Longitudinal study to investigate variation in results of repeated ELISA and culture of fecal samples for *Mycobacterium avium* subsp *paratuberculosis* in commercial dairy herds

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Objective—To determine sources and amounts of variation in a kinetics ELISA (KELA) and results of culture of fecal samples for *Mycobacterium avium* subsp *paratuberculosis* (MAP) in repeated tests of individual cows.

Animals—112 cows on 6 commercial dairy farms in New York.

Procedure—A nonrandom longitudinal study was conducted from January 2001 to March 2002. A KELA was performed monthly, and MAP culture was performed bimonthly. Cow- and herd-level data were collected. The KELA and culture results were analyzed by use of models that corrected for clustering within herds and repeated measures on cows.

Results—Cows of second or higher lactation had increased KELA values, compared with values for first-lactation cows. Cows had lowest KELA values during the first 15 days in milk; KELA values increased until 60 days in milk and then stabilized. Moderate and heavy shedders had significantly higher KELA values than culture-negative cows, and KELA values of shedders progressively increased over time. On average, the KELA value was significantly increased 132 days after a cow was first detected to be a moderate shedder and 236 days after a cow was first detected to be a low shedder.

Conclusions and Clinical Relevance—Analysis suggests that KELA results vary on a cow-level on the basis of lactation number and stage of lactation. High KELA values indicate heavy fecal shedding, but the KELA is not useful in identifying low and moderate shedders that can require up to 236 days to have a significant increase in KELA value. (*Am J Vet Res* 2003;64:479–484)

Control-and-eradication programs have been developed in several countries to eliminate ***Mycobacterium avium* subsp *paratuberculosis* (MAP)** infection in cattle. Such programs are based on repeated testing of blood and manure samples and voluntary disposal of infected cows. It is difficult to diagnose MAP infection during various stages of the disease.[1,2] The available tests for MAP have low **sensitivity** and moderate to high **specificity**. The reliability of detection of MAP infection improves when a combination of tests is used or when cows and herds are tested repeatedly. Results of ELISA and culture of fecal samples for individual cows seem to vary considerably over time, which complicates classification of the infection status of a cow or a farm.[3,4] However, it is of great importance for control or eradication of the disease to be able to correctly identify cattle that are shedding larger numbers of MAP or are likely to develop clinical disease. These cattle are contagious and will pose the largest threat for transmitting infection in the herd.

Few longitudinal studies have used ELISA and culture of fecal samples on a routine basis for a group of cattle. In 1 study,[2] investigators mainly focused on culture of fecal samples and culture of tissues obtained at slaughter, but they also stated that most of the infected cattle that were not detected by culture of fecal samples also had negative results for the serologic tests performed. In another study,[3] investigators compared results of an ELISA, γ-interferon test, and culture of fecal samples during a 2.5-year period. They found considerable variation in the responses of cattle to MAP for the various tests and suggested that other herd- and cow-factors may govern the differing response patterns. In another study,[1] investigators performed repeated serologic testing and found great variation in the ELISA values of subsequent samples that were independent of the time interval between collection of those samples.

The study reported here was designed to investigate the longitudinal patterns for results of culture of fecal samples and serologic tests for MAP in cows on commercial dairy farms in New York. The objective of the study was to determine the sources and amounts of variation in results for ELISA and culture of fecal samples in individual cows.

Material and Methods

Sample population—A nonrandom longitudinal study was conducted from January 2001 to March 2002. During that period, samples used for testing were obtained from 16 cows in each of 5 herds and 32 cows in a sixth herd. The 112 cows were initially selected on the basis of age, lactation number, and the fact that MAP was not previously detected

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by culture of fecal samples and that the cows had been tested previously by use of a **kinetics ELISA (KELA)** for MAP. Cows had to be likely to remain in the herd for at least 1 more year; thus, they were cows that had higher milk production. Moreover, half of the cows in each herd that were selected for the study were in their first lactation, and half were in at least their second lactation. In each lactation group, half of the cows had a result on the previous KELA < 40, and half had a result on the previous KELA ≥ 40.

