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Research Article

Elevated Infections of Lyme Disease Patients with *Cryptosporidium parvum*

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ABSTRACT

We report for the first time a relationship between clinical cases of Lyme disease diagnosed in a US patient population in a Virginia clinical facility and intestinal infections with *Cryptosporidium parvum* identified from fecal samples collected from the same patients tested in an Arizona Parasitology Center (PCI). Infections with *C. parvum* in the populations of Lyme disease patients were at a considerably higher prevalence rate than in the general non-Lyme US population. *Bartonella*, *Babesia*, *Ehrlichia*, and *Borrelia* co-infections with the Lyme disease have been well established. All these infections are resident in the *Ixodes* tick vector system and can be transmitted to human *via* tick bite. Infections with *C. parvum*, however, are transmitted by the water/food vehicle and not by tick bites and therefore do not lend themselves to the tick bite transmission pattern. This is the first such report involving a relationship between two pathologies with very different epidemiology. Our findings are more similar to the reported association between HIV-AIDS and *Cryptosporidium* infections as both conditions can often be associated with the fecal contamination *via* certain sexual practices. Our report addresses 456 cases studied for Lyme disease and *C. parvum* infections by sex and age tested in the same facility.

Keywords: Lyme disease; *Cryptosporidium parvum*; Associated infections

INTRODUCTION

Lyme disease is caused by *Borrelia burgdorferi* which is usually transmitted by the bite of the ixodid tick *Ixodes scapularis* endemic to the Northeastern and Upper Midwestern regions of the United States. Tick-borne infections with *Babesia*, *Bartonella*, *Ehrlichia*, *Anaplasma*, *Rickettsiae*, among others, have been increasingly recognized as important co-infections in Lyme disease pathology over the last few decades [1-4]. These co-infections also co-exist in the same tick vector, *I. scapularis*. Patients presenting with symptoms of multiple chronic infections underlying suppressed immune system may not test positive for Lyme disease. Patients infected with Lyme disease are at risk of developing co-infections with tick-borne illnesses that can increase the severity and/or the duration of Lyme clinical symptoms [5]. Tick-borne co-infections are not routinely tested for in Lyme patients.

Intestinal parasites are not tested for in Lyme patients as part of their Lyme testing protocol. Intestinal parasites are usually food/liquid-borne infections transmitted *via* the fecal-oral route and are not related to the tick-borne route through which Lyme and its co-infections are transmitted [6,7]. A Virginia clinical facility that treated Lyme cases also tested its patients for intestinal parasites at our Scottsdale Arizona Parasitology Center (PCI). Correlations were made between the status of Lyme and parasitic infections. A relationship was found between Lyme cases and infections with the intestinal protozoan *Cryptosporidium parvum*. A markedly higher prevalence rate in this study among Lyme disease patients was observed and is reported herein.

MATERIALS AND METHODS

Examination of fecal specimens for *Cryptosporidium parvum* infections

A total of 970 fecal specimens from 485 patients (two specimens per patient) were collected, preserved, and transported to Parasitology Center, Inc. (PCI) in Proto-fix ™ (Alpha-Tec Systems, Inc. Vancouver, Wash.) or SAF (sodium acetate–acetic acid–formalin mixture) in plastic vials provided in mailable kits. Patients, mostly with Virginia street addresses, were referred to PCI by the Virginia Clinical facility from April, 2008 to January,

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2012. Repeat patients were only counted once bringing the total number to 456 patients considered for this study. Specimens were processed and stained as reported in Amin with CONSED™ according to the manufacturer's (Alpha-Tec Systems) directions [8,9]. The processing procedure was previously evaluated and described in Amin and is routinely used in thousands of fecal specimens at PCI annually. Briefly, specimens are filtered, mixed with CONSED and ethyl acetate, vortexed, centrifuged, and decanted. The resulting fecal plug was mixed with CONSED diluting reagent, transferred to, and mounted on a slide for microscopic examination as wet mounts. All samples were evaluated by the same observer blinded to patient information, e.g., symptoms, travel history, etc. The reliability of diagnosis is indicated by the consistency of detection of different parasites at different levels of infection during the same period of time. Positive results were quantified (number of organisms per high power field on a scale of 1-4) from duplicate samples from each patient [8,9]. The term prevalence rate used in this study refers to the number of patients infected over the number of patients examined.

