Borrelia burgdorferi, the causal organism of Lyme disease, has been isolated from a black-legged tick, Ixodes scapularis, that was removed from a dog at Kenora, Ontario. This is the first time that B. burgdorferi spirochetes have been isolated from a black-legged tick on mainland Ontario.

On 28 October, 1993, a live, partially engorged, female black-legged tick was removed from a dog in Kenora, 50 km east of the Manitoba border. The dog had never been out of the town. The Kenora Veterinary Clinic submitted the tick to the Vector-borne Diseases Laboratory, British Columbia Centre for Disease Control, Vancouver, British Columbia, for identification and spirochetal analysis and it was positively identified as Ixodes scapularis.

In the laboratory, the tick was surface sterilized. Using micro-surgery, the midgut contents were put on Barbour-Stonner-Kelly (BSK) II culture medium. Within 2 weeks, motile spirochetes were isolated and observed by dark-field microscopy. The isolate was immunostained with monoclonal antibodies and was positive for OspA, OspB, P39, and flagellin of B. burgdorferi. The spirochetes were also positive for OspA gene using the polymerase chain reaction testing. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of this isolate was compared with B31 (type specimen) and eight B. burgdorferi isolates from British Columbia separated on the same gel and was found to be similar. DNA sequencing of the 16S r RNA gene of this isolate shows similarity to that of the B. burgdorferi B31 strain. On the basis of all the tests on this isolate, the spirochete was confirmed to be B. burgdorferi sensu stricto.

Blood samples taken from the dog at 4, 17, and 28 weeks after the tick was removed tested positive for B. burgdorferi using the indirect immunofluorescence assay; the titre values were 1:256, 1:256 and 1:512, respectively. The Western blot (WB) result for the sample taken 4 weeks after the tick was removed was negative; the WB for the 28-week sample was positive. No WB test was done on the middle sample.

From 1984 to the end of 1994 there were 205 cases of Lyme disease reported to the Ontario Ministry of Health. Of these 205 cases, 105 were contracted in Ontario, and only 14 of these 105 patients had been at Long Point, Ontario, which is a known endemic area for Lyme disease (Dr. C. LeBer, Disease Control Service, Ontario Ministry of Health, Toronto: personal communication, 1995).

There are over 65 locations across Ontario (as of the end of 1994) where black-legged ticks have been found and documented. The black-legged tick from Kenora is the farthest north that one has been reported in Ontario. Three engorged adult female ticks collected in and near the city of Thunder Bay, Ontario, were identified and reported as I. dammini in 1991.

In Canada, as of the end of 1994, black-legged ticks have been reported in over a total of 250 locations in Manitoba, Ontario, Quebec, Nova Scotia, New Brunswick, Newfoundland, and Prince Edward Island. The first black-legged tick was reported in 1904 on a human in Bracebridge, Ontario.

Birds are known to carry ticks hundreds of kilometres on their migratory flight path. Not only do they carry infected ticks, some birds carry B. burgdorferi infection systemically in their bodies. Robins and house wrens are known to be competent hosts of B. burgdorferi.

In 1993 and 1994, the Lyme-Borreliosis Support Group of Ontario conducted a tick collection project asking veterinarians to submit any specimens from their practices. These ticks were recorded and sent for spirochetal analysis. This study is continuing in 1995.
A rabbit was hit by a vehicle on a road through a wooded area just outside the city limits of Grande Prairie in Alberta on 21 June, 1994. The injured rabbit was taken by the driver to the Grande Prairie Animal Hospital for treatment. The veterinarian judged the injuries to be fatal and euthanized the animal.

During the examination of the animal, six ticks of "varying sizes" were found on the face and neck area. The veterinarian put the ticks in a plastic pill box along with a piece of moistened cotton and sent them to the Provincial Laboratory in Vancouver for identification and testing for the presence of Lyme spirochetes.

There were four engorged female and two engorged nymphal Haemaphysalis leporispalustris ticks. Each tick was sterilized and their gut tissues were cultured separately in BSK medium with antibiotics on 24 June. Cultures were examined 5 days later and motile spirochetes were found in one of the adult female tick cultures. Dead spirochetes were seen in three other adult female tick guts and one of the nymphal tick cultures.

