A hypothesis for explaining the geographical distribution of Crohn’s disease

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Crohn’s disease (CD) is an inflammatory bowel disease primarily affecting people in temperate zones. The etiology remains unknown, although it likely involves a combination of genetic and environmental influences. The hypothesis that Mycobacteria may be involved in the etiopathogenesis of CD is not new. The role of Helicobacter pylori in peptic ulcer disease underscores the importance of searching for infectious agents in idiopathic gastrointestinal disorders (1,2). Recently, several independent investigators (3) have isolated Mycobacterium paratuberculosis from CD patients. The organism is of particular interest because it causes a chronic enteritis in animals called paratuberculosis, a condition with many clinical and pathological similarities to CD. This finding adds support to the implication of M. paratuberculosis in the etiology of CD.

Key Words: Crohn’s disease, Epidemiology, Etiology, Mycobacterium paratuberculosis

Hypothèse sur la distribution géographique de la maladie de Crohn

RÉSUMÉ : L’étiologie de la maladie de Crohn demeure inconnue, bien que certaines données épidémiologiques soient indicatrices d’une influence environnementale. Les récentes techniques d’investigation moléculaires, y compris l’amplification génique, ont réveillé l’intérêt à l’endroit du rôle possible de Mycobacterium paratuberculosis, qui a été isolé chez plus d’un patient. L’organisme pathogène provoque chez l’animal une entérite chronique appelée paratuberculose, qui ressemble à plusieurs points de vue cliniques et pathologiques à la maladie de Crohn. Cette synthèse compare l’épidémiologie de la paratuberculose chez l’animal à celle de la maladie de Crohn chez l’humain. On note un chevauchement considérable des régions où les cas de paratuberculose et de maladie de Crohn sont nombreux. Cette observation appuie le rôle de M. paratuberculosis dans l’étiologie de la maladie de Crohn.
creased epidemiological literature concerning both diseases, few attempts have been made to record the similarity between the geographical distributions of the two diseases. This review compares such existing information. It furthers the hypothesis that M paratuberculosis may cause CD by showing that, in certain regions, large reservoirs of host animals susceptible to or infected by M paratuberculosis exist close to urban regions having the highest CD prevalences. The mechanism by which infection may be transmitted from animals to humans may then be reasonably conjectured.

**EPIDEMIOLOGY OF CD**

Although CD has been reported in many countries, it is most prevalent in northern areas, particularly North America, Scandinavia and Great Britain (5,6). The highest prevalence figures are from Edmonton, Alberta (7); Olmsted county, Minnesota (8); central and southwestern Sweden (9-11); Cardiff, Wales (12,13) and Aberdeen, Scotland (14). It is more prevalent in urban than rural inhabitants (14-18), a feature that cannot be explained by migration patterns (17), but which may be due to the following features of urban areas: better access to medical care, exposure to other infections because of crowding conditions or exposure to possible contributing factors, eg, industrial pollution (18). The increasing incidence of CD in some areas over the past several decades suggests an environmental factor because the genetic makeup of humans is not expected to change so rapidly. CD may be more common in Caucasians (19). One study demonstrated an inverse correlation between CD prevalence and lactose intolerance (adult-onset) (20). Although milk has been long discounted as a possible cause of CD, this correlation is interesting for reasons discussed later in this review.

**M PARATUBERCULOSIS: IDENTIFICATION IN CD PATIENTS**

M paratuberculosis is a subspecies of the Mycobacterium avium complex (3). Although the M avium-intracellulare complex has been found in warm, wetty environments (21,22), M paratuberculosis has not yet been found as a free-living organism. It may exist in a cell wall-deficient form that does not stain acid-fast (4,23) and grows poorly, if at all, in culture (4,21,24-26). Its DNA is 98% homologous with M avium (26,27), which is ubiquitous in the environment. Therefore, studies attempting cultural or serological identification of M paratuberculosis from CD patients may be fraught with difficulty. Recently, a genomic determinant unique to M paratuberculosis, called IS900, has been identified (28). By using PCR (the ultrasensitive technique of DNA amplification), IS900 has been used in studies of CD patients. PCR is ideally suited for detecting organisms in low numbers that grow slowly (29). The technique has been well described for identification of Mycobacteria from standard pathological specimens (30). The main downfall of PCR is its exquisite sensitivity; this necessitates stringent laboratory technique to avoid false positive tests (31).

When unique genetic determinants are sought, serological (32) and PCR evidence (25,33-36) of M paratuberculosis has been found in up to 84% of CD patients. However, some investigators have not found M paratuberculosis in CD tissue samples (3,37,38), while others report finding M paratuberculosis DNA in non-CD controls (39,40). To date, it appears no PCR samples have been sent to a neutral third party for confirmation of PCR status.