Diagnosis of Lyme disease and observed considerations

Patients referred by the Virginia facility whose fecal specimens were tested at the Arizona Parasitology Center, Inc. in Scottsdale were tested for Lyme disease using igenex: 188 IgM and 189 IgG western blot (IGENEX, Milpitas, CA). The interpretation is based on Internal validation studies. The criteria are considered positive if two or more of the double two-starred bands are present. Positive results indicate exposure to *B. burgdorferi* but not confirmatory of active Lyme disease cases; see Harris [10]. Considerable inter and intra-laboratory variability occurs in Lyme disease testing necessitating a separate evaluation of IgM and IgG; see Ma et al. and Aguero-Rosenfeld whose studies also demonstrated excellent sensitivity and specificity for IgM and IgG using any two of the following bands:23-25 kDa (Osp C), 31 kDa (Osp A), 34 kDa (Osp B), 39 kDa and 41 kDa [11,12]. The conservative CDC/ASPHLD consensus criteria for Western blot (CDC, 1995) required 5 of 10 antibody bands for IgG positivity, performing both IgG and IgM procedures, and testing of paired acute and convalescent-phase samples [13]. An IgM antibody response to *B. burgdorferi* may be detected in 60-70% of the patients within two to four weeks after infection. This may be followed by a specific IgG response which may remain detectable for a few months to a few years in some cases [10]. The above procedures and considerations have been observed in the Lyme disease testing reported by the Virginia facility.

RESULTS

Fecal samples and blood smears from a total of 456 patients from the Virginia facility were tested for parasites between April, 2008 and January, 2012 at the Parasitology Center, Inc. in Arizona. The most prominent gastrointestinal symptoms caused by *C. parvum* infections included abdominal pains and cramps, bloating, diarrhea, and gas.

Extraintestinal symptoms included fatigue, brain fog, and neurological symptoms (blurred vision, depression, lack of concentration, loss of motor skills, memory loss, itchy skin, and tingling sensations [14]. Symptoms of Lyme disease experienced by our subject patients were comparable to those described by Singleton and Steere et al. [15,16]. Our present results show that of our total Lyme population of 215 patients, 77 (36%) were infected with *C. parvum* (Figure 1). By the same token, of our 161 *C. parvum* cases, 77 (48%) were infected with Lyme disease (Figure 2).

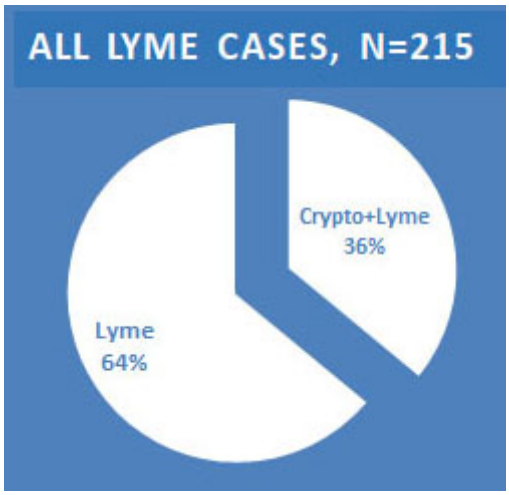

Figure 1: The prevalence of concurrent Lyme disease and *Cryptosporidium parvum* infections in 215 male and female Lyme disease patients.