Collection of data—For the study reported here, serologic samples were obtained on a monthly basis and tested by use of a KELA, and fecal samples were obtained on a bimonthly basis for culture of MAP organisms. Testing of samples was performed at the New York Animal Health Diagnostic Laboratory at Cornell University.

The KELA used a protoplasmic antigen and was performed in accordance with a standard indirect ELISA protocol.[4] The KELA measured the slope of the reaction rate that resulted in development of color, which was directly proportional to the amount of antibody in the reaction vessel. Samples were assayed in duplicate to assess repeatability, and 4 samples of control serum were used in each run of the assay to establish a standard curve. Multiple thresholds were used to determine the status of each cow with regard to risk of being infected. Four risk categories were distinguished: low (KELA < 40), moderate (KELA ≥ 40 but < 54), moderate high (KELA ≥ 54 but < 165), and high (KELA ≥ 165). For comparison, values for the positive-negative threshold used as a single cutoff for commercial ELISAs are typically in the range of 80 to 90.

The diagnostic laboratory used a liquid-culture method[a] for rapid detection of MAP in bovine fecal samples.[5,b] A 2-g aliquot of each fecal sample was decontaminated by use of a double-incubation and centrifugation technique.[6] Results were available within 5 to 6 weeks. Results of culture of fecal samples were categorized on the basis of the number of days of growth in liquid media until detection of the organisms,[c] which relates to the number of **total colony forming units (TCFU)**/2 g of sample on solid media (Herrold egg yolk media). Samples with heavy growth (> 300 TCFU), moderate growth (30 to 300 TCFU), and low growth (< 30 TCFU) were detected in < 22 days, 22 to 28 days, and 29 to 35 days, respectively. The liquid-culture method has a Se that is 13% better than the Se for the laboratory's previously used culture system (double-incubation technique with Herrold egg yolk media). In the study reported here, a cow was considered infected with MAP when the liquid-culture method yielded a positive result, and MAP was confirmed by acid-fast staining and results of a polymerase chain reaction technique for detection of IS900. The IS900 is an insertion sequence in the genome of MAP that is believed to be unique for MAP, and detection of IS900 as evidenced by a positive result for the polymerase chain reaction technique is considered confirmatory evidence for MAP.[7] A fecal sample was classified on the basis of the results of culture as a dichotomous variable (0 when MAP was not detected and 1 when MAP was detected). In addition, the cows were assigned an infection status on the basis of whether bacteria were ever detected by culture of fecal samples (infected) or not detected (noninfected).

Cow- and herd-level data were collected during every sample collection date from a management information system,[d] which was used by all farms. A categoric variable was made for days in milk and consisted of 8 categories (cows that were ≤ 15 days after parturition; cows that were 15 to 30, 31 to 60, 61 to 90, 91 to 120, and ≥ 121 days in milk, respectively; cows during the early portion of the nonlactating period; and cows in the final 14 days of the nonlactating period. The number of lactations was aggregated to 3 categories (lactation 1, lactation 2, and > 2 lactations). Several variables were created to investigate thresholds at which the KELA response was associated with the infection status determined from the results of culture of fecal samples. The KELA categories that were considered were the 4 described previously, as well as 3 dichotomous variables (positive-negative) with a single cutoff threshold for KELA values of 65, 80, and 90, respectively.

Longitudinal data were also used to determine the prognostic value of previous results of culture of fecal samples with regard to results of the KELA, and vice-versa, by including those data in multivariate models. Each **95% confidence interval (95% CI)** was calculated as the mean ± (1.96 × SE).

