ENVIRONMENTAL CONTAMINATION ON DAIRY FARMS WITH CATTLE INFECTED WITH MYCOBACTERIUM PARATUBERCULOSIS*

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Introduction:

The extent and persistence of Mycobacterium paratuberculosis environmental contamination on farms with a known history of Johne's disease is a commonly asked question by the agricultural community. Prospective buyers of farms following a dispersal sale often inquire about the possibility of newly acquired cattle becoming infected with M. paratuberculosis from the environment where Johne's cattle had previously been located. Thus, based on continuing inquiries from cattlemen, we developed a prospective research proposal to assess the extent of environmental contamination with M. paratuberculosis associated with known levels of infection in the herds as determined with whole herd fecal culture tests of adult cattle using a sensitive technique utilizing centrifugation[1].

Previous studies on the survival of M. paratuberculosis have been largely conducted under standardized laboratory conditions and not from samples taken from the immediate farm environment[2,3,4]. Those studies suggested that M. paratuberculosis is somewhat long lived in a variety of environmental conditions. Non-laboratory based studies have focused on the survival of M. paratuberculosis in slurry and no studies have focused on the extent of soil and environmental contamination on farms with Johne's disease.

The frequency of occurrence of M. paratuberculosis in the environment of farms with known infection of cattle with M. paratuberculosis has not been reported. But, the survival of M. paratuberculosis in water, slurry, urine and other media has been published[2,3,4,5]. Previous authors often used laboratory-grown isolates then inoculated a variety of materials and monitored the recovery rate over time. Vishnewskii[6] first reported that bovine urine markedly reduced the survival time of M. paratuberculosis to less than 10 days. The inhibitory effect of urine on tubercle bacteria growth has also been recognized for some time but the factor responsible for the inhibition has not been isolated[7,8]. Urine has a similar inhibitory effect on salmonella[9].

Experimental Design:

Eleven herds previously known to have had multiple animals infected with M. paratuberculosis were selected because of the willingness of the herd owners to cooperate by allowing the investigators to take the environmental samples and the farms were located within 150 miles of New Bolton Center, Kennett Square, PA. The herds were believed to represent a cross-section of dairy management practices in Pennsylvania. The herd size ranged from 40 to 125 milking cows in a stanchion barn or free-stall design barn. The herds were either of the Holstein or Guernsey breed.

Approximately 50-60 individual predetermined sites were selected from which to obtain the environmental sample specimens. Samples from sites inside the barn were scraped with a clean plastic cup and the placed in a four ounce specimen container. Soil samples from outside the barn were collected with a clean metal trowel and then placed in a covered specimen container. Obvious manure samples were avoided. Maternity stalls, calf hutches, mangers, feed bank areas and heifer pens provided sites for 20-25 samples from inside the barn. The cow pasture, exercise lots, heifer pastures, silage feeding areas both moist and dry represented the predetermined sites outside the barns. All environmental samples were collected between June and August, 1990.

Culture procedures:

A two gram aliquot of each environmental sample was processed using the double incubation described[10]. Each of the four tubes containing mycobactin and pyruvate (4.1 gm/liter) was inoculated with 0.20 ml of resuspended pellet. The tubes of Herrold's egg yolk media was incubated at 37°C for 16 weeks, with the tubes usually being evaluated every two weeks for colonies compatible with M. paratuberculosis and contaminants. Colonies morphologically compatible with M. paratuberculosis were sub-cultured onto two tubes, one with and one without mycobactin. The organisms were stained with Ziehl-Neelsen stain and evaluated microscopically to be acid-fast and club or dumbbell shaped.

Results:

Five of the eleven farms sampled had samples positive for M. paratuberculosis. Four of the five farms had two or fewer positive samples. Only one farm had extensive environmental contamination with 13 of 59 samples positive, many with high colony counts. Of the total of 672 environmental samples obtained, 20 or 3% were culture positive for M. paratuberculosis. Except for the heavily contaminated farm all positive cultures were from the pastures and exercise lots and not inside the barn.

Discussion:

Previous attempts to isolate M. paratuberculosis from soil utilizing the techniques reported to be adequate for fecal samples resulted in extensive contamination primarily from fungi but also included bacteria[11,12,13,14]. The contamination rate often ranged between 70-100% of the tubes inoculated. Therefore, until techniques were developed to control the excessive contamination of the culture tubes the project was delayed. The development of the double incubation technique by Shin and co-workers at Cornell represented a major advance in the culture methodology since this technique virtually eliminates both bacterial and fungal contamination in fecal cultures for mycobacteria[10]. The contamination rate is further enhanced by centrifugation which concentrates the fungal elements and bacterial spores along with the mycobacteria.

