

Crohn's disease in people exposed to clinical cases of bovine paratuberculosis

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SUMMARY

Mycobacterium avium subspecies *paratuberculosis* (*Map*), the cause of ruminant paratuberculosis, has been proposed as the causative agent of Crohn's disease. The objective of this study was to determine whether exposure to clinical cases of bovine paratuberculosis was a risk factor for Crohn's disease. A questionnaire was sent to dairy farmers living on premises where the occurrence or absence of clinical cases of bovine paratuberculosis had previously been determined. The prevalence of Crohn's disease was found to be similar to that reported in other studies in the United Kingdom and showed no association with bovine paratuberculosis. There was, however, a univariate association with geographical region. Ulcerative colitis showed univariate associations with age, frequency of contact with cattle and with smoking. The results do not support the hypothesis that *Map* plays a causative role in the aetiology of Crohn's disease.

INTRODUCTION

Over recent decades, the incidence of Crohn's disease has increased in many developed countries and is currently ~50–70 cases/1 000 000 per year [1]. Despite considerable research efforts, the aetiology of the disease remains unclear. A genetic aetiology has been implicated in studies showing increased concordance in monozygotic twins compared with dizygotic twins,

familial aggregation and different prevalences among ethnic groups living in the same geographical region. The recent discovery that mutations in CARD15 (formerly NOD2) are associated with Crohn's disease, possibly as a result of impaired responses to bacteria, may partially explain the genetic susceptibility to the disease [2, 3]. However, environmental factors also play an important role. Smoking is strongly associated with inflammatory bowel disease (IBD), being a risk factor for Crohn's disease and protective for ulcerative colitis [4], whilst the use of oral contraceptives has been associated with increased risk of Crohn's disease in some studies [5] but not others [6]. An infectious aetiology has also been considered and many bacteria,

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viruses and yeasts have been investigated as potential aetiological agents. Of these agents, the strongest evidence points to *Mycobacterium avium* subspecies *paratuberculosis* (*Map*), the causative agent of ruminant paratuberculosis. Whilst several recent reviews have dismissed or ignored its possible role [7–9], others have argued convincingly that the organism plays a central role in the aetiology of Crohn's disease [10].

Crohn's disease and paratuberculosis are pathologically similar but, unlike paratuberculosis, acid fast-staining organisms are not visible in the tissues taken from Crohn's disease patients. However, in 1984, Chiodini and colleagues isolated three strains of *Map* from Crohn's disease patients [11–13] and, over subsequent years, a total of 10 *Map* isolates were cultured from 26 patients with Crohn's disease but not from 13 patients with ulcerative colitis nor from 13 patients with other bowel disorders [14–16]. These results appeared to implicate *Map* as the causative agent of Crohn's disease but subsequent studies have failed to corroborate the findings since *Map* has been isolated from both Crohn's disease patients and from controls [17, 18]. Other experimental approaches have also failed to produce a definitive solution. Several studies have identified significantly increased antibody responses to *Map* antigens in Crohn's disease patients [19, 20] but a similar number have failed to identify significant differences in either humoral [21, 22] or cellular [23, 24] responses. Similarly, the identification of the *Map*-specific insertion sequence, IS900, by polymerase chain reaction (PCR) has identified associations between *Map* and Crohn's disease in several studies [25, 26] but, once again, a similar number have failed to confirm the findings [27].

An apparent association between *Map* and Crohn's disease does not imply a causal relationship. *Map* has been isolated from both Crohn's disease and control tissues and may be associated with macroscopically inflamed tissues [28]. The issue of causality can only be addressed by examining disease onset in relation to exposure to the putative pathogen. In cattle, *Map* infection occurs within the first few weeks of life but clinical signs do not develop until several years of age. If *Map* were to behave in a similar manner in humans [29], it would be extremely difficult to associate exposure events occurring during childhood with the subsequent development of disease, perhaps many years later. In the absence of suitable animal models, epidemiological studies are the most effective means of determining whether *Map* plays a causative role in the aetiology of Crohn's disease.

Map can be shed in large numbers during both the subclinical and clinical phases of bovine paratuberculosis and can survive for long periods in the environment. As a result, farms that have seen clinical cases of the disease are likely to be contaminated with large numbers of the organism for many years. We hypothesized that if *Map* plays a causative role in the aetiology of Crohn's disease, people living on contaminated farms would be at higher risk of developing the disease than people living on uncontaminated premises. Therefore, the aim of the current study was to use a postal questionnaire to determine the prevalence of Crohn's disease in people living on dairy farms where the occurrence or absence of clinical cases of bovine paratuberculosis had previously been determined [30, 31]. Information about the prevalence of ulcerative colitis was also collected as a control.

