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Lack of association between the occurrence of Crohn's disease and occupational exposure to dairy and beef cattle herds infected with *Mycobacterium avium* subspecies *paratuberculosis*

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ABSTRACT

A cross-sectional survey was conducted to identify associations between Crohn's disease (CD) and *Mycobacterium avium* ssp. *paratuberculosis* (*Map*) exposure. A questionnaire was used to collect information on exposure to cattle infected with *Map*, and personal and family history of CD in dairy and beef cattle producers with and without *Map*-infected herds, and in veterinarians who did or did not have contact with *Map*-infected herds. Cases of CD were selected from respondents and matched 1:4 with controls on occupation, age, and sex. Multivariable conditional logistic regression was used to assess associations between *Map* exposure and CD. There were 3 cases of CD in 702 producers and 4 cases in 774 veterinarians, yielding a prevalence of 0.47%. No association was found between exposure to JD and CD in any phase of the analysis. However, the number of cases of CD is not large and limits the power to detect important differences.
Key words: Johne's disease, Crohn's disease

INTRODUCTION

Crohn's disease (**CD**), a chronic inflammatory disease of the human intestine, shares some characteristics with Johne's disease (**JD**) in cattle, a chronic inflammatory disease of the bovine intestine caused by infection with *Mycobacterium avium* ssp. *paratuberculosis* (***Map***). Given the similarity of the 2 diseases and evidence of *Map* in CD patients (McFadden et al., 1987; Chiodini, 1989), considerable work has been conducted to draw causal associations between *Map* infection and CD in human patients, but different studies have yielded different results. Findings from one review (Behr and Schurr, 2006) indicated that the methods of most studies into causal associations between *Map* and CD lack sufficient standardization to generate reproducible prevalence estimates and strengths of association, which further fuels the ongoing controversy.

Many studies have found evidence for *Map* in CD patients (Lisby et al., 1994; Bull et al., 2003; Naser et al., 2004), but other studies have found no such evidence of *Map* (Van Kruiningen, 1999; Freeman and Noble, 2005; Feller et al., 2007), and some studies have found *Map* in patients that do not have CD (Bull et al., 2003; Naser et al., 2004). Findings that a combination of genetic mutations (CARD15, formerly NOD2) and *Map* infection are associated with the development of CD suggest that *Map* alone may not be sufficient to cause infection, but increased susceptibility due to mutation is needed to cause infection (Behr and Schurr, 2006). Because *Map* has been detected in pasteurized milk in the United States and the United Kingdom (Ellingson et al., 2005), any causal associations between *Map* and CD may represent a considerable public health concern, which will have a significant negative economic impact on the nation's cattle industry.

Ecological studies have been undertaken to determine whether associations exist between *Map* and CD, given the conflicting evidence of studies examining the presence or absence of *Map* in human CD patients. One study found spatial associations between areas of higher CD downwind of the Taff River in Wales, where bovine strains of *Map* have been cultured (Pickup et al., 2005), but other studies of exposure to *Map* through contact with cattle affected by JD found no associations between *Map* or JD and CD in the United Kingdom (Jones et al., 2006; Abubakar et al., 2007). To further explore the hypothesis that exposure to *Map*, through exposure to *Map*-infected cattle or dairy products, increases risk for CD in the United States, a cross-sectional study was conducted to 1) obtain information on the occurrence of Crohn's disease in dairy and beef cattle producers, their families, and veterinarians in Iowa, Michigan, and Wisconsin, and 2) determine if there were any associations between exposure to cattle infected with *Map* and the occurrence of CD in these populations.

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MATERIALS AND METHODS

Study Population

Study participants were selected based on their possible exposure to *Map* through contact with cattle, and consisted of 774 veterinarians and 702 dairy and beef producers from Michigan, Wisconsin, and Iowa. In Michigan, lists of potential participants were randomly selected from a list of veterinary practitioners provided by the Michigan Veterinary Medical Association, and from producer list frames created by the Michigan Agricultural Statistical Service. Dairy and beef producers and veterinarians in Wisconsin and Iowa were recruited at producer meetings and professional conferences and meetings.

Case and Exposure Definitions

Cases of CD in producers or veterinarians were defined as physician-confirmed diagnoses of the disease, as reported by the study respondent. Cattle producers and veterinarians were classified as being either exposed or unexposed to *Map*, based on their reported contact with cattle diagnosed as having JD, confirmed by fecal culture or typical histopathologic lesions, and supported by positive serology, PCR, or both, for *Map*.

