

Results of Serological Testing of AABP Members for Antibody to *M. paratuberculosis*

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Background

Mycobacterium paratuberculosis causes Johne's disease in a variety of animal (primarily ruminant) species. Some recent reports in medical literature suggest that this pathogen may also infect humans. The organism has been incriminated as a possible cause of Crohn's disease, a chronic inflammatory bowel disease in humans with marked clinical and pathological similarity to Johne's disease.

Objective

The objective of the research was to determine if humans with a high risk of exposure to *M. paratuberculosis* had different levels of serum antibody to this infectious agent than persons with low risk of exposure.

Subjects

Representing a group with a high *M. paratuberculosis*-exposure risk, 191 members of the American Association of Bovine Practitioners (AABP) attending the 1996 AABP convention volunteered to participate. The moderate exposure group was represented by 193 serum samples collected in 1990 from farmers in Barron County, Wisconsin. All types of farming enterprises, dairy and crop production were represented. The low exposure group was 242 normal healthy blood donors who visited the Red Cross Center in Madison, Wisconsin in January and February, 1996.

Serologic assay

The commercially available (IDEXX Laboratories, Inc.) ELISA for bovine paratuberculosis was used with minor modification. The anti-bovine immunoglobulin conjugate supplied with the kit was replaced with goat anti-human-immunoglobulin and optimized for conjugate dilution, serum dilution and stop time. The negative control serum was a commercial pool of normal human serum (Binding Site). The positive control was serum from a veterinarian who had been accidentally

inoculated with the vaccine for bovine paratuberculosis on two separate occasions. Both inoculations resulted in a strong immunologic response as indicated by pathology at the inoculation site. ELISA optical density readings for all samples were transformed into a standardized score (ELISA values) by comparison to both the negative and positive controls such that values of 0 were equivalent to the OD of the negative control and values of 100 were equivalent to that of the positive control.

Results

Mean \pm standard deviation ELISA values for Red Cross donors, farmers and AABP members were 18.5 ± 21.1 , 20.8 ± 67.8 , and 34.3 ± 39.0 , respectively. These ELISA values were not normally distributed, thus non-parametric methods were used for statistical comparison of the groups. The Kruskal-Wallis nonparametric ANOVA indicated that the differences among ELISA values for the three human populations was extremely significant ($p < 0.00001$). Dunn's multiple comparisons test showed that both farmers and AABP members had significantly higher ELISA values than the controls ($p < 0.001$), and AABP members had ELISA values significantly higher than farmers ($p < 0.001$). If a cut-off for a positive ELISA was established as the mean plus three standard deviations above the mean of normal controls, as is conventional (assay specificity = 99% by definition), then those persons with ELISA values > 81.8 would be classified as positive for serologic response to *M. paratuberculosis*. By this standard of interpretation, 21 AABP members (11.7%), 22 farmers (11.4%), and 8 control subjects (3.3%) tested "positive" for evidence of past or present *M. paratuberculosis* infection. Fourteen (7.8%) of AABP members had ELISA results greater than the positive control (a veterinarian twice inoculated with Johne's vaccine).

Interpretation / implications

Persons in occupations that are likely to bring them

in close contact with animals infected with *M. paratuberculosis*, such as farmers and bovine veterinarians, have a significantly higher probability of having elevated levels of serum antibody to this veterinary pathogen. This evidence of serological response suggests that infection with *M. paratuberculosis* has occurred. These findings support the theory that *M. paratuberculosis* has the capacity to infect humans. The findings do not ad-

dress the ability of this bacterial agent to cause disease in humans but document the importance of conducting research on this question.

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