METHODS

Study design

An anonymous, pre-tested questionnaire, designed to determine the prevalence of IBD in dairy farmers and their immediate families, was sent to farmers in November 1998. The study population was 2897 dairy farmers who had previously participated in a questionnaire survey of bovine paratuberculosis [30, 31]. Of these farms, 954 were in the north, 913 in the central region and 1030 were in the south. The questionnaire relied on self-reporting of Crohn's disease and ulcerative colitis although the instructions accompanying the questionnaire emphasized that only diagnoses that had been confirmed by a doctor should be included. Data were also collected about age, sex, time spent on the farm, smoking, diet, the consumption of tea, coffee, milk and alcohol, and contact with cattle. The response rate was maximized by sending non-responders a postcard reminder followed by a duplicate questionnaire and finally a second postcard reminder. Questionnaires received after 23 April 1999 were not included in the study.

Statistical analysis

Data were entered into a computer spreadsheet application (Excel, Microsoft Corporation, Redmond, WA, USA) and examined for typing errors and inconsistencies. Some questions were not relevant to young children and, in many cases, parents left the questions blank. As a result, only individuals who

Table 1. Univariate associations between the occurrence of inflammatory bowel diseases and personal variables (odds ratios are given relative to the lowest category)

Variable	Category	Crohn's disease			Ulcerative colitis		
		Yes	No	OR (95% CI)	Yes	No	OR (95% CI)
Age*	<40 years	1	2692	1.00	3	2688	1.00
	40 to <60 years	3	1994	4.05 (0.32–212.7)	9	1984	4.06 (1.01–23.36)
	≥60 years	2	694	7.76 (0.40–457.8)	6	692	7.77 (1.65–48.08)
		<i>P</i> = 0.146			<i>P</i> = 0.004		
Sex*	Male	2	2825	1.00	13	2812	1.00
	Female	5	2575	2.74 (0.45–28.82)	5	2571	0.42 (0.12–1.26)
		<i>P</i> = 0.269			<i>P</i> = 0.102		
Still living on current farm?*	No	1	1045	1.00	2	1044	1.00
	Yes	6	4118	1.52 (0.18–70.09)	16	4101	2.04 (0.48–18.28)
		<i>P</i> = 1.000			<i>P</i> = 0.555		
Time spent on current farm*	<20 years	1	1667	1.00	3	1661	1.00
	20 to <40 years	3	2152	2.32 (0.19–122.1)	8	2144	2.07 (0.49–12.11)
	≥40 years	2	876	3.81 (0.20–224.6)	6	869	3.82 (0.81–23.66)
		<i>P</i> = 0.418			<i>P</i> = 0.131		
Frequency of contact with cattle*	Occasional	3	1744	1.00	1	1741	1.00
	Frequent	4	3617	0.64 (0.11–4.39)	17	3604	8.21 (1.28–343.3)
		<i>P</i> = 0.689			<i>P</i> = 0.011		
Smoking*	Never/occasional	4	4305	1.00	12	4295	1.00
	Previous	2	495	4.35 (0.39–30.41)	6	490	4.38 (1.34–12.66)
	Current	1	404	2.66 (0.05–26.99)	0	403	0.00 (0.00–3.42)†
		<i>P</i> = 0.070			<i>P</i> = 0.009		
Use of oral contraceptives‡	Never	2	611	1.00	2	611	1.00
	Previous	0	552	0.00 (0.00–2.13)†	2	552	1.11 (0.08–15.32)
	Current	2	301	2.03 (0.15–28.10)	1	300	1.02 (0.02–19.63)
		<i>P</i> = 0.204			<i>P</i> = 1.000		

OR, Odds ratio; CI, confidence interval.

* The survey provided information on 5604 individuals who could be confirmed to be ≥15 years; any reduction in the total number of subjects included in each analysis was due to missing values in one or both variables.

† 95% CI calculated using Cornfield approximation since exact CIs could not be calculated with zero count cells.

