Association between soil type and paratuberculosis in cattle herds

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Objective—To determine the association between soil type and paratuberculosis in cattle.

Sample Population—Soil samples and test results for paratuberculosis in 92 Indiana cattle herds.

Procedure—Testing records from herds in which ≥ 20 cattle were tested for paratuberculosis by use of an ELISA between 1998 and 2002 were identified. Soil type was characterized on the basis of herd location. Clusters of herds with seroprevalence greater than the median seroprevalence were identified. Association between clusters and soil types was estimated by logistic regression, adjusted for herd type (dairy or beef).

Results—A spatial cluster of greater than the median seroprevalence was identified in northeast Indiana. Soils with low silt content were associated (odds ratio [OR], 7.2; 95% confidence interval [CI], 2.1 to 24.5) with this cluster. Adjusting for herd type did not substantially alter this association (OR, 6.7). Herds located in areas with sandy loam (OR, 6.2; 95% CI, 1.4 to 27.4) and loam (OR, 3.6; 95% CI, 1.0 to 13.2) soils were also more likely to be within the cluster of greater than the median seroprevalence. Herds located in areas of silt loam soils were less likely (OR, 0.2; 95% CI, 0.1 to 0.7) to be included in this cluster.

Conclusions and Clinical Relevance—Spatial distribution of herds with greater than the median seroprevalence of paratuberculosis was associated with soil characteristics. Survival of *Mycobacterium avium* subsp *paratuberculosis* may be enhanced by silt or sand content in loamy soils. These results may be used to modify paratuberculosis control programs. (*Am J Vet Res* 2004;65:10–14)

Paratuberculosis (ie, Johne's disease) in cattle is characterized by chronic enteritis attributable to infection with ***Mycobacterium avium* subsp *paratuberculosis* (MAP)**. It has important economic impacts on cattle production, including direct, indirect, and inapparent losses.[1,2] In 1999, costs attributable to reduced productivity were estimated at $200 million to $250 million/y for the US dairy industry.[3] When the prevalence of clinically affected cattle among culled cows in a herd is ≥ 10%, the average cost may be > $200/cow.[3] Consequently, control of paratuberculosis is considered a high priority in the United States.[4]

An association between high prevalence of MAP infection in ruminants and soil type has been recognized. However, some reports[5,6] were based on anecdotal information without inclusion of control herds and lacked quantitative estimation of the risk. Paratuberculosis in sheep and goats in Spain was associated with entisol soils.[7] Although the reason for the association is unclear, the authors of that study suggested that a combination of low soil pH and lack of nutrients in the soil may be responsible. An association between paratuberculosis, low soil pH, and soil iron content was found in dairy herds in Michigan.[8] To our knowledge, that was the first report in which soil-specific characteristics were significantly associated with paratuberculosis.

Soils are complex structures. Soil formation is influenced by climate, parent material, landscape position, living organisms, and human activity. Soils with similar properties have the same characteristic behavior in terms of agricultural use, regardless of the geographic location of the soil. Soil properties are numerous and varied, including texture, slope, drainage class, permeability, pH, and frost potential.[9] Analogous to other agricultural variables, survival of microorganisms is likely to be influenced by more than 1 soil property.

Understanding the relationship between soil factors and paratuberculosis would allow at-risk herds to be identified and the expected impact of control activities to be assessed. For example, the dairy survey conducted in 2002 by the USDA National Animal Health Monitoring System included an assessment of risk factors associated with paratuberculosis. That assessment was designed to quantitatively estimate the risk of paratuberculosis in a herd; however, it did not consider soil-related factors. Assuming that soil type is associated with paratuberculosis, the accuracy of risk assessments would be improved by incorporating information on soil type for herd locations. The objective of the study reported here was to assess the association between soil characteristics and the spatial distribution of paratuberculosis in cattle herds in Indiana.

Materials and Methods

Study population—Results of diagnostic testing conducted between January 1998 and December 2002 to detect paratuberculosis in 359 cattle herds in Indiana were available. Herds (n = 112) in which ≥ 20 cattle had been tested by use of an ELISA were selected for the study. For 20 of these herds, it was not possible to identify an address, and data from these herds were excluded from analysis. Results of the ELISA for the remaining 92 cattle herds were analyzed. Number of cattle tested in each herd ranged from 20 to 2,936

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(median, 50 cattle), and prevalence of paratuberculosis in those herds ranged from 0% to 81% (median, 8.8%). Mean number of times each herd was tested was 6. In the 65 herds in which cattle were tested 2 or more times, the median interval between tests during the study period was 1.7 years.