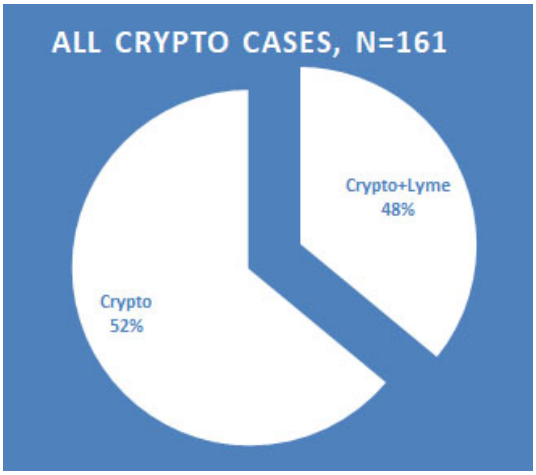

Figure 2: The prevalence of concurrent Lyme disease and *Cryptosporidium parvum* infections in 161 male and female *Cryptosporidium* cases.

These relationships were most demonstrable for the 46-60 years age group: of 70 cases of Lyme, 34 (49%) were infected with *C. parvum* and of 58 cases of *C. parvum* infections in the same age group, 34 (59%) were infected with Lyme. Single and cross infections in this, and other age groups are demonstrated for males and females (Figures 3 and 4).

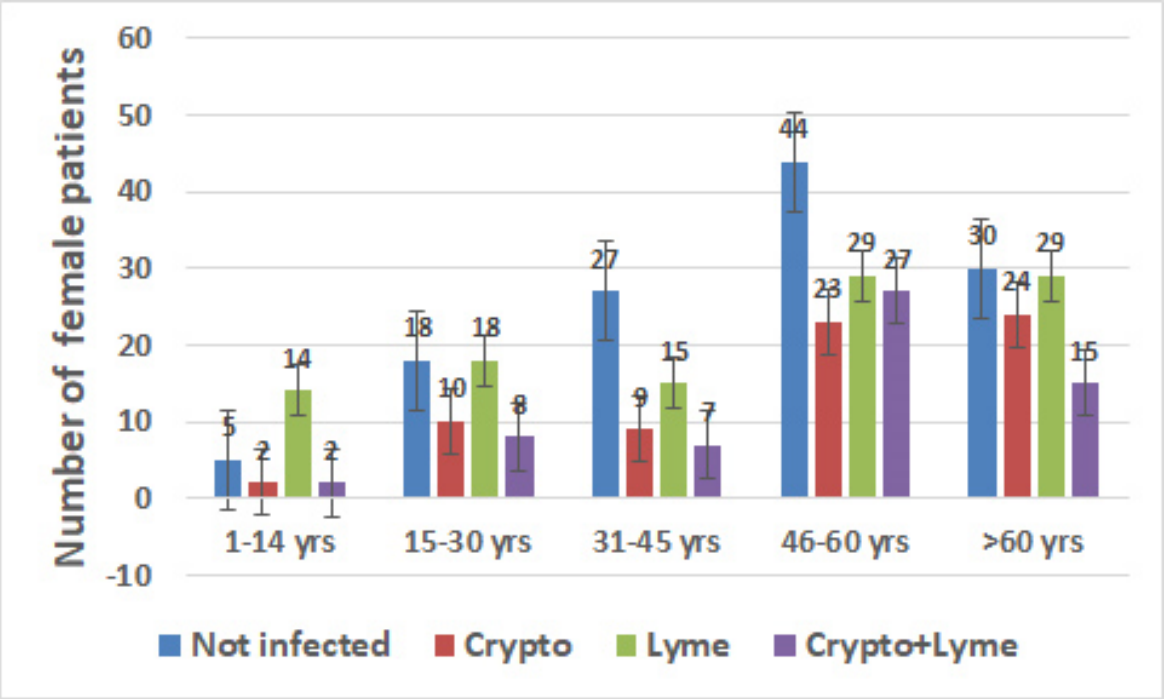

Figure 3: The number of 356 female patients infected singly and concurrently with Lyme disease and *Cryptosporidium parvum* by age. Margins of error are marked.