‡ The survey provided information on 2662 respondents who could be confirmed to be females who were ≥15 years. Only 1468 subjects were used to calculate ORs because the use of oral contraceptives was not reported in the remainder.

could be confirmed to be 15 years or older were included in the final analysis. Continuous variables were categorized because the log_e odds did not show linear relationships with the outcome variables. Data were analysed using Stata statistical software, release 8.0 (Stata Corporation, College Station, TX, USA).

The crude prevalence of each gastrointestinal disease was calculated as the number of cases per 100 000 population together with binomial exact 95% confidence intervals (CIs). The univariate associations between disease and exposure were performed using algorithms that extend Fisher's exact test from 2 × 2 tables to more general *r* × *c* tables. Odds ratios (ORs) and exact 95% CIs were calculated where possible. The results of univariate analyses are shown in Tables 1–3.

RESULTS

Respondents

A total of 1857 (64.1%) farmers responded to the survey and 1686 (58.2%) returned usable questionnaires. The response rates within the north, central and southern regions were 57.4, 57.2 and 59.3% respectively. The survey provided information on 6641 individuals, of whom 5604 (84.4%) could be confirmed to be 15 years or older. In total, 47.5% (95% CI 46.2–48.8) of these respondents were female and 51.8% (95% CI 50.5–53.1) were male; the remainder (0.7%) did not report a gender. The mean age of the respondents was 41.1 years (s.d. = 15.3 years). There was a significant association between sex and age (*P* < 0.001) with the proportion of males in

Table 2. Univariate associations between the occurrence of inflammatory bowel diseases and dietary variables (odds ratios are given relative to the lowest category)

Variable	Category	Crohn's disease			Ulcerative colitis		
		Yes	No	OR (95% CI)	Yes	No	OR (95% CI)
Diet*	Meat	7	5254	1.00	18	5240	1.00
	Vegetarian/vegan	0	100	0.00 (0.00–29.09)†	0	97	0.00 (0.00–11.59)†
		<i>P</i> = 1.000			<i>P</i> = 1.000		
Tea and coffee consumption*	No	0	240	1.00	0	241	1.00
	Yes	7	5118	–(0.09, –)†	18	5100	–(0.22, –)†
		<i>P</i> = 1.000			<i>P</i> = 1.000		
Weekly milk consumption*	0–3 pints	1	2454	1.00	6	2441	1.00
	> 3 pints	5	2654	4.62 (0.52–218.7)	12	2649	1.84 (0.64–5.99)
		<i>P</i> = 0.221			<i>P</i> = 0.245		
Frequency of drinking unpasteurized milk*	Never/occasional	3	1770	1.00	7	1764	1.00
	Frequent	3	3217	0.55 (0.07–4.11)	11	3206	0.86 (0.31–2.64)
		<i>P</i> = 0.673			<i>P</i> = 0.807		
Weekly alcohol consumption*	0–8 units	7	3971	1.00	17	3957	1.00
	> 8 units	0	981	0.00 (0.00–2.22)†	1	977	0.24 (0.01–1.52)
		<i>P</i> = 0.358			<i>P</i> = 0.230		

OR, Odds ratio; CI, confidence interval.

* The survey provided information on 5604 individuals who could be confirmed to be 15 years or over; any reduction in the total number of subjects included in each analysis was due to missing values in one or both variables.

† 95% CI calculated using Cornfield approximation since exact CIs could not be calculated with zero count cells.

the sample steadily increasing with age. Farmers who had reported a case of paratuberculosis on the farm 'ever' were more likely to return a usable questionnaire (OR 1.31, 95% CI 1.07–1.61) but this association was not found with farmers who reported paratuberculosis on the farm in the 10-year period between 1985 and 1994.

Crohn's disease

Seven cases of Crohn's disease were identified among the 5439 individuals whose disease status was known (crude prevalence = 128.7/100 000, 95% CI 51.8–265.0). There was no association between the reporting of Crohn's disease in farmers and their families and the occurrence of paratuberculosis on the farm. None of the cases lived on farms that had reported clinical cases of paratuberculosis in the 10-year period between 1985 and 1994 but two cases lived on farms that reported having clinical cases of the disease 'ever'.