All spirochete cultures were immunostained by four monoclonal antibodies specific for Borrelia burgdorferi, viz., OspA (31 kilodalton [kD] protein), OspB (34 kD), flagellin (41 kD) and P39 (39 kD). All spirochetes were positive in these tests. The motile spirochetes were further tested for OspA gene by polymerase chain reaction and found to be positive. This culture has been rendered axenic by passing through a 0.2 μ filter and antibiotic treatment. Further studies of the SDS-PAGE protein profile of these spirochetes and DNA sequencing of the 16S r RNA gene found them to be similar to B. burgdorferi.

Antisera to B. burgdorferi were tested against this isolate and found to be positive by indirect immunofluorescence assay. It is concluded that the spirochete isolated from the rabbit tick H. leporispalustris in Alberta is indeed B. burgdorferi, the etiologic agent of Lyme disease (LD) and is identical to the spirochetes isolated in British Columbia (1,2). This isolation of B. burgdorferi from rabbit ticks in Alberta is the first such discovery of LD spirochetes in Canada. Rabbit ticks rarely bite humans and this may be one of the reasons why Alberta has not reported human LD.

The rabbit is well known as a host of LD spirochetes in the United States (3-5) Ixodes dentatus larvae, nymphs and adults retrieved from trapped cottontail rabbits in the New York Botanical Garden yielded B. burgdorferi spirochetes (5). Drs. Burgdorfer (6) and Lane (7) found two of 174 H. leporispalustris ticks infected with spirochetes indistinguishable from B. burgdorferi. Dr. Rawlings has also isolated B. burgdorferi spirochetes from rabbit ticks in Texas (8).

It is important to note that B. burgdorferi isolated from rabbit ticks (I. dentatus) showed antigenic variation when compared with the B31 strain although they were positive by monoclonal antibody tests. However, similarities were sufficient to lead Dr. Anderson et al (9) to conclude that borreliae in rabbits and I. dentatus were B. burgdorferi.

The isolation of B. burgdorferi from H. leporispalustris in Grande Prairie, Alberta, demands further investigation of local canines, other pets and domestic animals for Lyme-like symptoms in clusters of animals that may have gone unnoticed. It would be important to extend the studies to include wild rabbit and rodent populations and ticks retrieved from these trapped animals for isolation of LD spirochetes.

The antigenic variations seen in rabbits and rabbit ticks may modify the manifestations of LD in infected patients and pets. Serologic evaluations of these patients for LD using B. burgdorferi antigens may be negative for the disease. We would like to mention here two such patients, one of whom may have been infected by an unknown tick bite in Calgary, Alberta, in 1986. This patient developed a multisystem disease in 1986 following a tick bite in his backyard. A second patient was bitten by H. leporispalustris ticks at Fort Fraser, British Columbia, in 1985 while mining for gold. He developed intense generalized polyarthralgias, recurrent headache and memory loss. Both of these patients had symptoms mimicking chronic Lyme borreliosis but serologic tests for LD were negative.

We would like to emphasize the importance of rabbit and rabbit ticks in the dissemination of LD in Alberta and would encourage further research in this area.

References


**Source:** SN Banerjee, PhD; M Banerjee, PhD; K Fernando, MSc; MY Dong, MD; JA Smith, MD; Vector-borne Diseases Laboratory, BC Centre for Disease Control, Vancouver, BC; D Cook, DVM, Grande Prairie Animal Hospital, Grande Prairie, Alberta.

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**A CASE OF CYCLOSPORA INFECTION IN AN ALBERTAN TRAVELLER**

**Case Description**

A 47-year-old male presented to his family physician with a one-month history of intermittent watery diarrhea, nausea, anorexia, flatulence, abdominal cramps, and a weight loss of approximately 2.5 to 4.5 kg. Two weeks prior to seeing his physician, the patient had returned from a 2-year teaching assignment in Indonesia. His wife, who had accompanied him on his travels, was asymptomatic.

Two stool samples were submitted for routine enteric culture and ova parasite examination, followed by a third specimen for routine enteric culture only. All three specimens for enteric culture and ova parasite examination, followed by a third specimen for assignment in Indonesia. His wife, who had accompanied him on his travels, was asymptomatic.

Two stool samples were submitted for routine enteric culture and ova parasite examination, followed by a third specimen for routine enteric culture only. All three specimens for enteric culture were negative for *Salmonella, Shigella, Yersinia, Campylobacter, Aeromonas,* and *Escherichia coli* O157:H7.

Stool samples for ova and parasite examination were submitted in sodium acetate-formalin (SAF) preservative. When stained using a modified Kinyoun stain, large numbers of *Cyclospora* oocysts were detected. Upon this discovery, the third stool sample which was submitted in enteric pathogen transport media (modified Cary-Blair) was stained; although the stain was performed on the sample one week following collection, large numbers of relatively well-preserved oocysts of *Cyclospora* were again demonstrated.