Analysis of results of KELA—Results of the KELA were skewed (skewness, 3.1) and normalized by transformation (**natural logarithm KELA [LNKELA]**); the LNKELA values were then used in multivariate analyses. Values to characterize the distribution of the LNKELA were as follows: mean, 3.3; median, 3.0; and skewness, –0.8. Analyses were performed by use of a multivariate procedure for a commercially available statistical program.[e]

Two models were used to analyze **LNKELA at time t ($LNKELA_t$)**: a transition model that generated population average effects and a random-effects model that generated a farm-specific effect.[8] For both models, several covariance structures were tested (eg, variance components, first-order autoregressive structure, and compound symmetry) to correct for within-cow and -herd variation as a result of the repeated measures at cow- and herd-level. The model with a random cow-nested-in-herd effect with a compound symmetry covariance structure had the lowest Akaike information criteria and, thus, was considered the best model.[9] The general structure of the multivariable model that was used for analyis of the data was as follows:

$$LNKELA_t = \alpha_t + (\beta_t \times Z_t) + (\delta_t \times herd[cow]_t) + \varepsilon_t$$

where α_t is the intercept, β_t is the estimate of covariates, Z_t is the matrix of covariates, δ_t is the cow-nested-in-herd random error (normally distributed with a mean of zero and cow-nested-in-herd specific variance), and ε_t is the residual error (normally distributed with a mean of zero and variance of σ_e^2).

Analysis of results of culture of fecal samples—A fecal sample was considered to have negative results when MAP organisms were not cultured and positive results when MAP organisms were cultured from the sample. **Results of culture of a fecal sample at time t (CF_t)** were modeled by use of a generalized linear model in accordance with a binomial distribution. A generalized estimating equation was used to model clustering within herds and repeated measures on cows.[10,e] The general structure of the model was as follows:

$$CF_t = \alpha_t + (\beta_t \times Z_t) + \Sigma_t$$

where α_t is the intercept, β_t is the estimate of covariates, Z_t is the matrix of covariates, and Σ_t is the variance-covariance matrix adjusted for the correlation in CF_t between samples at time t by use of the random error for the correlation structure matrix (normally distributed with a mean of zero and variance of σ_e^2).

Results

Descriptive results—General descriptive statistics were calculated to describe the cows in the study (**Table 1**). Mean KELA for all observations of the cows was 39. Mean 305-day milk production of cows in the study (12,091 kg) was higher than the mean 305-day milk production of a typical cow in New York (7,900 kg).[f] Results of the KELA were significantly correlated (r, –0.08; $P = 0.05$) with 305-day milk production predicted

on the basis of monthly test-day records. Number of days in milk was significantly correlated (r, 0.09; $P = 0.04$) with results of culture of fecal samples.

Ninety (80%) cows had negative results for culture of fecal samples throughout the study. Five cows became low shedders, 5 became moderate shedders, and 12 became heavy shedders by the end of the study or at the time of removal of the cow from the study.

Results of culture of fecal samples and classification on the basis of KELA results were tabulated (**Table 2**). Twenty-five percent of the samples of nonshedders had moderate to high KELA values at the time of sample collection. Thirty-nine percent of the moderate and 19% of the heavy shedders had a low-risk classification on the basis of the KELA result. One of the 2 heavy shedders that had KELA values < 40 was culled before the KELA increased. The other heavy shedder had a KELA value of 60 at the next test. Fifty-two percent of the low shedders had a KELA value in the moderate- to high-risk categories.

Table 1—Results for continuous variables of 112 cows on 6 commercial dairy farms in a longitudinal study for the detection of *Mycobacterium avium* subsp *paratuberculosis*

Variable	n	Mean	SD	Minimum	Maximum
KELA value	912	39	37	0	333
No. of days in milk	808	204	125	0	612
Predicted 305-day milk production (kg)	808	12,091	2,174	4,626	18,428
Daily milk yield (kg)	694	37.7	10.4	2.3	74.9
Somatic cell count (× 1,000 cells/mL)	615	268	759	6	9,999

KELA = Kinetics ELISA.