Indiana counties were grouped into 9 agricultural districts.[10] The northern, central, and southern regions contain 31%, 28%, and 41% of the state's cattle herds, respectively.[11] Of the 92 herds included in the study, 47 (51%), 37 (40%), and 8 (9%) were located in the northern, central, and southern regions, respectively. Sixty-six (72%) herds included in the study were dairy herds, and 21 (23%) herds were beef herds. In the remaining 5 (5%) herds, it was not possible to identify the breed or production type of the cattle. Data on management practices were not available.

Data collection—Data collected for each herd included address and zip code as well as date, number, and result (positive or negative) for cattle tested by use of the ELISA. Locations of the study herds were geographically coded[a] by use of roads maps from the 2000 national census.[b] Soil characteristics for each herd were identified from information included in a database.[c] For each herd, recorded soil data included a mean value for the range of slope of a soil component within a map unit (ie, slope), texture, mean value for the range of depths to the seasonally high water table during the months specified (ie, depth of water table), mean value for the range of depths for surface water that could form a pond on the soil (ie, ponding), rating of the susceptibility of the soil to frost (ie, frost), frequency and duration of periods when the soil was free of saturation (ie, drainage), hydric soil rating (ie, hydric), rating of the susceptibility of concrete to corrosion when in contact with the soil (ie, concrete corrosion), rating of the susceptibility of uncoated steel to corrosion when in contact with the soil (ie, steel corrosion), and rating of the soil for nonirrigated agricultural use (ie, agricultural use).

Statistical analysis—A test for clustering of disease (ie, the scan statistic[d]) was used to detect spatial clustering of paratuberculosis.[12] The scan statistic is defined by the maximum number of cases detected in a predefined spatial window. The window is moved systematically across the study area. In each window, the number of cases of disease is compared with the number expected (assuming that cases are randomly distributed) such that aggregations of cases (ie, clusters) can be identified. In the data analyzed, the overall proportion of cases (cattle seropositive on the ELISA) was < 10%, and results of preliminary analyses were unaffected by the distribution (Bernoulli or Poisson) of expected cases. Subsequently, the distribution of cases was assumed to be a Poisson distribution[12] such that each herd was considered a census tract, with the total number of cattle tested during the study period as the at-risk population.[13] The data set was scanned for clusters of herds with seroprevalence greater than the median (ie, > 8.8%) and seroprevalence less than or equal to the median. Scanning windows that comprised up to 50% of the study area were used to identify clusters, and a likelihood-ratio test statistic was calculated for each cluster identified. Distribution of the likelihood ratio was obtained by use of a Monte-Carlo simulation such that type-1 error was maintained at 5%.[d]

When the identified clusters were associated with soil type, then clustering should have become weaker when soil type was included as a covariate. Therefore, additional analyses including slope (herds with greater than the median seroprevalence and herds with less than or equal to the median seroprevalence); soil content of silt (> 50% or ≤ 50%), sand (> 50% or ≤ 50%), or clay (> 30% or ≤ 30%); depth of the water table (herds with greater than the median seroprevalence and herds with less than or equal to the median seroprevalence); ponding (herds with greater than the median seroprevalence and herds with less than or equal to the median seroprevalence); frost (high or low to moderate); drainage (poor or worse and moderate or better); hydric (yes or no); concrete corrosion (high or low to moderate); steel corrosion (high or low to moderate); or agricultural use (herds with greater than the median seroprevalence and herds with less than or equal to the median seroprevalence) were performed. The association between these independent variables and MAP seroprevalence was assessed by use of logistic regression models.[e] Herds within the clusters that had greater than the median seroprevalence were considered case herds, and the herds excluded from the clusters of greater than the median seroprevalence were considered control herds. Bivariate logistic regression was initially performed, and only those variables significantly ($P < 0.20$) associated with herd seroprevalence were made available for selection into a stepwise, multivariate model (value to enter model, $P < 0.05$; value to leave model, $P < 0.10$). Model fit was assessed by use of the Hosmer-Lemeshow statistic.[e]

Possible confounding of the association between soil type and paratuberculosis on the basis of herd type (beef or dairy) was investigated by use of the Mantel-Haenszel adjusted **odds ratios (ORs)**.[f] A change in crude versus adjusted OR > 10% was considered evidence of confounding.