Data Collection

Data were collected via pretested, self-administered questionnaires that were mailed (Michigan) or distributed at conferences and meetings (Wisconsin and Iowa) in 2000. Second and third mailings to nonrespondents in Michigan were conducted to improve response rates. The questionnaires collected information about respondent (age and sex), diagnosis of Crohn's disease or symptoms indicative of CD (self or family members), and any exposure to raw milk, dairy cattle, and cattle with JD, either through employment or from living on a farm.

Statistical Analysis

The prevalence of CD was computed as the number of cases of CD divided by the total number of respondents overall and within exposure groups (producer exposed/not exposed to *Map*, veterinarian exposed/not exposed to *Map*).

Descriptive statistics were generated for each exposure group. To determine whether associations existed between exposure to *Map* and CD, multivariable analyses were conducted using a matched case-control approach. Cases of CD were matched to randomly selected, noncase respondents, and matched 4:1 to each case by occupation (producer, veterinarian), state (Iowa, Michigan, Wisconsin), age category (10-yr age groups), and sex (male, female).

To quantitatively describe the association between exposure and risk factors on CD, conditional logistic regression models for m:n matching (PROC PHREG, SAS 9.3.1) were developed for risk factors associated with exposure to *Map* (raw milk, dairy cattle, cattle with JD). Models were developed for each risk factor individually, and those found to be significantly associated with CD at $P \leq 0.05$ were considered for inclusion in multivariable analysis. A model including all potential risk factors was created and subjected to a backward model building approach to achieve the most parsimonious model, by repeatedly removing the risk factor with the highest P-value from the model until all risk factors were significant at $P \leq 0.05$. If removal of a risk factor resulted in changes in odds ratio of the major risk factor of interest (exposure to *Map*), the variable was considered a confounder and was maintained in the model. The final model was determined by the best combination of significance of risk factors retained in the model and model log-likelihood scores.

RESULTS

A total of 702 producer and 774 veterinarian surveys were successfully completed from 1,526 returned surveys (Table 1). The majority of producers surveyed were dairy producers (95%), and 66% of producers (67% of dairy and 39% of beef) and 56% of veterinarians reported exposure to cattle infected with *Map*. The highest levels of exposure to cattle with JD were seen in respondents from Wisconsin (71%), followed by Iowa (59%), and Michigan (46%). Dairy farmers had the highest exposure to cattle infected with JD (67%), followed by veterinarians (56%) and beef cattle farmers (39%).

There were 7 cases of CD out of 1,476 study respondents, yielding a prevalence of 474.2 cases per 100,000 (Table 1). The highest prevalence of CD was seen in veterinarians (516.8 per 100,000), followed by dairy producers (448.4 per 100,000). The highest rates by state were seen in Iowa (854.7 per 100,000), followed by Wisconsin (407.7 per 100,000) and Michigan (395.3 per 100,000). The prevalence of CD in respondents exposed to *Map* was 446.9 per 100,000 (4 of 895 respondents) versus 516.4 per 100,000 in respondents with no reported exposure to *Map* (3 of 581). Study respondents also reported 41 cases of inflammatory bowel disease (1 beef, 9 dairy, and 31 veterinary cases).

Most dairy farmers (89.5%) were raised on dairy farms, whereas 45.5% of beef farmers were raised on

Table 1. Numbers of study participants with Crohn's disease, by state, sex, occupation, and potential exposure to *Mycobacterium avium* ssp. *paratuberculosis* (*Map*)[1]

			Iowa		Michigan		Wisconsin	
Occupation	Sex	*Map* exposure	n	CD	n	CD	n	CD
Dairy producer	Female	Positive	4	0	11	0	30	1
		Negative	1	0	9	0	8	0
	Male	Positive	41	0	132	0	233	1
		Negative	11	1	94	0	95	0
Beef producer	Female	Positive	1	0	0	0	0	0
		Negative	1	0	0	0	2	0
	Male	Positive	7	0	0	0	5	0
		Negative	6	0	0	0	11	0
Veterinarian	Female	Positive	6	0	12	0	66	0
		Negative	21	0	41	1	52	1
	Male	Positive	79	1	78	1	190	0
		Negative	56	0	129	0	44	0

[1]n = number of study participants; CD = number of cases of Crohn's disease.

beef or other types of farms, and 41% of veterinarians were raised on farms (Table 2). Potential exposure of respondents to *Map* through consumption of unpasteurized milk was common, with 94% of producers and 62% of veterinarians reporting this practice. Contact with cattle demonstrating clinical signs of JD (chronic, nonresponsive, intermittent diarrhea, progressive weight loss, normal appearance but unthrifty) was common: 59% of veterinarians, 56% of dairy producers, and 42% of beef producers reported contact with cattle with clinical signs of JD, and 59% of veterinarians, 71% of dairy producers, and 42% of beef producers reported contact with cattle with confirmed cases of JD.

