significantly less than that under O_3 exposure, however.

The concentrations of trace gases that we have used in these experiments are high but not unrealistic. Concentrations of H_2S of several parts per million occur in the vicinity of sources such as oil fields, pulp mills, and sewage treatment plants. In any case, our previous results demonstrate that sulfidation involving H_2S scales with total exposure over a wide range of concentrations (*4*), so that lower concentrations and longer exposure times produce equivalent sulfidation of copper. Ozone concentrations of 0.2 to 0.3 ppm do not often occur, but such concentrations have been measured in locations as diverse as Detroit (*26*), Houston (*26*), Los Angeles (*26*), New Haven (*27*), Sydney (*28*), and Toronto (*29*). High concentrations outside buildings can produce reduced but still high ($\sim$ 0.2 ppm) O_3 concentrations indoors (*30*). The high O_3 concentrations in urban areas are generally the result of photochemical generation from anthropogenic precursors (*26*), although high O_3 concentrations can occur anywhere as a result of the injection of stratospheric air into the troposphere (*31*). Once high concentrations of trace atmospheric gases are present in the air, they can be transported many hundreds of kilometers (*32*).

Our results and these considerations support the view that atmospheric O_3 participates in a wide range of corrosion processes on a variety of materials, both indoors and out. The diverse items affected must surely include open-wire telephone lines, copper roofing, and bronze statuary. With the involvement of O_3 in atmospheric corrosion now established, it is reasonable to anticipate that other atmospheric oxidizing species are involved as well. Among those species are H_2O_2 and the OH, hydroperoxyl (HO_2), and alkylperoxyl (RO_2) radicals. It is clear that atmospheric chemistry and atmospheric corrosion of materials are inextricably intertwined and that a new degree of complexity has been added to corrosion research.

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Amblyomma americanum: A Potential Vector of Lyme Disease in New Jersey

Abstract. Amblyomma americanum *is a likely secondary vector of Lyme disease in New Jersey. Ticks of this species were removed from the site of the characteristic skin lesion known as* erythema chronicum migrans *on two patients with the disease, and the Lyme disease spirochete was isolated from nymphs and adults of this species. That* A. americanum *is a potential vector is supported by its similarities to* Ixodes dammini, *the known tick vector, in seasonal distribution and host utilization. The extensive range of* A. americanum *may have great implications for potential Lyme disease transmission outside known endemic areas.*

Lyme disease, first described in Connecticut in 1975, is an inflammatory disorder with a characteristic annular lesion, *erythema chronicum migrans* (ECM), which may be followed by arthritic, cardiac, or neurological manifestations (*1*). Initial epidemiologic evidence suggested that Lyme disease was transmitted by the nymphal stage of the tick *Ixodes dammini* (*2*). That the etiologic agent of Lyme disease is a spirochete was first proposed in 1982 (*3*). Spirochetes were subsequently isolated from adult and immature *I. dammini* from New York, Connecticut, and New Jersey (*4–6*). Past evidence indicated that Lyme disease in the United States and cases of ECM in Europe were transmitted solely by ticks in the genus *Ixodes* (*7*). In New Jersey, populations of *Amblyomma americanum* ticks temporally coexist with *I. dammini* and equally infest a variety of mammalian hosts including the white-footed mouse (*Peromyscus leucopus*) and white-tailed deer (*Odocoileus virginianus*) (*8–10*).