Results

Significant ($P = 0.01$) clusters of greater than the median seroprevalence (1 cluster) and less than or equal to the median seroprevalence (2 clusters) were identified by use of the spatial scan statistic **(Fig 1)**. The cluster of

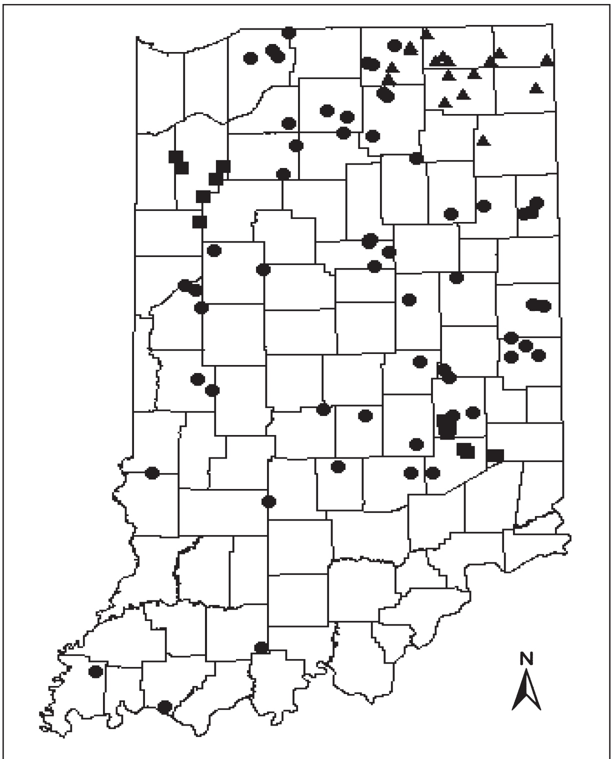

Figure 1—Spatial distribution of cattle herds in Indiana in which ≥ 20 cattle were tested between 1998 and 2002 to detect antibodies against *Mycobacterium avium* subsp *paratuberculosis*. Each herd tested is indicated by a dot. Median seroprevalence in herds was 8.8%. Notice the clusters of herds with greater than the median seroprevalence (ie, > 8.8%; triangle) and less than or equal to the median seroprevalence (square).

Table 1—Soil factors associated with cattle herds in Indiana within a spatial cluster of greater than the median seroprevalence (ie, > 8.8%) for antibodies against *Mycobacterium avium* subsp *paratuberculosis* (17 case herds), compared with herds not included in a cluster (75 control herds)

Variable	Category	Controls	Cases	Odds ratio	*P*
Silt content*					0.002
	≤ 50%	23	13	7.20	
	> 50%	51	4	1.0†	
Sand content*					0.059
	≤ 50%	63	11	0.32	
	> 50%	11	6	1.0†	
Clay content					0.801
	≤ 30%	5	0	—	
	> 30%	69	17	1.0†	
Frost					0.323
	Low to moderate	38	11	1.74	
	High	36	6	1.0†	
Concrete corrosion					0.791
	Low to moderate	55	13	1.18	
	High	20	4	1.0†	
Steel corrosion					0.612
	Low to moderate	36	7	0.76	
	High	39	10	1.0†	
Drainage*					0.051
	Poor or worse	38	4	0.30	
	Moderate or better	37	13	1.0†	
Hydric					0.784
	No	63	17	—	
	Yes	12	0	1.0†	
Slope‡					0.866
	Low (≤ 1%)	38	9	1.09	
	High (> 1%)	37	8	1.0†	
Ponding‡					0.793
	No (≤ 0)	64	17	—	
	Yes (> 0)	11	0	1.0†	
Depth of water table‡					0.219
	Low (≤ 3)	39	6	0.50	
	High (> 3)	36	11	1.0†	
Limitations of agricultural use‡					0.503
	Few (≤ 2)	61	15	1.72	
	High (> 2)	14	2	1.0†	

*Variable is significantly ($P < 0.20$) associated with paratuberculosis in cattle. †Reference category for comparison. ‡Based on median value for the variable. — = Not determined (zero denominator). Ponding = Mean value for the range of depth of surface water that could form a pond on the soil.

