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Short communication: Progression of Johne's disease curtailed by a probiotic

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ABSTRACT

The naturally occurring inflammatory bowel disease Johne's, caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP), has many clinical manifestations in common with the human inflammatory bowel disease Crohn's disease. In addition, both lack preventive and curative therapies. Because a high percentage of Crohn's patients harbor MAP, it is not surprising that MAP is at the center of controversy as to its contribution. Special concern is being raised as to what role, if any, food animals play in transmission of MAP to humans. Because management practices, presently considered the best way to control the spread of MAP, have not and most likely will not eliminate MAP from food animals, other preventive or curative measures are needed. The results presented herein show that a unique bacterium, *Dietzia* ssp. C79793–74, used as a probiotic, was therapeutic for adult paratuberculosis animals, and resulted in a cure rate of 37.5%.
Key words: Johne's disease, probiotic, *Dietzia*, inflammatory bowel disease

The naturally occurring inflammatory bowel disease (**IBD**) found predominantly in ruminants called Johne's disease is caused by *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**). For paratuberculosis to be manifested in cattle, both infection with MAP and inflammation of the intestine are required. Infection that results in disease can occur via any of the following, primarily at the neonatal or early postnatal stage: 1) in utero (Seitz et al., 1989; Sweeney et al., 1992a; Whittington and Windsor, 2009), 2) colostrum or milk (Taylor et al., 1981; Sweeney et al., 1992b; Streeter et al., 1995), and 3) ingestion of fecal contaminated material (Chiodini et al., 1984; Sweeney, 1996). Interestingly, Johne's disease has many manifestations in common with a human subtype IBD, Crohn's disease, including the most clinically notable, debilitating diarrhea (Chiodini, 1989; Clarke, 1997; Scanu et al., 2007). Because a high percentage of Crohn's patients, relative to the general population, are infected systemically with MAP, it not surprising that MAP is at the center of controversy with regard to its role in this disease (Chiodini, 1989; Clarke, 1997; Hermon-Taylor et al., 2000; Chamberlin et al., 2001; Harris and Lammerding, 2001; Hermon-Taylor and Bull, 2002; Chamberlin and Naser, 2006; Feller et al., 2007; Scanu et al., 2007; Abubakar et al., 2008; Behr and Kapur, 2008).

As Johne's disease has become an increasingly worldwide problem, due in part to the absence of a preventive vaccine or drug or curative treatment, there is concern as to what role, if any, animals play in the transmission of MAP to humans (McDowell and McElvaine, 1997; Grant et al., 2002; Ayele et al., 2005; Ellingson et al., 2005; Pickup et al., 2006; Abubakar et al., 2007; Antognoli et al., 2008). Presently, sound management practices and stringent culling are considered the best means to reduce the spread of MAP from animal to animal, as well as from farm to farm (Wells and Wagner, 2000; Kennedy and Benedictus, 2001; McKenna et al., 2006; Benedictus et al., 2008; Lu et al., 2008; Tavornpanich et al., 2008). However, because such practices have yet to (and most likely will not) completely eliminate MAP from food animals, other preventive or curative measures are needed. The research presented herein was undertaken to assess whether the bacterium *Dietzia* ssp. C79793–74, reported to inhibit growth of MAP under specific in vitro conditions (Richards, 1988), would have any therapeutic benefit as a probiotic for adult paratuberculosis dairy cows.

Animals for the present experiments included adult dairy cows that (a) tested negative for all parameters throughout their lifetime or (b) were at an early stage of Johne's disease as defined by clinical evaluation, serum agar gel immunodiffusion (**AGID**) negativity, low or negative fecal shedding, and low positive ELISA values of 1.5 to 2.5. Our final classification of an animal as positive was based on whether she tested both ELISA-positive and positive, but not necessarily concurrently, for one of the following parameters—fecal shedding, AGID, end-stage disease—or tested ELISA

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positive multiple times. An emaciated animal was defined as having end-stage disease based on the presence of "pipestream" diarrhea and depressed appetite. As an additional means to document Johne's disease of specific animals, complete pathological postmortem analysis and culture determination of MAP in tissues was done at the University of Minnesota Veterinary School Diagnostic Laboratory (St. Paul). The autopsies were designed to confirm Johne's disease status only; they were not intended to define specific aspects or extent of disease or to be compared with other antemortem parameters. Recumbent, emaciated, or cachectic end-stage animals were humanely euthanized by a veterinarian when they no longer could get up and stand on their own by intravenous injection of a sodium pentobarbital solution (Fatal Plus, 6 g/mL, Vortech Pharm, Dearborn, MI). Animals that showed life-threatening ramifications from non-paratuberculosis conditions were also euthanized.