**MECHANISMS OF INFECTION**

The mechanism by which M paratuberculosis infects humans is not clear. The strain is non-acid-fast (4,23) and grows slowly (29). The technique has been well described for identification of Mycobacteria from standard pathological specimens (30). The main downfall of PCR is its exquisite sensitivity; this necessitates stringent laboratory technique to avoid false positive tests (31).

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**EPIDEMIOLOGY OF PARATUBERCULOSIS IN DOMESTIC AND WILD ANIMALS**

In 1895 paratuberculosis was first described in cattle. It is a chronic wasting granulomatous enteritis affecting mostly young animals, particularly ruminants such as domestic cattle and sheep, bighorn sheep, domestic and mountain goats, deer, moose, bison and others (4,21,24,26,41-44). Cross-contamination of species may occur (24). Like CD, paratuberculosis has a similar maturity index, is characterized by relapse and remission, affects primarily the ileum (although any part of the gastrointestinal tract can be involved), and causes stricture formation, diarrhea and malnutrition. M paratuberculosis strains isolated from a CD patient have caused paratuberculosis when inoculated into goats (45). There is no cure for paratuberculosis and no adequate method to detect infected animals because the majority remain asymptomatic. The latency period may be up to 15 years in cattle, during which organisms may be shed fecally.

The complement-fixing antibody test has failed to detect serological evidence of exposure to M paratuberculosis in domestic cattle and sheep, and other free-ranging wild herbivores in Kenya (46). In animals testing positive (eight camels and one goat), cross-reaction with antibodies to a wide variety of organisms may have occurred (21,46). In contrast, it has been estimated that 25% to 30% of all cattle in the United Kingdom are latently infected (47). The disease has an affinity for dairy cattle (21,44,48,49).

Among domestic cattle, there appears to be a north to south gradient for paratuberculosis in the United States. In particular, the following American states have been recognized (43,44,48,50-52).

**New England states:** Connecticut, Massachusetts, New Jersey, New York, New Hampshire and Vermont collectively showed a prevalence of paratuberculosis in 18% of cattle from 100 culture specimens of slaughterhouse cattle (44).

**Wisconsin:** Eleven per cent of 1000 ileocecal valves from slaughterhouse samples cultured M paratuberculosis (52). This was considered an underestimate. Another study ranked Wisconsin fourth in overall prevalence (48).

**Florida:** Based on a serological study, 17% of Florida cattle tested positive for paratuberculosis (51).

**Pennsylvania and Minnesota** In one of the most recent surveys (48), 7540 ileocecal lymph nodes were cultured from apparently healthy slaughtered cattle from 32 states. Only Pennsylvania and Minnesota were significantly higher than the overall mean prevalence (P<0.01). The actual prevalences were probably underestimated due to methodology.
Ohio: Ohio was ranked third overall in the study cited just above (48).

The rough distribution outlined here has been known for at least 20 years (50). It is unknown whether apparent increases in prevalence from 1949 to 1971 are real or due to better surveillance and diagnosis, although the latter is probably at least partially responsible.

Within Wisconsin, an uneven geographical distribution of paratuberculosis-infected dairy cattle herds exists (43,53). The majority of infected cattle are found in the western, southern and southwestern portions of that state. This is interesting because Olmsted county, Minnesota (noted earlier for its unusually high CD prevalence) lies immediately adjacent to these areas. One small study from Alberta suggests that the prevalence of paratuberculosis in Alberta’s domestic cattle is similar to the highest American figures (54).

The overlap of regions with paratuberculosis and CD becomes clearer when a study using national Medicare records of CD is considered. Using these records, Sonnenburg et al (55) defined the geographic distribution of CD in the United States for all persons aged 65 and older. Because Medicare is provided universally to this population, accessibility to medical care should not have influenced the results.

Among Caucasians, the observed number of CD cases was higher than expected for 12 states. These states, in order of highest rate to lowest rate, are Massachusetts, Pennsylvania, New Jersey, New York, Florida, Ohio, Arizona, Michigan, Washington, Illinois, Connecticut and Maryland.

Comparison of the leading states for paratuberculosis and CD is noteworthy on two accounts. First, the overlap of the two lists is obvious. Second, Florida and Arizona appear as unique exceptions to the north to south gradient generally observable for both diseases. Sonnenburg and colleagues (55) suggest that Florida’s climate may attract many northerners. Similarly, Arizona is a popular destination for many northwesterners. Therefore, the prevalence of CD in those states may be artificially inflated because the study population consisted only of persons aged 65 and over. The question of CD prevalence among young persons in these states is interesting, although presently unanswered.

Among wild animals, paratuberculosis has been reported in fallow deer and red deer in the United Kingdom (called elk in North America) (56-60), free-ranging bighorn sheep and Rocky Mountain goats in Colorado (21,41,42), and moose (61). All these species are noteworthy because they occur in large numbers in the protected national parks of Alberta, which are close to Edmonton. Tuberculosis is prevalent in Alberta’s wildlife; an epizootic of Mycobacterium bovis infection in domestic elk has likely infected humans (62).