Table 2—Infection status for *Mycobacterium avium* subsp *paratuberculosis* determined on the basis of results of culture of fecal samples and risk category*

Risk category on the basis of KELA results	Category for result of culture of fecal samples				
	Negative	Low shedder	Moderate shedder	Heavy shedder	Total
Low risk	363 (75)	13 (48)	7 (39)	3 (19)	386 (70)
Moderate risk	59 (12)	3 (11)	2 (11)	3 (19)	67 (12)
Moderate-high risk	61 (12)	10 (37)	8 (44)	7 (44)	86 (16)
High risk	4 (1)	1 (4)	1 (6)	3 (19)	9 (2)
Total	**484 (100)**	**27 (100)**	**18 (100)**	**16 (100)**	**548 (100)**

Numbers in parentheses represent percentages within each column.
*Four risk categories were created on the basis of KELA results: low (KELA < 40), moderate (KELA ≥ 40 but < 54), moderate high (KELA ≥ 54 but < 165), and high (KELA ≥ 165).

Results of the KELA and culture of fecal samples for individual cows during the study period were plotted on graphs (**Fig 1**). Evaluation of the graphs revealed that the KELA value increased throughout lactation, especially for an infected cow. Moreover, the KELA value appeared to decrease slightly around the time of parturition. Other events that were recorded during the study, such as changes in pen or feed, mastitis, acetonemia, and retained placenta, were not significantly correlated with LNKELA values or results of culture of fecal samples.

Mean KELA values for cows that never had a positive result, as well as those that had positive results, for culture of fecal samples were plotted for the 6 categories of number of days in milk (**Fig 2**). The KELA values for cows that always had negative results on culture of fecal samples were lowest within 15 days after parturition, increased until 60 days in milk, and then appeared to remain constant throughout the remainder of lactation. The 95% CI for the cows with positive results on culture of fecal samples was fairly wide because of the low number of cows, and the KELA value for cows with positive culture results was only significantly different from the KELA value for cows with negative culture results for the category of > 120 days in milk.

Mean KELA values for cows with negative and positive results of culture of fecal samples were plotted for lactations 1, 2, and > 2 (**Fig 3**). Mean KELA value of cows with positive culture results increased during subsequent lactations, and cows in the second and higher lactation had significantly higher KELA values than first-lactation cows (univariate analysis). The univariate analysis revealed that mean KELA values of cows with negative culture results were similar among lactations.

Correlations—Mean interval (ie, number of days) between previous sample collections for results of KELA and culture of fecal samples was calculated (**Tables 3 and 4**). Pearson correlation coefficients between the current test value and previous test results were summarized (**Tables 5 and 6**). All correlation coefficients were significant ($P = 0.01$) for a 2-tailed test. The KELA result (ie, LNKELA) was compared with the KELA results of the preceding month and with results for 2, 3, and 4 tests earlier.

Correlation between KELA values was moderately high and varied between 0.66 and 0.77. The correlation decreased slightly over time as indicated by the fact that the current KELA value had a higher correla-

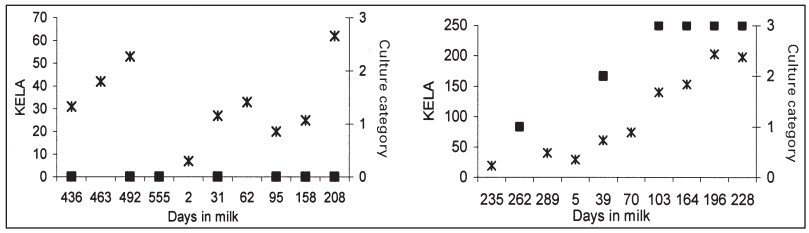

Figure 1—Results of kinetics ELISA (KELA; asterisk) and culture of fecal samples (solid square) for a representative noninfected cow (never had a positive result for culture of fecal samples) and an infected cow (at least 1 positive culture result) at the end of a lactation and during the subsequent lactation (day 0 = start of each lactation). There were 4 categories for culture results, ranging from 0 (no colony-forming units) to 3 (> 300 total colony-forming units).