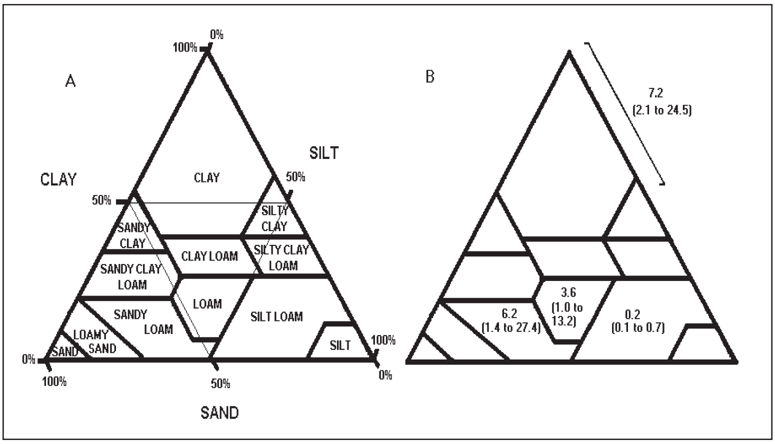

Figure 2—Soil textures determined on the basis of silt, clay, or sand content (A) and risk of paratuberculosis in cattle herds in Indiana that were tested between 1998 and 2002 by use of an ELISA, stratified on the basis of soil texture (B). Values reported are the odds ratio (95% confidence interval). Notice the significant ($P < 0.05$) odds ratio for herds located on soil types being within a spatial cluster of herds with greater than the median seroprevalence (ie, > 8.8%). Notice that there is also a significant ($P < 0.05$) odds ratio for herds located on soil types that were < 50% silt.

greater than the median seroprevalence (217 seropositive cattle; 103 expected) included 17 herds (case herds). The clusters of less than or equal to the median seroprevalence included 6 herds (157 seropositive cattle; 297 expected) and 9 herds (30 seropositive cattle; 65 expected), respectively. Adjustment for soil variables reduced the size of the cluster of greater than the median seroprevalence to 11 herds (165 seropositive cattle; 123 expected) and accounted for the clusters of lower seroprevalence. We did not detect a significant (Wilcoxon rank-sum test statistic, 0.24; $P = 0.81$) difference between the number of cattle tested per herd within this cluster, compared with the number of cattle tested per herd in other herds in the study.

Results of bivariate logistic regression analyses comparing herds within the cluster of greater than the median seroprevalence (17 case herds) to the herds excluded from the cluster (75 control herds) revealed that clustering was significantly ($P = 0.20$) associated with 3 soil variables (silt content, sand content, and drainage; **Table 1**). Risk of being within the cluster of greater than the median seroprevalence was significantly greater for herds located within areas of soil with low silt content (OR, 7.2; 95% **confidence interval [CI]**, 2.1 to 24.5), loam soil (OR, 3.6; 95% CI, 1.0 to

13.2), or sandy loam soil (OR, 6.2; 95% CI, 1.4 to 27.4), and risk was significantly lower for herds located in areas of silt loam soil (OR, 0.2; 95% CI, 0.1 to 0.7; **Fig 2**). Only low silt content was significantly associated with greater than the median seroprevalence in the multivariate model. This model fit the data adequately (Hosmer-Lemeshow χ^2 statistic, 0.81; $P = 0.67$), and the status (case or control herd) of 81.1% of herds was correctly classified. We did not detect a substantial (> 10%) change in the association between risk of a herd being in the greater than the median seroprevalence cluster and silt content when the OR estimate was adjusted for herd type (Mantel-Haenszel OR, 6.7).

Discussion

Clustering of herds with greater than the median seroprevalence for paratuberculosis in Indiana can be explained on the basis of soil characteristics for herd locations. Loamy (silt loam, loam, or sandy loam) soils are rich in organic matter and therefore the most productive soil types for agricultural use. Lack of a significant association between paratuberculosis and other soil types may be attributable to the fact that few herds with these characteristics were included in the study. Herds located in areas of silt loam soils were, typically, 80% less likely to be included in the cluster of greater than the median seroprevalence, compared with herds located in areas with other soil types. Herds located in areas of loam and sandy loam soils were, typically, 4 and 6 times more likely, respectively, to be included in the cluster of greater than the median seroprevalence than herds located in areas with other soil types. Soils associated with low (silt loam) and high (loam and sandy loam) risk of paratuberculosis can be classified with respect to silt content (Fig 2). Herds located in areas of low silt soils (< 50% silt) were significantly ($P = 0.01$) more likely (typically, 7 times more likely) to be included in the cluster of greater than the median seroprevalence than herds located in areas of silty soils (> 50% silt). Although not significantly different, herds located in areas of sandy soils were 3 times more likely to be included in the greater than the median seroprevalence cluster. Thus, as silt was replaced by sand within loamy soils, the risk of being included in the cluster of higher seroprevalence increased. This association may have been related to the reported[8] association between soil pH and MAP.