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Larsen[2] first reported that bovine urine inhibited growth of M. paratuberculosis in the laboratory with decreased growth directly related to increasing concentrations of urine. With 2% urine in the media no

growth occurred. The critical value seemed to be between 2% with no effect and at 5% urine in the media with complete cessation of growth. The addition of bovine feces also inhibited growth in a proportional manner. Thus, it would seem that recovery of M. paratuberculosis in a slurry media would be inhibited, however, the slurry storage experiments would suggest otherwise.

Larsen[2] found that M. paratuberculosis survived in tap water or saline for up to 17 months, with the pH adjusted to either be more acid at 5.5 or more alkaline up to 8.5; with added 1% gelatin the survival time was still 14 to 17 months. Suspension of organisms in bovine urine marked reduced survival to less than a month. The constituent in urine inhibiting growth or survival of M. paratuberculosis was not identified.

The survival of M. paratuberculosis in both swine and bovine slurry was studied experimentally by inoculating 0.1 mg (3.3×10^6 viable units) per ml then storing the slurry in sealed vials at 5^0 or 15^0C under anaerobic conditions[3]. Viable organisms were detectable up to 252 days in both swine and cattle slurry at 5^0C while survival was reduced to 182 days for swine slurry and 98 days for cattle slurry when stored at 15^0C. The pH of the slurry from both swine and cattle was quite alkaline (8.3 to 8.5) which may favor survival in as much as M. paratuberculosis grows better in an alkaline environment. The initial dose of viable organism used per ml of slurry was likely in excess of the concentration in even the environment from a farm with many infected cattle. The concentration used was nearly 10,000 fold greater than present in the manure of a cow with an advanced stage of infection, but not yet showing clinical signs. Jorgensen's results were similar to those of Lovell[5] who found that naturally infected cattle feces contained viable organisms after exposure to atmosphere conditions for 246 days.

Test cultures of M. paratuberculosis placed in bovine manure silage and corn silage became negative by the fifth day after ensiling. The pH of the silages were less than 4.2 while media above 4.6 supported growth of M. paratuberculosis[4]. The recovery of mycobacteria from environmental samples is a function of several independent processes including sample size, decontamination procedures, sample preparation procedures, inoculum size per tube and recovery medium composition. We believe the process utilized in this current study to be state-of-the-art but not necessarily optimal. The double incubation step nearly eliminated both bacterial and fungal contamination yet did not impair growth of M. paratuberculosis. (unpublished data) However, it is possible and perhaps likely that the environmental contamination is higher and more extensive than the data described. Although M. paratuberculosis was not detected in the samples, it is not proof the organism was not present. Similarly infected cattle likely shed the organism in the feces for many months prior to detection by standard methods. In all likelihood the organism was present but the culture techniques did not have the sensitivity to detect M. paratuberculosis.

Conclusions:

Although previous published data suggested that M. paratuberculosis can survive for prolonged time periods (12-16 months) under a variety of experimental condition, the authors are unaware of any previously published information determining the prevalence of positive cultures from known infected herds. Therefore, we believe this study to represent the first report describing the extent of environmental contamination on dairy farms with cattle infected with M. paratuberculosis. The environmental contamination was very closely correlated to the herd prevalence of Johne's disease and the number of colony forming units of M. paratuberculosis present in the manure of infected cattle.

Ergebnisse

In 5 von 11 Milchbetrieben, die bakteriologisch untersucht wurden, konnte Mycobacterium paratuberculosis nachgewiesen werden. Im durchschnitt wurden je betrieb 50 -60 proben von boden, wanden und aus der Erde entnommen. Bei 4 der 5 Betriebe waren 1-2 Proben bakeriologisch positiv. Ein Betreib wies mit 13 von 59 bakeriologisch positiven Proben eine intensive Umwelt-Kontaimation auf. Von diesen positiven Proben wiesen viele hohe Anzahl koloniebilden-der Erreger auf. In 20 (3%) von 672 insgesmat entnommenen Umweltproben wurde M. paratuberculosis nachgewiesen. Mit Ausnahme des stark kontaminierten Betriebes wurden die bakteriologisch positiven Proben auf den Weiden und in den Auslaufen, jedoch nicht innerhalb der Stallen gefunden.

El resumen:

Cinco de las once granjas de las caules se tomaron muestras tienen el cultivo bacteriano positivo de M. paratuberculosis. Cuatro de las cinco granjas tuvieron dos o menos cultivos positivos. Sólo una granja tuvo contaminación ambiental extensa, con treee de cincuenta y nueve muestras positivas, muchas con una alta suma de las colonias bacterianas. Del total de las seiscientos setenta y dos muestras ambientales, veinte, o tres por ciento, probaron positivas de la bacteria M. paratuberculosis, excepto la granja con la contaminación extensa. Todas los cultivos positivos vinieron de los pastos y los pistas de ejercicio, y no dentro de los establos.

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