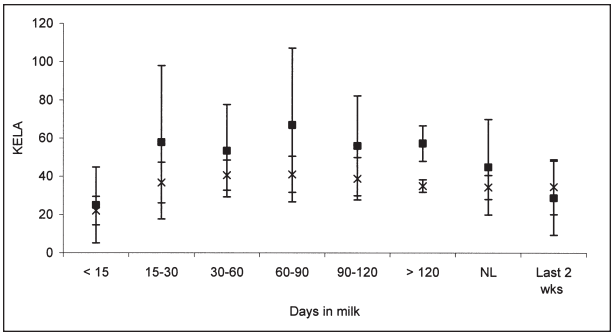

Figure 2—Mean value and 95% confidence interval (CI) for KELA results of noninfected (asterisk) and infected (solid square) cows for each of 8 categories of days in milk. NL = Nonlactating. Last 2 weeks = Final 14 days of the nonlactating period prior to parturition.

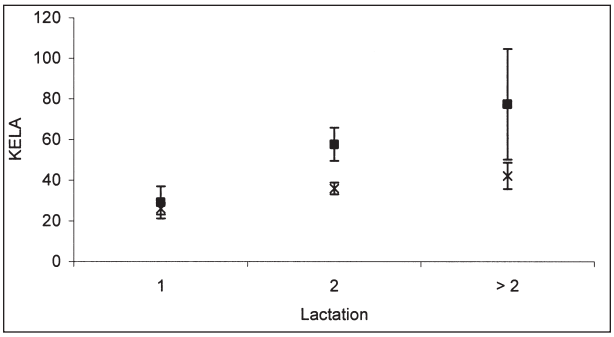

Figure 3—Mean value and 95% CI for KELA results of noninfected (asterisk) and infected (solid square) cows based on the number of lactations.

Table 3—Interval (number of days) between KELA tests of a cow for detection of *Mycobacterium avium* subsp *paratuberculosis* in 6 commercial dairy herds

Sample interval from:	n	Mean	SD	Minimum	Maximum
Preceding test	784	43	22	13	338
2 tests previously	679	85	30	48	366
3 tests previously	574	127	38	77	386
4 tests previously	471	167	44	111	421
5 tests previously	373	204	49	139	339

Table 4—Interval (number of days) between collection of fecal samples for use in microbial culture to detect *Mycobacterium avium* subsp *paratuberculosis* in 6 commercial dairy herds

Sample interval from:	n	Mean	SD	Minimum	Maximum
Preceding test	463	65	36	13	293
2 tests previously	359	132	55	61	385
3 tests previously	259	187	50	103	385
4 tests previously	166	236	28	180	312
5 tests previously	92	298	22	215	359

Table 5—Pearson correlation coefficients between the current KELA value ($KELA_T$) and the KELA value obtained 1, 2, 3, 4 or 5 tests previously ($KELA_{T1}$ to $KELA_{T5}$, respectively)

Test	$KELA_T$	$KELA_{T1}$	$KELA_{T2}$	$KELA_{T3}$	$KELA_{T4}$	$KELA_{T5}$
$KELA_T$	—	0.77	0.66	0.59	0.53	0.48
$KELA_{T1}$	0.77	—	0.76	0.63	0.53	0.48
$KELA_{T2}$	0.66	0.76	—	0.74	0.56	0.49
$KELA_{T3}$	0.59	0.63	0.74	—	0.70	0.52
$KELA_{T4}$	0.53	0.53	0.56	0.70	—	0.66
$KELA_{T5}$	0.48	0.48	0.49	0.52	0.66	—

— = Not applicable.

tion with the KELA value of the preceding month (correlation between 0.66 and 0.77) than with the results of 2 to 5 months earlier (correlation between 0.48 and 0.66). Tabulation of data revealed that 87% of the cows in the low KELA category and 74% of the cows in the higher KELA categories remained in these categories for the subsequent sample.