Infiltration of water and leaching is higher in sandy soils, decreasing the pH and low base saturation.[14] Good drainage, correlated with lack of water retention and possible leaching, was also associated with paratuberculosis seroprevalence. Unlike many other bacteria, *Mycobacterium* spp survive better in acidic environments. A low pH provides a competitive advantage for the survival of *Mycobacterium* organisms, compared with survival for other microbes. Subsequently, environments rich in organic matter and that have a low pH, such as sandy loam soils, may provide conditions that enhance survival of *Mycobacterium* organisms and therefore environmental persistence.[15] In another study in Spain,[7] a higher risk of paratuberculosis was detected in sheep flocks and goat herds kept on sandy, low pH soils, compared with herds kept on soils with higher pH and higher capacity for water retention.

The ability of MAP to survive in the environment is an important component of the epidemiologic characteristics of paratuberculosis. Pastures contaminated with MAP may remain sources of infection for at least 1 year, depending on environmental conditions.[16] Reducing the exposure of calves to soils in which MAP is more likely to survive, as well as avoiding the application of manure and preventing grazing of infected cattle on these types of soils, may reduce transmission rates of MAP within herds and improve the success of paratuberculosis control programs.

Herds included in the study reported here were not randomly selected. Cattle herds in southern Indiana (where a relative higher proportion of beef herds are located) were underrepresented. The inclusion of a greater proportion (74%) of dairy herds in this study, compared with the proportion of dairy herds in Indiana (17% of all cattle herds), may be explained by the importance of paratuberculosis to the dairy industry and, consequently, increased participation in paratuberculosis control programs. Differing management practices may affect the timing of exposure of cattle to contaminated pastures in various production systems. However, herd type did not substantially affect the estimated risk associated with silt content in soils.

A minimum number of cattle tested (ie, 20) was used as an inclusion criterion in the study. The MAP ELISA lacks sensitivity, although sensitivity increases with progression of clinical paratuberculosis. Sensitivity of the ELISA has been estimated at 45%.[17] Herd-level sensitivity is positively correlated with number of cattle tested in each herd. Assuming an ELISA sensitivity of 45%,[17] ELISA specificity of 99%,[17] prevalence of MAP-infected herds of 20%,[17] and a minimum of 20 cattle tested in each herd, minimum herd-level ELISA sensitivity in our study was approximately 87%. On the basis of the median number of cattle tested in each herd (ie, 50 cattle/herd), herd-level sensitivity was 99%. Herd-level sensitivity was considered sufficient and was a compromise between detection of MAP infection and inclusion of a sufficient number of herds to provide study power to detect potentially important associations between paratuberculosis and soil type.

Herd-level specificity depends on the animal-level specificity of a test and is negatively correlated with the number of cattle tested. On the basis of the median number of cattle tested in each herd, the herd-level specificity (estimated as approx 61%) was low in this study. However, because we did not detect a significant difference between the number of cattle tested for each herd within the identified clusters, compared with the number of cattle tested for other herds in the study, it is unlikely that differences in herd specificity could have caused the detected clustering.

Because of a lack of data to suggest the contrary, we assumed that diagnostic test errors were nondifferential (ie, false-positive and false-negative herds were independent of soil type); therefore, the soil associations we found were not biased. However, the estimated association between paratuberculosis and soil type may have resulted from other geographically related risk factors. For example, trade in cattle is expected to be more common between neighboring herds than among herds located long distances apart. Thus, infected cattle sold to neighboring herds with a similar type of soil could have resulted in a spurious association between paratuberculosis and soil type.

We conducted a hypothesis-generating study; therefore, results of the study reported here should be confirmed by controlling these potential biases. Longitudinal studies of identified clusters, including testing of soil samples, may provide more definitive information regarding the association between paratuberculosis and soil type.

Although the specific nature of the association between paratuberculosis and soil type remains unclear and possible confounding factors (including herd and host factors, such as management practices, cattle breed and genetics, and methods adopted by producers to control paratuberculosis) need to be considered, to our knowledge, this is the first report in which identified geographic clusters of paratuberculosis in cattle have been associated with soil type. The strong association between paratuberculosis and soil type estimated in the study reported here suggests that follow-up studies, particularly those involving the use of a prospective study design, should be conducted. Additional investigations should be directed at estimating the magnitude of the association more accurately, investigating the role of other soil factors, and determining the possible impact of this association on the design and evaluation of regional control programs for endemic paratuberculosis.

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