The patient’s diarrhea resolved without treatment one week following his presentation, but he continued to experience some anorexia and mild nausea.

**Discussion**

Previously known as cyanobacterium-like or coccidian-like bodies (1,2,3) or CLBs, this new protozoan pathogen of humans has been assigned to the coccidian genus, *Cyclospora.* Ortega et al (4) have proposed the name *Cyclospora cayetanensis* n. sp. for this emerging pathogenic coccidian. Knowledge of the epidemiology and life cycle of *Cyclospora* in both humans and animals is still in its early stages. Various species have been found in reptiles, moles, rodents (5), and chimpanzees (6), but it remains unclear if any of these species play a role in human disease.

*Cyclospora* has been implicated in a number of sporadic cases and epidemic outbreaks of diarrheal illness in Nepal (1,2,7), Peru (5,8), Solomon Islands (9), Morocco (10), and other countries in North, Central, and South America (6,9,11,12), Eastern Europe (5), and Asia (5,11), including India (5,9,10,11), Pakistan (9), and Cambodia (9).

Most infections were associated with travel to or residence in developing countries, while others were acquired in more temperate climates. Several cases have also been reported in the United States (8,11,12) and Britain (13). Diarrhea caused by *Cyclospora* appears to display a seasonal variability with the incidence peaking during the more hot and humid months of the year (2,5,8).

The overall prevalence of *Cyclospora* infection appears to vary depending on the region and population studied. In Nepal, it has been estimated to range from between 10% to 20% in individuals with diarrhea to 1% in asymptomatic populations (4). The prevalence in Lima, Peru, was reported to be 6% to 18%, while an American laboratory-based study estimated its prevalence as approximately 0.2% (12).

The route of transmission is most likely through ingestion of contaminated water. In Chicago, Illinois, an outbreak involved a hospital dormitory where several individuals became ill after the failure of the dormitory water pump (7,14). One report linked the disease to the accidental ingestion of water from an aquarium (8). Other studies reported isolation of *Cyclospora* in the tap water (1) and on vegetables in the households of patients diagnosed with *Cyclospora* diarrhea (5). A number of cases were also reported in residents and tourists in Kathmandu, Nepal, and were linked to the consumption of contaminated water and milk, which was prepared with contaminated water. Of the infected patients, only 28% drank the untreated water or milk; this indicates that there may be other modes of transmission that require consideration (1).

The pathogenesis of *C. cayetanensis* diarrhea is not fully understood. The characteristic absence of white and red blood cells in stool specimens most likely indicates a non-invasive pathologic mechanism (1,12). The diarrhea appears to be secretory in nature, as indicated by excessive production of fluid by the affected intestinal epithelium. Duodenal and jejunal biopsies reveal variable degrees of villous blunting (10), villous atrophy, and crypt hyperplasia (5), with impaired D-xylose absorption documented in some cases (2,10).

A study from Lima, Peru, demonstrated that only 11% to 28% of children excreting *Cyclospora* in their stools presented with diarrhea (9), and this may be considered by some to be evidence of its low pathogenicity. However, a similar situation has been noted for *Cryptosporidium* (15); asymptomatic carriage of pathogens, such as *Giardia lamblia* and *Entamoeba histolytica* is well recognized.

*Cyclospora* may be detected in preserved stool specimens and jejunal aspirates or biopsies by using a modified Ziehl-Neelsen or Kinyoun stain (1,2,10), which is used in some laboratories to identify *Cryptosporidium parvum*. The organisms appear as red spheres, 8 µm to 10 µm in diameter with well defined, non-refractile double...
walls with some demonstrating internal sporocysts\(^{(10)}\). Some organisms may not take up the Kinyoun stain as readily and appear as round clear "ghosts" against the background material\(^{(3,5,10,11)}\). 

*Cyclospora* can also be detected by its ability to auto-fluoresce a blue-green color when viewed under ultraviolet illumination\(^{(3,5,10,12)}\).

Patients with *Cyclospora* infection develop serum antibodies against the organism, with up to a 10-fold rise in antibody titre detected in some cases\(^{(3)}\). However, it is not known if these antibodies confer immunity to subsequent infections, or for how long the antibody levels persist in the serum.