Correlations between the shedding status for subsequent fecal samples were determined (Table 6). The current result of culture of a fecal sample had a slightly higher correlation with the culture result of the preceding bimonthly sample (correlation between 0.90 and 0.94) than with the culture results of 4 to 8 months earlier (correlation between 0.72 and 0.85). Tabulation of data revealed that 9% of the cows with negative results for culture of fecal samples and 90% of cows with positive culture results again had negative or positive results, respectively, for the next sample.

Multivariate analysis of LNKELA values—A mixed-model multivariate analysis was conducted on LNKELA values by use of 492 complete records that contained results of a KELA and culture of fecal samples, as well as cow-level data (**Table 7**). The compound symmetry covariance structure corrected best for the correlation in the data; it had the lowest Akaike

Table 6—Pearson correlation coefficients between results of the current culture of a fecal sample (CF_T) and results for culture of fecal samples performed 1, 2, 3, or 4 tests previously (CF_{T1} to CF_{T4}, respectively) on samples obtained 2, 4, 6, or 8 months previously

Test	CF_T	CF_{T1}	CF_{T2}	CF_{T3}	CF_{T4}
CF_T	—	0.90	0.72	0.85	0.72
CF_{T1}	0.90	—	0.91	0.76	0.85
CF_{T2}	0.72	0.91	—	0.94	0.74
CF_{T3}	0.85	0.76	0.94	—	0.91
CF_{T4}	0.72	0.85	0.74	0.91	—

— = Not applicable.

Table 7—Multivariate analysis of factors related to values for the natural logarithm of a kinetics ELISA (LNKELA) for detection of *Mycobacterium avium* subsp *paratuberculosis* in 6 commercial dairy herds

Variable	Estimate	SE	*P*
Intercept	2.20	0.19	0.01
Lactation			
1	0.00	NA	NA
2	0.33	0.08	0.01
≥ 3	0.52	0.12	0.01
No. of days in milk			
< 15	0.00	NA	NA
15 to 30	0.77	0.21	0.01
30 to 60	0.84	0.18	0.01
60 to 90	0.74	0.18	0.01
90 to 120	0.63	0.18	0.01
> 120	0.72	0.16	0.01
Nonlactating period	1.02	0.19	0.01
Final 2 weeks of nonlactating period	0.60	0.23	0.01
Categories for culture of fecal samples			
Negative	0.00	NA	NA
Low shedder	0.17	0.15	0.28
Moderate shedder	0.29	0.20	0.16
Heavy shedder	0.74	0.20	0.01

NA = Not applicable.

information criteria of 933. The –2 log-likelihood of the null model was 1,220, which decreased to 929 for the saturated model. The variance component of the repeated measures on a cow within a herd was 35%, and the variance component of the residual was 24%; thus, the intraclass correlation for cow within a herd was 59%. Analysis of the results revealed that cows in lactation 2 or higher had increased KELA values, compared with cows in the first lactation. Cows that were within 15 days after parturition (ie, ≤ 15 days in milk) had the lowest KELA value, which rapidly increased until 60 days in milk and then remained similar throughout the remainder of lactation.

Heavy shedders had significantly ($P = 0.01$) higher KELA values than cows with negative culture results (Table 7). Results of culture of previous fecal samples were also included in the model, and all were found to be significantly correlated. Estimates for correlation on the basis of categories of culture results were calculated (**Table 8**). In models that included culture results of 3 to 5 months previously, lactation number was not significant anymore, but it was allowed to remain in the model for better comparison of estimates. Analysis of the results revealed a general trend that KELA values of shedders increased over time. Heavy shedding can be predicted from current KELA values, although the results of samples obtained 1, 2, or 3 sample collection periods earlier were more strongly related to the current KELA value. However, the current KELA value was increased when a cow was classified as a moderate shedder in the culture performed 2 samples previously, which was, on average, 132 days previously (Table 4). The KELA value was increased when a cow was classified as a low shedder in the culture performed 4 samples previously, which was, on average, 236 days previously.