Ages were reported for six of the seven cases of Crohn's disease and ranged between 36 and 69 years (mean = 52.8, s.e.m. = 5.0). The odds of disease increased with age but the association was not

statistically significant ($P = 0.146$). There was also no evidence that milk consumption or the consumption of unpasteurized milk were associated with Crohn's disease. There was a significant univariate association between Crohn's disease and geographical region ($P = 0.028$) with the prevalence in the north being 112.9/100 000 (95% CI 13.7–407.1), in the central region being 299.6/100 000 (95% CI 97.3–697.7) and zero cases being reported in the south (0/1998). Four of the cases of Crohn's disease had never smoked or were only occasional smokers whilst two cases were previous smokers (OR 4.35, 95% CI 0.39–30.41) and one case was a current smoker (OR 2.66, 95% CI 0.05–26.99); the association between smoking category and Crohn's disease was not significant ($P = 0.070$).

Ulcerative colitis

Eighteen cases of ulcerative colitis were identified among the 5433 individuals whose disease status was known (crude prevalence 331.3/100 000, 95% CI 196.5–523.1). There were no significant associations between ulcerative colitis and the occurrence of paratuberculosis in cattle. The mean age of people

Table 3. Univariate associations between the occurrence of inflammatory bowel diseases and farm variables (odds ratios are given relative to the lowest category)

Variable	Category	Crohn's disease			Ulcerative colitis		
		Yes	No	OR (95% CI)	Yes	No	OR (95% CI)
Region*	North	2	1770	1.00	3	1768	1.00
	Central	5	1664	2.66 (0.43–27.95)	8	1663	2.84 (0.68–16.61)
	South	0	1998	0.00 (0.00–1.70)†	7	1984	2.08 (0.47–12.48)
		<i>P</i> = 0.028			<i>P</i> = 0.277		
Case of paratuberculosis ever?*	No	5	4173	1.00	12	4160	1.00
	Yes	2	1026	1.63 (0.15–9.95)	4	1024	1.35 (0.32–4.48)
		<i>P</i> = 0.631			<i>P</i> = 0.538		
Case of paratuberculosis from 1985–1994*	No	7	5021	1.00	17	5005	1.00
	Yes	0	291	0.00 (0.00–9.50)†	0	291	0.00 (0.00–3.89)†
		<i>P</i> = 1.000			<i>P</i> = 1.000		
Size of farm (no. of cattle)*	< 50	4	1108	1.00	5	1107	1.00
	50 to < 100	1	2102	0.13 (0.00–1.33)	8	2093	0.85 (0.24–3.30)
	≥ 100	2	2090	0.27 (0.02–1.85)	5	2084	0.53 (0.12–2.31)
		<i>P</i> = 0.095			<i>P</i> = 0.512		
Predominant breed of cattle*	Black & white	7	5150	1.00	17	5134	1.00
	Channel Island	0	159	0.00 (0.00–17.87)†	0	158	0.00 (0.00–7.37)†
	Other	0	78	0.00 (0.00–36.64)†	0	78	0.00 (0.00–14.98)†
		<i>P</i> = 1.000			<i>P</i> = 1.000		

OR, Odds ratio; CI, confidence interval.

* The survey provided information on 5604 individuals who could be confirmed to be ≥ 15 years; any reduction in the total number of subjects included in each analysis was due to missing values in one or both variables.

† 95% CI calculated using Cornfield approximation since exact CIs could not be calculated with zero count cells.

reporting ulcerative colitis was 53.7 years (S.E.M. = 3.2). There was a significant univariate association between the disease and age ($P=0.004$), with older age categories having significantly increased odds of disease compared with the youngest group. Ulcerative colitis was also significantly associated with smoking status ($P=0.009$) and previous smokers had a significantly higher odds of disease than non- or occasional smokers (OR 4.38, 95% CI 1.34–12.66); no cases of ulcerative colitis were identified as current smokers. Frequency of contact with cattle was also associated with ulcerative colitis and people who had frequent contact with cattle had significantly higher odds of disease than people who had only occasional contact (OR 8.21, 95% CI 1.28–343.3).

DISCUSSION

In the current study, the prevalence of Crohn's disease in dairy farmers and members of their families aged 15 years and older was 128.7 (95% CI 51.8–265.0) cases/100 000. The results of other studies in the

United Kingdom have reported prevalences (per 100 000) of 35.0, 47.0, 85.0, 147.0, 75.8 and 144.8 between 1977 and 1995 respectively [32–37]. The 95% CI for the present study includes the prevalences reported in the other studies with the exception of the two earliest papers. The most recent estimates of Crohn's disease prevalence are in close agreement with the current study, suggesting that the prevalence of Crohn's disease in dairy farmers is not different from the general population. There was, however, no association between Crohn's disease and the occurrence of bovine paratuberculosis.