The incubation period following exposure to *Cyclospora* ranges from 1 to 7 days, with the onset of symptoms being abrupt (68\% of cases) or more gradual (32\%)\(^{(3)}\). Patients usually present with intermittent but self-limited diarrhea, consisting of up to eight or more loose watery stools per day\(^{(2,5,7)}\). Other associated symptoms include nausea, anorexia, abdominal cramps, bloating, flatulence, fatigue, myalgia, and mild to moderate weight loss; fever, however, is rarely present\(^{(1,2,7)}\). In the immunocompetent host, the diarrhea may last between 4 to 107 days\(^{(1,2,5,7,8,11)}\), with shedding of the organism lasting up to 7 to 70 days\(^{(5,8)}\). In the immunocompromised host, particularly AIDS patients, the duration of symptoms can range from weeks to several months with outcomes of either full recovery\(^{(12)}\), persistent symptoms\(^{(2,12)}\), or death\(^{(17)}\).

Currently, there is no recommended treatment for *C. cayetanensis*; however, a recent report documented that oral trimethoprim-sulfamethoxazole has been shown to eliminate the organism in the stool\(^{(10)}\), and under reported.

Although rare, *C. cayetanensis* should be considered in the differential diagnosis of diarrhea in travellers once other etiologies have been ruled out. Since it has only recently been recognized as a pathogen, it is possible that its geographic distribution may include Canada. Both physicians and laboratory personnel across the country should be aware of its existence and pathogenic potential.

### References


Source: DB Purcyd, MD, IL Perry, AR, D Bulawka, RT, KT Kowalewska-Grochowska, MD, Microbiology and Public Health, University of Alberta Hospitals, Edmonton; BL Oldale, MD, Athabasca Medical Centre, Athabasca, Alberta.

### Editorial Comment: Cyclospora cayetanensis infection

Cyclospora cayetanensis infection, frequently associated with the drinking of contaminated water, occurs worldwide, but is particularly notable in Nepal, where outbreaks have been reported during the rainy season\(^{(1)}\), and in Peru\(^{(2)}\). The majority of isolates from humans have been identified in stool samples from residents of developing countries or from travellers returning from such countries. This organism has been found in North American travellers returning from Haiti, Mexico, Puerto Rico, Pakistan, and India.

Although there does not appear to be any documented reports of *Cyclospora* infection in Canadians in the literature, several parasitology laboratories do identify cases annually (Dr. D. MacPherson, Regional Parasitology Laboratory, Hamilton: personal communication, 1995). *Cyclospora* infection is unusual and requires special techniques for detection; moreover, it is not a notifiable condition in Canada. Therefore, it is probably under detected and under reported.

### References


LYME DISEASE — UNITED STATES, 1993

In 1982, CDC initiated surveillance for Lyme disease (LD), and in 1990, the Council of State and Territorial Epidemiologists adopted a resolution making LD a nationally notifiable disease. This report summarizes surveillance data for LD in the United States during 1993.

LD is defined as the presence of an erythema migrans rash or at least one objective sign of musculoskeletal, neurologic, or cardiovascular disease and laboratory confirmation of infection (1). In 1993, 8,185 cases of LD were reported to CDC by 44 state health departments, 1,492 (15%) fewer cases than were reported in 1992 (9,677). Most cases were reported from the northeastern, mid-Atlantic, north-central, and Pacific coastal regions. Six states (Alaska, Arizona, Colorado, Mississippi, Montana, and South Dakota) reported no cases. The overall incidence rate was 3.3 per 100,000 population. Eight states in established LD-endemic northeastern and upper north-central regions reported rates of more than 3.3 per 100,000 (Connecticut, 41.3; Rhode Island, 27.3; Delaware, 21.0; New York, 15.5; New Jersey, 10.1; Pennsylvania, 8.9; Wisconsin, 8.2; and Maryland, 3.8); these states accounted for 6,962 (85%) of the cases reported nationally. Of the total cases, 6,132 (75%) were reported from 81 counties that had at least five cases and had rates of at least 10 per 100,000 population.

Most (83%) of the decrease in 1993 resulted from reductions in the numbers of case reports from four states in which LD is endemic (California, Connecticut, New York, and Wisconsin). New York, which reported 34% of the U.S. cases in 1993, accounted for 41% of the decrease (609 cases), and Connecticut accounted for 27% of the decrease (410 cases). Thirteen states reported small increases in the number of cases. New Jersey had the largest increase (786 cases, compared with 681 in 1992).

The age distribution of persons reported with LD was bimodal, with peaks occurring for children aged 5 to 14 years (1,098 cases) and adults aged 30 to 49 years (2,298 cases). Males (51%) and females were nearly equally affected.

Reference