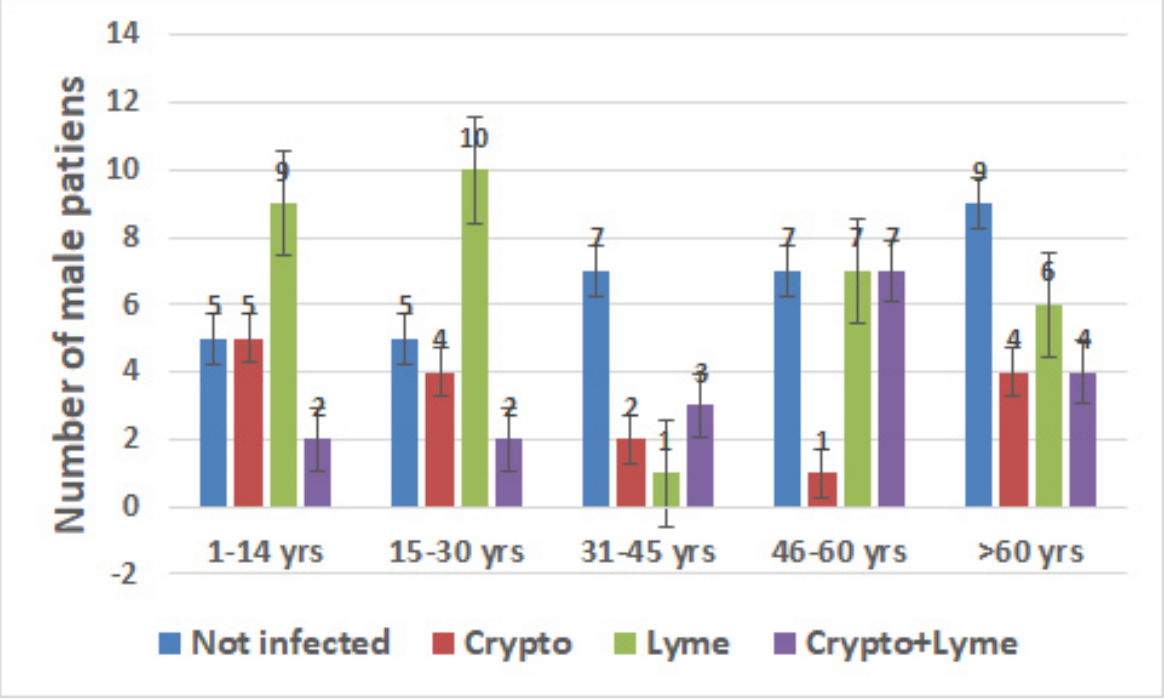

Figure 4: The number of 100 male patients infected singly and concurrently with Lyme disease and *Cryptosporidium parvum* by age. Margins of error are marked.

DISCUSSION

There is clearly a numerical association between Lyme disease cases and higher prevalence rates of *C. parvum* infections, especially in the upper age groups. Our present results show that of our total Lyme population of 215 patients, 77 (36%) were infected with *C. parvum*. Of our 161 *C. parvum* cases, 77 (48%) were infected with Lyme disease. This relationship is more demonstrable in females where a larger sample size was available (Figures 1-4). Cross sectional studies of the prevalence of *C.*

parvum infections in the USA run at our Arizona PCI showed a prevalence rate between 2 and 13% (usually 4-6) [9,14,17]. Prevalence rates were considerably lower elsewhere in the USA (0.6-4.3%) and in Europe (1-2%); see for example Current and Garcia and Garcia, and other references quoted therein [18,19]. The question is, considering the different epidemiology of these two infections, would C. *parvum* (an intestinal parasite) in Lyme (a tick-borne disease) patients qualify as co-infection [20-31].

CONCLUSION

A relationship between C. *parvum* and HIV infections especially among men who have sex with men is more understandable and well known; see for example Adesiji et al. as both conditions have a common epidemiological denominator. This is not the case between Lyme disease and *Cryptosporidium* infections.

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