Univariate analyses identified a significant association between Crohn's disease and geographical region with the highest prevalence in the central region and the lowest prevalence in the South. No other associations were identified.

Univariate analyses for ulcerative colitis identified significant associations with age, frequency of contact with cattle and smoking. Again, these associations may have been confounded by other variables but the literature suggests that the association with smoking may have been real. It is well established that smoking

is associated with a decreased risk of ulcerative colitis [4] although Boyko et al. [38] reported that previous smokers actually had increased risk of disease.

The lack of an association between bovine paratuberculosis and Crohn's disease does not preclude *Map* from the aetiology of Crohn's disease. Failure to identify a real association (type II error) may have occurred for several reasons, the most important of which is the low power of the study resulting from the small number of cases. To a certain extent, this was anticipated before the study was undertaken. Nevertheless, the reported study design was used because the research group already had information on the paratuberculosis status of a large number of dairy farms. Case-control designs are often more appropriate for rare diseases but identifying clinical cases of Crohn's disease in people living on dairy farms would have required the involvement of a large number of hospitals over a long period of time and, as such, would not have represented the most efficient use of available resources. Future studies, however, will need to address this issue and alternative study designs will need to be employed.

The absence of an association with smoking, an established risk factor for Crohn's disease, may be seen to indicate that the power of the study was too low to detect even reasonably large differences. However, the effect of smoking on Crohn's disease in rural, farming populations specifically has not been reported in the United Kingdom. In the absence of an established mechanism by which smoking affects the incidence of Crohn's disease, it may be inappropriate to extrapolate results obtained from the general population to a predominantly farming population. Moreover, ulcerative colitis was found to be associated with variables that had previously been identified as being important predictor variables, despite a similarly small number of cases. Such observations provide an important source of external validation and give some degree of confidence that the results obtained for Crohn's disease were valid.

A further possible explanation for the failure to identify an association between Crohn's disease and bovine paratuberculosis relates to age at exposure. In cattle, young animals are susceptible to infection with *Map* whereas adults are relatively resistant. As a result, it is generally accepted that infection with *Map* occurs within a few months of birth and that animals enter a long subclinical phase before developing clinical signs of paratuberculosis at several years of age. If patients with Crohn's disease were indeed

suffering from human paratuberculosis, it would not be surprising to find a similar age-related resistance in humans [29]. As a result, exposure to *Map* during childhood may be more important in terms of the subsequent development of Crohn's disease whereas exposure during adulthood, as determined in the current study, may be less important.

The diagnosis of Crohn's disease is difficult and relies on clinical and histopathological findings. It has been suggested that Crohn's disease may not be a single disease entity but merely a clinical description of several conditions, one of which could feasibly be human paratuberculosis. If this were the case, attempting to identify the causal agent of a subset of cases in the absence of an appropriate case definition would, almost certainly, result in failure.

Finally, it is possible that an association between bovine paratuberculosis and Crohn's disease was not identified, despite a causative role for *Map* in the aetiology of Crohn's disease, because the respective strain of the organism is maintained within the human population and is not associated with paratuberculosis in farm animals. Experimental evidence, however, is currently not available to support this hypothesis. Restriction fragment length polymorphisms (RFLP) have been used to characterize over 1000 isolates of *Map* from around the world, including nine isolates from Crohn's disease patients [39]. The nine strains were classified as four RFLP types, all of which were also represented in isolates from cattle. Such results do not support the hypothesis of a human-specific strain of *Map* and may simply indicate that the strains isolated from Crohn's disease are merely secondary, opportunistic pathogens.

The possibility of an association between *Map* and Crohn's disease remains controversial. Culture and DNA techniques have been used to examine the association between *Map* and Crohn's disease but inconsistent results, even within the same patient, have made it impossible to definitively confirm or deny a causal relationship. Nevertheless, the need for a definitive answer remains a priority, especially with the recent finding of viable *Map* organisms in retail milk [40]. Further studies are required but using experimental designs similar to those used previously may not be the most appropriate means of confirming a causal relationship. In the future, stronger support for a causal link, if it exists, will come from epidemiological studies that aim to identify the time of exposure to the pathogen and show a subsequently increased risk of developing the disease.

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