Table 8—Multivariate analysis of the prognostic value for LNKELA values of culture of fecal samples obtained 1 to 5 samples previously for detection of *Mycobacterium avium* subsp *paratuberculosis* in 6 commercial dairy herds

Variable	n	Estimate	SE	*P*
Culture categories of previous fecal sample				
Negative	456	0.00	NA	NA
Low shedder	26	0.23	0.16	0.18
Moderate shedder	17	0.36	0.20	0.08
Heavy shedder	10	1.08	0.24	0.01
Culture categories of 2 samples previously				
Negative	402	0.00	NA	NA
Low shedder	26	0.27	0.17	0.14
Moderate shedder	14	0.66	0.22	0.01
Heavy shedder	6	1.46	0.30	0.01
Culture categories of 3 samples previously				
Negative	333	0.00	NA	NA
Low shedder	22	0.17	0.20	0.41
Moderate shedder	12	0.61	0.25	0.03
Heavy shedder	2	1.52	0.51	0.01
Culture categories of 4 samples previously				
Negative	277	0.00	NA	NA
low shedder	20	0.39	0.17	0.05
Moderate shedder	9	1.07	0.23	0.01
Heavy shedder	0	NA	NA	NA
Culture categories of 5 samples previously				
Negative	217	0.00	NA	NA
Low shedder	14	0.46	0.23	0.08
Moderate shedder	6	0.84	0.31	0.03
Heavy shedder	0	NA	NA	NA

NA = Not applicable.

Table 9—Results of multivariate analysis for detection of fecal shedding of *Mycobacterium avium* subsp *paratuberculosis* corrected for the cow-within-herd correlation

Effect	Estimate	SE	*P*
Intercept	–2.628	0.412	< 0.001
LNKELA	0.287	0.088	0.001
Lactation			
1	0.000	NA	NA
2	–0.415	0.167	0.013
> 2	–1.216	0.433	0.006

NA = Not applicable.

Multivariate analysis of results of culture of fecal samples—Results of a multivariate analysis for the MAP infection status of cows, as determined on the basis of results of culture of fecal samples, were tabulated (**Table 9**). The cow-within-herd effect had a correlation of 0.77. Every unit of increase in LNKELA value increased the risk of a positive culture result. The chance of becoming a shedder was highest in first-lactation cows and decreased in cows during the second and higher lactations. None of the other variables was found to be significantly correlated.

Continuous KELA values provided the best prediction of culture-determined infection status. The KELA was not a significant variable when entered in the model as a 4-category variable or a dichotomous variable with thresholds of 65, 80, and 90. Moreover, previous KELA values did not significantly influence the odds of being classified as a shedder. Higher lactation cows (≥ 3 lactations) were less likely to become shedders than were first-lactation cows.

Discussion

In the study reported here, results of the KELA could be used to predict impending fecal shedding, but the relationship was weak and mainly caused by increased KELA values of heavy shedders. The increased antibody concentrations of moderate and, in particular, heavy fecal shedders were in agreement with results of other studies.[1,5] It is generally believed that the serologic response follows fecal shedding,[11] and results of the study reported here confirmed that assumption. Shedding precedes the humoral immune response as indicated by analysis with models that evaluated previous culture results. In this study, 81% of the heavy shedders were detected on the basis of their current KELA value. However, the KELA value was significantly increased, on average, 236 and 132 days after MAP was first cultured in fecal samples of low and moderate shedders. This has important implications for management programs that are intended to reduce MAP prevalence. Shedders cannot be detected by use of KELA results until they are shedding large numbers of MAP; thus, they continue to contaminate the environment for a prolonged period. Therefore, when the