The presence of family members with CD was assessed as an indicator of other possible exposures associated with CD (Table 2). Although 1 beef producer, 28 dairy producers, and 41 veterinarians reported having relatives with CD, only 1 veterinarian with CD reported any family members with CD. Stress was reported in 45% of veterinarians, 43% of dairy producers, and

Table 2. Numbers of study participants with Crohn's disease with potential risk factors for Crohn's disease or exposure to *Mycobacterium avium* ssp. *paratuberculosis*, by type of respondent[1]

	Beef (n = 33)		Dairy (n = 669)		Vet (n = 774)	
Risk factor	n	CD	n	CD	n	CD
Drank unpasteurized milk as adult	30	0	630	3	477	2
Drank unpasteurized milk as child	28	0	583	3	395	2
Consumed only once	1	0	4	0	36	0
Consumed rarely	7	0	52	0	201	0
Consumed often	21	0	574	3	246	2
Raised on dairy farm	12	0	599	3	205	3
Raised on other cattle farm	15	0	3	0	109	0
Contact with cattle with clinical signs of Johne's disease (JD)	14	0	437	2	458	2
Contact with cattle with confirmed JD	14	0	474	2	458	1
Respondent diagnosed with CD	1	0	9	3	31	4
Family member with CD	1	0	30	9	41	1
Spouse/child	0	0	9	0	3	0
Parent/sibling	1	0	3	0	11	4
Other	0	0	16	0	26	0
History of smoking	9	0	157	1	224	0
History of other tobacco use	4	0	100	0	141	0
Reported stress in life	12	0	285	2	349	4
Hunter	16	0	352	2	388	2
Hunted deer, elk	14	0	327	2	271	1
Hunted game birds	14	0	198	2	322	2
Hunted small game	14	0	246	1	297	2
Hunted predators	11	0	170	1	104	1

[1]n = number of study participants; CD = number of cases of Crohn's disease.

Table 3. Multivariable logistic regression for matched analysis for occurrence of Crohn's disease for 7 cases and 28 controls matched by respondent occupation, age, sex, and state

Risk factor	*P*-value	Odds ratio	95% CI
Exposure to *Map*-infected cattle[1]	0.3910	2.5	0.3–20.7
Relative with Crohn's disease	0.2356	2.8	0.5–14.8
Model log-likelihood = 17.36			
Likelihood ratio χ^2 = 1.96, 2 df, $P = 0.3756$			

[1]*Map* = *Mycobacterium avium* ssp. *paratuberculosis.*

36% of beef producers: all 4 CD cases in veterinarians and 2 of 3 CD cases in dairy producers reported stress. Cigarette smoking was not common (<30% in all 3 respondent groups), and only 1 case of CD (dairy producer) reported a history of smoking. Hunting was reported by 53% of beef producers, 48% of dairy producers, and 50% of veterinarians, with 2 of 3 CD cases in dairy producers and 2 of 4 CD cases in veterinarians reporting hunting in the past. The majority of dairy producers and veterinarians were 40 to 49 yr of age (Figure 1), whereas the majority of beef producers were 60 or older. No statistically significant associations were found between CD and any of the risk factors evaluated in this study at the univariable level.

A multivariable conditional logistic regression model was developed using a backward model building approach (Table 3), and the best regression model developed suggested that occupational exposure to cattle with JD and having a relative with CD tended to increase the odds of the respondent having the disease, but these associations were not statistically significant.

DISCUSSION

The prevalence of CD in this study (474 per 100,000) was higher than that reported in a survey of dairy producers with JD in the United Kingdom (128.7 per 100,000; Jones et al., 2006) and in kibbutz dwellers in Israel (65.1 per 100,000; Niv et al., 1999), but agreed with these studies in finding no association between the occurrence of CD and exposure to *Map* through cattle with JD. In one study (Jones et al., 2006), the prevalence of CD in dairy producers was not different from the prevalence in the general population, whereas the study conducted in Israel found a lower prevalence of CD in residents of the agricultural setting of the kibbutz compared with the rest of the country (Niv et al., 1999).

There were no statistically significant associations observed between exposure to cattle herds with JD and occurrence of CD in producers or veterinarians in this study, which may be because of the low number of cases of CD reported. The use of the matched case-control study approach was intended to provide additional power to the study, but it yielded no significant associations between risk factors and the occurrence of CD.