We first became aware of the vector potential of *A. americanum* in May 1982 when a tick removed from the site at which ECM developed in an 87-year-old male from Medford, New Jersey, was identified as a female of this species. As this case predated the publication of the isolation of the Lyme disease spirochete (*3*), serological confirmation was not attempted at the time of illness. A serum sample drawn 4 months after the date of onset showed a titer of 1:128 by indirect immunofluorescence (IFA) (Table 1). In June 1983, a second *A. americanum* as-

Table 1. Titers of antibody to the Lyme disease spirochete in serum samples from two patients. For antigen preparation, cultures of spirochetes from *I. dammini* found on Shelter Island, New York, were washed six times in sterile phosphate-buffered saline (PBS), *p*H 7.4, supplemented with $MgCl_2$. Immediately after the last wash, the spirochete pellet was resuspended in PBS and 10 μl of the suspension was applied to each well of microtiter slides, dried at 37°C, and stored at −70°C. Slides were overlaid with twofold serial solutions of patient serum and incubated (37°C) for 45 minutes, washed in PBS (*p*H 7.4), and stained (37°C for 45 minutes) with fluorescein isothiocyanate–labeled goat antiserum to human IgM (μ chain specific) and IgG (γ chain specific) (Cappel). Positive and negative controls were tested simultaneously on each slide. End points were defined as the highest dilution in which all spirochetes fluoresced dimly.

Patient	Date of onset of disease	Date serum collected	Antibody titer	
			IgM	IgG
No. 1	May 1982	3 September 1982		1:128
No. 2	28 June 1983	24 June 1983	< 1:16	1:16
		25 August 1983	< 1:16	1:16

sociated with a case of Lyme disease was reported from Barnegat, New Jersey, when a female tick was removed from the site where ECM developed. The 37-year-old female patient was a resident and part-time employee of the Boy Scout Reservation where we subsequently found the Lyme disease spirochete in *A. americanum*. Within 2 days of the appearance of ECM the patient began a 4-week course of tetracycline (500 mg four times a day) and the rash quickly resolved. Serum samples obtained during the acute and convalescent phases of the disease demonstrated no immunoglobulin M (IgM) antibody and weakly reactive immunoglobulin G (IgG) titers by IFA (Table 1). Early antibiotic therapy has been linked to earlier resolution of illness and minimal antigenic stimulation of the immune system (*5*). Failure of the patient's serum to show a change in antibody titer (seroconversion) was not surprising because she received tetracycline within 2 days of ECM and at double the recommended dosage and treatment interval.

We isolated and identified the Lyme disease spirochete from adult and nymphal *A. americanum*. Forty-four *A. americanum* were collected from the clothing of investigators who randomly walked through the Boy Scout Reservation in Barnegat, New Jersey, on 28 June 1983. These ticks were individually examined for Lyme disease spirochetes as previously described (*4*). Of these ticks, 9.1 percent contained spirochetes as determined by dark-field microscopy (Table 2), and the spirochetes were serologically identical to the original *I. dammini* spirochete by direct immunofluorescence (*11*). The sole larval tick did not yield spirochetes, but 22.2 percent of nymphs and 5.9 percent of the adults (11 percent of males and 4 percent of females) did. No motile spirochetes (therefore assumed dead) were observed in infected ticks. It is not known if nonmotile spirochetes were the result of examining dead or moribund ticks, loss of pathogen viability during transtadial passage, or from indigenous substances within the tick hostile to spirochete vigor or survival. However, nonmotile spirochetes are also observed in *I. dammini*.

Infection rates for nonengorged adult *A. americanum* were lower than for nonengorged *I. dammini* adults (61 and 78.9 percent, respectively) collected from Shelter Island, New York (*3*), and Colt's Neck, New Jersey (*6*), respectively. Spirochete burdens in *A. americanum* also appeared low. Similarly, infection rates for *A. americanum* nymphs (22.2 percent) were lower than for engorged and nonengorged subadult *I. dammini* (37 percent) removed from killed rodents (*4*) and fully engorged subadults (60 to 90 percent) allowed to drop from live feral rodents in New York (*12*).

New Jersey marks the northern extent of the occurrence of significant populations of *A. americanum*. Unlike other endemic areas in the Northeast, *A. americanum* and *I. dammini* share a common distribution throughout the southern two-thirds of the state (*9*). Immature stages of both species have similar seasonal distributions. Adult *I. dammini* exhibit spring and fall peaks whereas adult *A. americanum* are most abundant in June and July (*8*). Peak populations of adult *A. americanum* coexist with peak populations of nymphal *I. dammini*, the nymphal stage of the latter being thought to be important in Lyme disease transmission (*2*).