goal of a program is to reduce MAP prevalence by eliminating all shedders, producers and veterinarians should not rely solely on the KELA results but should use culture of fecal samples to test suspect cows. Methods for rapid culture of fecal samples can be used to decrease the time to detection of shedders, which will enhance the advantage of culture of fecal samples over ELISAs. The KELA can be a useful tool for producers and veterinarians who are only interested in detecting heavy shedders, especially in high-prevalence herds that have more cattle in advanced stages of infection. When KELA results are used to estimate MAP prevalence in herds, it is important to realize that these results do not have prognostic value for low to moderate shedders that are in the herd on the day of testing. The value of KELA results for prognostication of shedding cattle will be improved considerably when used repeatedly in a herd or in a subgroup of cows that are suspected of becoming shedders.

The best predictor for increased KELA values was a categoric variable for fecal shedding (negative, low shedder, moderate shedder, and heavy shedder); those categories were approximations determined on the basis of the number of days the sample took to yield positive results in the culture system. We also included categoric KELA values and standardized KELA values in the model, but none had a better prognostic value than the continuous KELA value.

The power of the study was slightly limited by the low numbers of samples, herds, and cows (ie, maximum of 6 fecal samples cultured/cow; 112 cows in 6 herds). Because of the limited sample size, results of the study should be interpreted with caution; it is not known whether results reported here will be valid for other cows and herds. However, we believe that the results are consistent with those reported in other studies and, thus, may be generalized to other cows in commercial dairy herds.

Variation in KELA values is strongly dependent on herd and cow factors. Random cow-within-herd effect explained 59% of the total variance. Part of the variation may be explained by the ELISA performance, which varies for herds that differ in prevalence.[12,13] In addition, KELA values for infected cows increased with an increasing number of lactations and also had a predictable pattern during a lactation. The KELA values were lower during the first 15 days in milk, increased until 60 days in milk, and then appeared to stabilize throughout the remainder of lactation. Therefore, increased KELA results in fecal shedders may be detected best after cows reach 60 days in milk.

The correlation between subsequent KELA results was fairly high, indicating a consistent pattern when cows are tested repeatedly. When previous and current KELA categories were tabulated, 76% of the cows remained in the same category; a similar result was found for all categories of culture results of fecal samples (data not shown). The correlation for subsequent culture results was high, which was a result of little intermittent shedding. Only 7% of the shedding cows had negative results on a concurrent culture of a fecal sample (data not shown).

Analysis of results of the study reported here confirmed the suspicion reported in other studies[3-5] that considerable cow- and herd-level variation exists in results of KELA and culture of fecal samples. Test interpretation will be improved when conducted on a herd-specific basis. Moreover, KELA results need to be interpreted for each cow by taking the number and stage of lactation into account. Increased KELA values indicate an increased chance of fecal shedding. However, the infection status of a cow should be verified by culture of a fecal sample.

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[a]TREK ESP Culture System II, TREK Diagnostic Systems Inc, Westlake, Ohio.

[b]Shin SJ, Kim SG, Miller LJ, et al. Further evaluation of ESP Culture System II for detection of *Mycobaccteria avium* subsp *paratuberculosis* in bovine fecal samples (abstr), in *Proceedings*. 44th Annu Meet Am Assoc Vet Lab Diagn, 201;39.

[c]Shin SJ. New methods for reduction in bacterial and fungal contamination from fecal-culture for *Mycobacterium paratuberculosis* (abstr), in *Proceedings*. 93rd Annu Meet U S Anim Health Assoc, 1989;381.

[d]Dairy COMP 05, Valley Agricultural Software, Tulare, Calif.

[e]SAS, version 8.2, SAS Institute Inc, Cary, NC.

[f]National Agriculture Statistics Services. Available at: www.nass.usda.gov/ny/bulletin/2001/01-bulle.htm. Accessed Mar 5, 2003.

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