Because it was not our purpose to confirm the prognosis that Johne's-positive animals succumb to their disease, many more animals were assigned to the treated group than to the nontreated group. As positive ELISA values were detected, 1 of 4 animals was randomly assigned to the nontreated group and the other 3 to the treated group. Once a cow was enrolled in the study, the different assays were repeated at various intervals over her remaining life. All animals were housed in a tie-stall facility as a single dairy herd under field conditions designed to mimic those on a typical dairy farm. By design, the positive animals were all at an early stage of disease before initiation of treatment. The treated and nontreated positive groups were closely matched for initial ELISA values and number of lactations. The treated group comprised almost equal numbers of Holsteins and Jerseys.

Fecal material collected directly from the rectum using individual disposable gloves and blood obtained aseptically from the tail vein were transferred to sterile containers and sent overnight to Allied Monitor Inc. (Fayette, MO) for serum ELISA, serum AGID, and fecal culture analysis. The majority of fecal and serum samples were obtained concurrently. The reliability, sensitivity, and specificity of these assays, as performed by Allied Monitor, for individual animals were previously established (our unpublished data). The most important finding was that a single positive ELISA value >1.4, including 12 of 14 suspect values (1.5 to 2.0), unequivocally identified 73 out of 75 animals as Johne's positive.

Dietzia (originally classified as *Mycobacterium gardonae*) was isolated from fecal material of a paratuberculosis sero- and fecal-positive cow (Richards, 1988). It was reclassified as *Dietzia* using the gold standard for bacterial identification, DNA sequence of 16S rRNA (Woese, 1987) by Midi Labs Inc. (Newark, DE). *Dietzia* were grown in 75-L fermenters at the University of Minnesota Biotechnology Institute for 3 to 4 d at 29°C in fructose-supplemented tryptic soy broth and then centrifuged, washed, and concentrated 20-fold before storage in 45-mL aliquots at –20°C. New lots were prepared as needed, approximately every 3 mo. Based on preliminary dosage experiments, Jerseys, Jersey × Holstein crosses, and Holsteins were treated by top dressing *Dietzia* on the morning feed at a daily dose of 2×10^{11}, 3×10^{11}, and 4×10^{11} cfu, respectively. If animals refused to eat, *Dietzia* were directly deposited into their mouths with a needleless syringe.

The basic parameters determined for the 3 different groups of animals—paratuberculosis-free, subclinical diseased animals that were not treated with *Dietzia*, and subclinical diseased positives that were treated with *Dietzia*—are shown in Table 1. Fecal shedding, ELISA, and AGID were routinely measured longitudinally over the lifetime of each animal. Also shown in the table is the number of months that each animal survived after the first positive ELISA value was detected (or after the first ELISA test for negative animals). Because several animals were terminated for health reasons unrelated to paratuberculosis, Table 1 includes a "+" sign after the number of months an animal survived to indicate that the actual survival time would have been longer.

As described by others, when ELISA values of nontreated, early-stage animals change over time, they do so by continuously increasing (Waters et al., 2003; Nielsen and Toft, 2006) and when positive change ceased, the ELISA values are reasonably stable (Nielsen et al., 2002). In contrast, fecal MAP shedding showed large variations over time (Sherman et al., 1995). Our results with *Dietzia*-treated animals were similar (our unpublished data). Because of 1) the lack of correlation of ELISA and MAP, 2) the high variability of MAP counts over time, and 3) the fact that only 4 of 21 initially ELISA-positive animals were also shedding MAP in feces, evaluation of any potential benefit of *Dietzia* treatment was based on longitudinal analysis of ELISA values. Shedding of MAP, positive AGID, appearance of clinical disease, and autopsy were parameters used to definitively identify paratuberculosis animals.

The average initial, maximum, and final ELISA values for the 3 experimental groups are depicted graphically in Figure 1, panel A. Results for cow 3056 were not included in the calculations because she is still alive and her maximum and final values are yet to be determined. The average initial ELISA values of the treated and nontreated positive groups were not significantly different ($P = 0.39$). The initial positive ELISA value obtained for each treated animal cannot