Table 2. Prevalence of spirochetes in *Amblyomma americanum*. Each tick was immersed in aqueous Merthiolate (1:1000), rinsed twice in distilled water, and triturated on a glass slide in one drop of sterile PBS (*p*H 7.4) supplemented with $MgCl_2$. When spirochetes were detected by dark-field microscopy, the contents of the slide were smeared, air-dried, fixed in methanol, and stained with fluorescein isothiocyanate–conjugated antiserum produced in a rabbit to the Shelter Island, New York, *Ixodes dammini* spirochete (*11*).

Tick stage	Number collected	Number infected*	Percent infected
Adult (male)	9	1	11.1
Adult (female)	25	1	4.0
Nymph	9	2	22.2
Larva	1	0	0
Total	44	4	9.1

*The adult ticks and one nymph were dead before they were tested; the remaining nymph was moribund when tested.

The *I. dammini* infestation rate for 265 *P. leucopus* from Colt's Neck, New Jersey, was 48.7 percent (*8*), similar to the 43 percent of 77 *P. leucopus* found at Shelter Island, New York (*4*). In New Jersey, 5.3 percent of *P. leucopus* were infested with *A. americanum*. All stages of *A. americanum* readily attacked humans, whereas immature *I. dammini* were rarely collected from us during 9 months of surveying free-ranging, nonengorged ticks (*13*). The failure to collect immature *I. dammini* by this survey technique resulted in a dramatic seasonal change in the tick species composition of our collections. In April, 63.9 percent of 681 ticks collected were *I. dammini*. In May, the majority of the collection (N = 473) was composed of *A. americanum*, with *I. dammini* representing only 32.3 percent. During the period of peak Lyme disease transmission, 97.3 percent (N = 283) of the June and 99.4 percent (N = 176) of the July collections, respectively, were composed of adult and nymphal *A. americanum*. The host-seeking behavior of all stages of *A. americanum* seems to favor the location of large mammals including humans.

We believe that *A. americanum* serves as a secondary vector of Lyme disease in endemic areas of New Jersey. The removal of *A. americanum* from the site at which ECM developed in two patients and the isolation and identification of the Lyme disease spirochete from *A. americanum* collected near the second patient's residence provide direct evidence in support of this hypothesis. Further, both *A. americanum* and *I. dammini* are active during periods of peak human infection. Neither tick is host-specific. In New Jersey, *P. leucopus* served as a principal host for immature stages of both species while all stages of *A. americanum* and *I. dammini* were found on *O. virginianus* (*8*). Both mammals are suspected natural reservoirs of the Lyme disease spirochete (*4*).

Further investigations are needed to define the precise role of this tick in Lyme disease transmission. Clarification is needed on the apparent low frequency of infection, low spirochete burden, and low survival rate in *A. americanum*. *Amblyomma americanum*'s ability to transmit Lyme disease may be of extreme importance because of the extensive range of this species. Efforts to identify vectors of Lyme disease in Texas, Arkansas, and Georgia (*7*) may benefit from this new information (*14*).

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14. Since acceptance of this report, live spirochetes have been recovered from two specimens of adult *Amblyomma americanum* collected from a known Lyme disease endemic area in New Jersey.
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Complete Development of *Cryptosporidium* in Cell Culture

Abstract. *Protozoan parasites of the genus* Cryptosporidium *cause a short-term, flu-like, gastrointestinal illness in immunocompetent persons and severe, persistent, life-threatening diarrhea in immunodeficient individuals. No effective therapy is available for the treatment of cryptosporidiosis in the immunodeficient host. Complete development (from sporozoite to sporulated oocyst) of a human isolate of* Cryptosporidium *was achieved in cultured human fetal lung cells and primary chicken kidney and porcine kidney cells. The growth of this newly recognized zoonotic agent in cell culture now provides a means of studying its behavior, development, and metabolism, and a mechanism for evaluation of potentially useful therapeutic agents.*