An association between CD and *Map* was not demonstrated in this study, and current research on the topic has been divided. Much of the argument for a causal association between *Map* and CD has been based on finding viable *Map* in intestinal lesions (McFadden et al., 1987; Bull et al., 2003; Autschbach et al., 2005; Sechi et al., 2005) and peripheral blood (Naser et al., 2004) of CD patients. One meta-analysis of 28 case-control studies of *Map* in CD patients found the odds of finding *Map* in tissue samples from CD patients to be 7.0 using PCR and 1.7 using ELISA to detect antibodies in serum (Feller et al., 2007). However, some studies have found *Map* in patients who did not have CD (Sechi et al., 2005; Feller et al., 2007), and other studies have found no *Map* in tissues or peripheral blood of patients with CD (Freeman and Noble, 2005; Lozano-Leon et al., 2006). Differences in these studies may be attributable to different biases within each study and to differences in *Map* culture methods (Naser and Collins, 2005). Although finding viable *Map* in CD patients suggests an association between the two, the temporal association

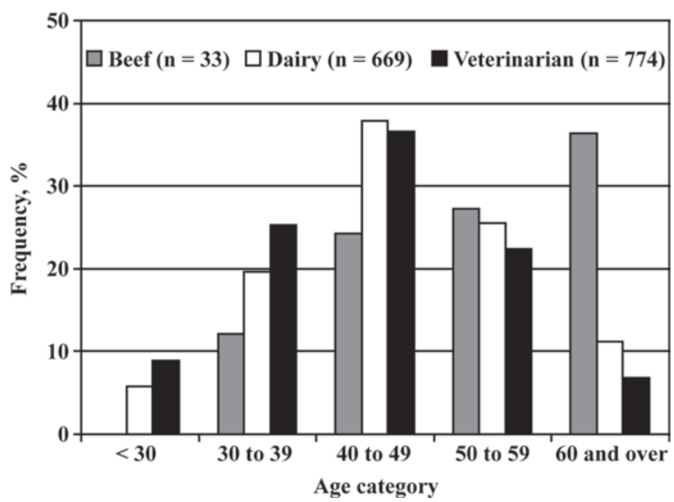

Figure 1. Age of respondents in years, by type of respondent.

between the presence of *Map* and later development of CD has been difficult to demonstrate conclusively (Sechi et al., 2005). Some researchers believe that the temporality of the association has been demonstrated, based on findings that show that *Map* harvested from CD patients can result in disease in goats (Uzoigwe et al., 2007), but comparable findings in humans have not been found. Recently, it has been suggested in several studies that *Map* itself may not be sufficient to cause CD, but that certain host mutations, including NOD2/CARD15 (nucleotide-binding oligomerization domain containing 2 or caspase recruitment domain family, member 15), serve a role in bacterial sensing and activation of innate immune responses, providing a possible link between CD and a bacterial trigger such as *Map* (Behr and Schurr, 2006). Others have concluded that the causal association is not clear but that *Map*-associated CD needs to be acknowledged even though the etiology of the process is not understood (Selby, 2004; Chamberlin et al., 2007).

Given conflicting evidence from studies based on bacterial detection in patients with CD, studies examining the association between exposure to *Map* and CD can provide a different insight into the association between *Map* and CD. One study in Israel found no difference in rates of CD between kibbutz residents and the rest of the country (Niv et al., 1999), which suggests that contact with livestock was not associated with increased CD risk. In a cross-sectional study of dairy farmers in the United Kingdom (Jones et al., 2006), no association was found between exposure to clinical cases of JD and the development of CD, but associations were found between ulcerative colitis and age, frequency of contact with cattle, and smoking. A UK case-control study of CD with exposure to drinking water and dairy products (potential sources of *Map*) with CD showed no significant association with measures of potential contamination of water sources with *Map*, water intake, or water treatment and CD (Abubakar et al., 2007). However, the same study found that consumption of pasteurized milk was associated with reduced risk of CD, indicating that dairy products potentially contaminated with *Map* were not important factors in the etiology of CD. Overall, these studies indicate no clear association between exposure to *Map* or JD and the development of CD.

CONCLUSIONS

There were no statistically significant associations observed between exposure to cattle with Johne's disease and Crohn's disease in producers and veterinarians. Despite the use of a cross-sectional survey to overcome the limitations of the low number of cases reported herein, there were insufficient cases for adequate power using this study design. Although the current study does not offer conclusive proof of a lack of association between *Map* and Crohn's disease, it adds to the body of evidence that, if such an association exists, there are probably other more important risk factors that are likely to be directly associated with the development of Crohn's disease.

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