves in the herd.

This repeated, short term, testing scheme reinforces the interpretation in place for the milk ELISA test results. As milk antibody titre increases, the relationship between milk ELISA and fecal culture becomes more consistent. When the milk ELISA result is positive, more than 50% of these cows are likely to be shedding MAP in manure. When the ELISA S/p ratio is over 1.0, it is likely that over 80% of cows will be fecal shedders. Less than a third of cows with low positive or suspect S/p ratios are expected to be shedding MAP. Among test-negative cows, less than 3% would be shedding.

A key feature of all Johne’s testing is the increased knowledge and confidence in cow and herd status

that is gained over time with repeated test result. In Denmark as of 2006 and the UK as of September 2008, formal herd programs are used where cows are tested three to four times annually using a milk ELISA test. When herd tests are repeated, greater definition of the true herd status with regards to Johne’s is achieved.