Protozoans of the genus *Cryptosporidium* (Phylum Apicomplexa; Suborder Eimeriorina) are small (2 to 6 μm) coccidian parasites that inhabit the microvillous region of epithelial cells of a variety of animals, including man (*1*). Until recently, *Cryptosporidium* infections were considered rare in animals, and in man they were thought to be the result of a little-known opportunistic pathogen outside its normal host range (*2*). Our concept of cryptosporidiosis has changed within the past several years to that of an important cause of gastroenteritis and diarrhea in several animal species, especially calves, lambs, goats, and humans (*1*).

In immunocompetent humans, *Cryptosporidium* may produce a short-term, flu-like, gastrointestinal illness (*3, 4*). This contrasts sharply with the prolonged, severe diarrhea in immunodeficient individuals who contract cryptosporidiosis (*3, 5*), especially those with acquired immunodeficiency syndrome (AIDS). Recent reports of respiratory (*6*) and biliary (*7*) infections demonstrate that *Cryptosporidium* is not always confined to the gastrointestinal tract of immunodeficient persons.

Experimental infections in farm and laboratory animals have established that *Cryptosporidium* has little or no host specificity, and that it is transmitted by ingestion of oocysts that are fully sporulated and infective at the time they are passed in the feces (*1, 3, 4*). Calves and perhaps companion animals such as kittens, puppies, and rodents may serve as sources of human infection (*3, 4*), and human-to-human transmission may occur (*8*). Thus, cryptosporidiosis has joined the list of more than 150 zoonoses; those diseases caused by agents that are naturally transmitted between other vertebrate animals and man (*9*).

We report here the complete development (from sporozoite to infective oocyst) of a human isolate of *Cryptosporidium* in cell culture. Cultivation of *Cryptosporidium* in vitro will enable investigators to determine precisely the biology of this intracellular parasite in the absence of other microbes found in the host intestine.

Cryptosporidium oocysts were obtained from the feces of a patient with persistent cryptosporidiosis and the clinical and laboratory features of AIDS, or from the feces of goats experimentally infected with this human isolate (*3*). Feces containing oocysts were stored in a 2.5 percent (weight to volume) solution of $K_2Cr_2O_7$ at 4°C for up to 12 months prior to use. Oocysts were purified and concentrated by flotation in Sheather's sugar solution (*3*), washed three times by centrifugation in phosphate-buffered saline [(PBS), *p*H 7.2], and incubated in PBS containing 0.1 percent (weight to volume) glucose, penicillin (5000 IU/ml), streptomycin (5 mg/ml), and amphotericin-B (20 μg/ml), for 3 hours at 37°C to kill microbial contaminants (*10*). The oocysts were then washed three times and incubated in PBS containing 0.25 percent trypsin and 0.75 percent sodium taurocholate (each weight to volume) for 30 to 60 minutes at 37°C to obtain free sporozoites (*10*).

Excysted (free) sporozoites were washed twice in PBS and resuspended in growth medium (*11*) and approximately 150,000 sporozoites were added to each Leighton tube containing a monolayer of cells growing on a 5 cm^2 plastic cover slip. After incubation for 4 hours at 37°C in a 5 percent CO_2 atmosphere to allow attachment and penetration of sporozoites, cell monolayers were rinsed vigorously with maintenance medium (*12*). Infected cell cultures were kept in maintenance medium at 37°C with a 5 percent CO_2 atmosphere throughout the study. Cells used to study the developmental cycle of *Cryptosporidium* were human fetal lung (HFL) cells (Flow 2000). Primary chicken kidney (PCK) and porcine kidney (PK-10) cells were also examined for their ability to support the growth of *Cryptosporidium*. The cell monolayers were examined at various times after inoculation of sporozoites for developmental stages of *Cryptosporidium* by