Located in northeastern Alberta, Wood Buffalo National Park is the only known reservoir of bovine tuberculosis and brucellosis in Canada, and the largest in North America (63,64). Bison and elk in Elk Island National Park, 30 km east of Edmonton, have been previously infected with tuberculosis and brucellosis (65,66). Paratuberculosis in Alberta’s wildlife has not been investigated. The presence of one of the world’s largest reservoirs of susceptible animals suggests that such investigation may be valuable, especially in light of the putative relationship of paratuberculosis to CD. Wild animals with paratuberculosis may be a reservoir of infection for domestic animals (67).

**DISCUSSION**

Interpreting the findings – A mechanism of infection: This review has compared existing data regarding CD and paratuberculosis. Similarities have been noted between their clinical and pathological characteristics and their geographic distribution, and there is direct evidence of Mycobacterium paratuberculosis presence in at least some CD patients. Despite this, several questions remain unanswered. Why does CD have an urban preponderance? What about CD patients far removed from large natural reservoirs of M. paratuberculosis? If M. paratuberculosis causes CD, how does it infect the individual? In a provocative editorial, Hermon-Taylor (68) forwarded the suggestion that M. paratuberculosis may cause CD, especially via ingestion of contaminated water or milk.

Heavy rains “will wash these organisms into the spate rivers” such as the Taff running into Cardiff, Wales, the Mississippi river on the Minnesota-Wisconsin border, the Dee river running into Aberdeen, Scotland or the North Saskatchewan river flowing through Edmonton, Alberta. In these regions, one can envision the large repositories of appropriate host animals – both domestic and wild – that reside upstream. Asymptomatically, the microorganisms are shed fecally and carried by the rivers into urban areas, where they are ingested with the drinking water. M. paratuberculosis can survive for 163 days in river water, for 11 months in feces or soil and for at least 12 months at −14°C (69,70). Mycobacteria, including the M. avium complex, are commonly isolated from chlorinated municipal drinking tap waters, particularly in North America, the United Kingdom and Scandinavia (71,72). There are no published studies that have specifically searched for M. paratuberculosis from these sources. Consider the case of Edmonton, where CD is unusually prevalent. Its drinking water is obtained from the North Saskatchewan river. Research has shown that spring run-offs from creeks feeding the river carry livestock wastes to the city, periodically causing serious contamination of the raw water supply (73). Alberta has approximately 4800 cattle feedlots, and a large one can produce wastes equivalent to a city of 20,000 people. Correlations exist among peak creek and river run-off during the spring thaw, high river bacterial counts and raw water colour in Edmonton. Studies on water quality in Alberta suggest that information regarding the influence of agriculture on water quality is inadequate.

Alternatively, M. paratuberculosis may be able to survive pasteurization (74,75) and so infect individuals, particularly through fluid milk. The distribution of dairy farms in Alberta is centred around Edmonton (76, personal communication). Of 11 milk marketing regions in Great Britain, South Wales has the greatest dependency on fluid milk production (77). Similarly, regions reporting the highest prevalences of CD correspond to the most area-intensive regions of dairy farming in the world (78). This may explain the correlation be-
tween CD incidence and lactose-tolerant populations consuming large quantities of cow’s milk (20). CD incidence has also been positively correlated with socioeconomic status (17). Among urban women, socioeconomic status is correlated with levels of fluid milk consumption (79). The risk of mycobacterial contamination of milk has been previously suspected (80).

CONCLUSIONS
Implications for future therapy: If CD is an infection, a cure is potentially available. Many studies have been conducted using antitubercular agents to treat CD, with varying success. Poor results may be due to small sample sizes, improper choice and/or duration of antibiotics, or incorrect diagnosis (81,82). Clarithromycin, a new macrolide antibiotic, has one of the highest in vitro activities against \textit{M} \textit{paratuberculosis} (83). Recent clinical experience suggests that clarithromycin may be useful for inducing remission in some CD patients (84). It may also play a role in the treatment of perianal fistula associated with CD (personal communication). However, its mechanism of action is unknown. An applicable prospective, double-blind, randomized, placebo controlled clinical trial using a combination of antibiotics such as clarithromycin, clofazimine and rifabutin at sufficient dosages for a period of at least 12 months is suggested as a potentially useful treatment regimen for CD.

Although there is some evidence from molecular medicine that \textit{M} \textit{paratuberculosis} may play a role in the etiology or pathogenesis of CD, this theory remains unproved. This review supports the hypothesis by showing a distinct overlap of some regions reporting high prevalences of CD and \textit{M} \textit{paratuberculosis}. It suggests that \textit{M} \textit{paratuberculosis} may be disseminated in these environments, perhaps via drinking water or dairy products, especially milk. This epidemiological review provides justification for further intensive investigation of the proposed hypothesis that \textit{M} \textit{paratuberculosis} plays a role in the etiology of CD.

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