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Lyme borreliosis: A neglected zoonosis in Egypt

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ABSTRACT

Borrelia burgdorferi, the causal organism of Lyme borreliosis. In Egypt, available data about the occurrence of Lyme disease are scarce and no structured studies documented the presence of Lyme borreliosis in Egyptian animals and tick reservoirs verifying its zoonotic evidence. Besides, no successful trials to isolate *B. burgdorferi* from clinical samples have occurred. This study was conducted to investigate *B. burgdorferi* infection as an emerging zoonosis neglected in Egypt. A total number of 92 animals, tick and human companion specimens were collected and subjected for culture, PCR and/or serodetection. *B. burgdorferi* has been detected and isolated from Egyptian animal breeds. We also detected the presence of outer surface protein A gene of *B. burgdorferi* by PCR as well as anti-*B. burgdorferi* IgM by ELISA in human contacts who were suffering from fever of unknown origin. This report represents the first systematic study on animals associated with patients suffering from febrile illness to confirm the emerging of such neglected zoonosis in Egypt.

1. Introduction

Borrelia burgdorferi, the etiologic agent of Lyme borreliosis, is transmitted to a mammalian host by any of several species of *Ixodes* ticks. The disease usually begins with a characteristic skin lesion, erythema migrans at the site of the tick bite. After several days or weeks, the spirochetes typically spread hematogenously, and patients may develop early-disseminated disease with dermatologic, cardiac, neurologic, and rheumatologic involvement. Late-stage disease can present chiefly as arthritis and/or neurological impairment (Steere et al., 1977).

The principal vector of *B. burgdorferi* are ticks of *Ixodes ricinus* complex including *Ixodes scapularis* and *Ixodes pacificus* in the US, *I. ricinus* in Europe and *Ixodes persulcatus* in Asia. Deer and small rodents especially mice, constitute main reservoir hosts (Lebech, 2002). *B. burgdorferi* was first isolated in culture in 1982 from *I. scapularis* tick vector recovered from Long Island, New York (Baron and Finegold, 1990; Burgdorfer et al., 1982).

In Egypt, available data about the incidence and prevalence of Lyme disease are scarce (Haberberger et al., 1989). Only two recent studies have pointed out the molecular evidence of *B. burgdorferi* in ticks (Adham et al., 2010) and serological screening of Lyme disease in children (Hammoud et al., 1995). Furthermore, no structured studies that documented the presence of Lyme borreliosis in Egyptian animals and tick reservoirs infesting such animals and then verifying its zoonotic evidence could be found. Besides, no successful trials to isolate *B. burgdorferi* from clinical samples in Egypt have occurred. Therefore, the objective of the current study was to investigate the occurrence of *B. burgdorferi* among bovine and canine species and their tick reservoirs in Egypt, as well as to document its zoonotic activity by detection of the pathogen among diseased human contacts. This study was occurred by isolation of the pathogen in a specific medium, BSK-H and detection of its DNA by PCR from animals, tick reservoirs and human contacts. Also a specific IgM-ELISA was done on febrile sera of companion humans.

2. Methods

The study was performed at the Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt under the recommendations of Institutional Animal Care and Use Committee Guidebook.

2.1. Sampling

From Summer 2008 to Autumn 2009, a total number of 77 non-human samples (51 blood and 26 ticks) have been collected from 51 animals (25 cattle and 26 dogs). The animals were feverish and showed off-food and loss of body weight. All the animals were distributed in Fayoum and Beni-Suif governorates. Moreover, 15 blood samples were withdrawn from 15 persons were in intimate

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Table 1
Culture, PCR and ELISA results.

Type of samples	Total number	Number of positive culture (%)	Number of positive PCR (%)	Number of positive ELISA (%)
Ticks from dogs	12	0	7(58.3)	NA
Ticks from cattle	14	0	3(21.4)	NA
Blood from dogs	26	0	6(23)	NA
Blood from cattle	25	1 (4%)	4(16)	NA
Blood from human	15	0	4(26.6)	15 (100)
Grand total	92	1 (1.08%)	24(26)	

contact to those animals and they suffered from acute febrile illness (AFI). A case of AFI was defined as any individual with a history of fever (temperature ≥38 °C) for 3 days or more. Exclusion criteria included the presence of obvious clinical signs for certain diseases such as diarrhea, pneumonia, typhoid fever, brucellosis, or established fever of unknown origin (Ismail et al., 2006). The people in such study were not hospitalized in a certain healthcare facility and the sporadic human cases were all farmers lived in rural areas. Animal blood specimens were subjected for culture and PCR, while human blood was subjected for culture, PCR and sera were separated for ELISA. All samples were taken prior any antibiotic administration.

2.2. Tick speciation

Ticks from bovine were *Hyalomma anatolicum excavatum*, while those from canine were *Rhipicephalus sanguineus*.

2.3. Culture

The growth of *B. burgdorferi* was observed by inoculation of the specimens (one drop of whole blood or gut contents of the collected ticks) into 2–3 tubes containing BSK-H medium (Sigma Co., St. Louis, MO). All the inoculated tubes were incubated at 33 °C for up to 8 weeks under a microaerophilic condition. Cultures were examined weekly under the dark-field microscope to observe the characteristic morphology and motility of *B. burgdorferi* indicating the viability of the pathogen (Pollack et al., 1993).

2.4. DNA extraction from whole blood

Total genomic DNA was extracted from whole blood using QIAamp® DNA blood Mini kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

2.5. DNA extraction from ticks

Briefly, tick specimens were cleaned by sonication for 3–5 min in ethanol and then washed twice in sterile distilled water. Afterwards, individual tick specimens were divided into pieces, placed in a microcentrifuge tube filled with 180 μL lysing buffer solution supplied in the Kit and then homogenized with a sterile tissue grinder. The homogenate was centrifuged at room temperature and the supernatant was further processed and DNA was extracted by using QIAamp® DNA Mini kit (Qiagen, Valencia, CA) in accordance with manufacturer's instructions.

2.6. DNA amplification by polymerase chain reaction

DNA extracted from blood and tick specimens as well as the positive control, *B. burgdorferi* B31 (ATCC# 35210) and negative control, *Leptospira interrogans* serovar Icterohaemorrhagiae (NVSL# 17) were used as template for PCR amplification. A specific novel primer sequence targeted *OspA* gene of *B. burgdorferi* sensu lato was designed which is F: atc gga tcc cct ggg gaa atg aaa gtt ctt g and R: cct gcc cat ggt act agt gtt ttg cca tc. All PCR reagents and Taq polymerase were obtained and used as recommended by the supplier (Sigma Co., St. Louis, MO). Briefly, a total of 0.2 μmol of the appropriate primers and various amounts of template DNA were used in each 50 μL reaction mixture. The PCR amplification was performed with a programmable thermocycler for 35 cycles with denaturation at 93 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min. Amplified DNA products were electrophoresed in 2% agarose gels in Tris–Borate–EDTA (TBE) buffer and visualized under ultraviolet (UV) light after staining with ethidium bromide. DNA marker (100–1000 bp) was obtained from Stratagen Co., USA, Cat. No. 201115. It was used for detection of the expected size of the target genes under test (230 bp).

2.7. ELISA

Human sera were tested for the presence of IgM class antibodies against *B. burgdorferi* using (ab108711—anti-*B. burgdorferi* IgM Human ELISA Kit, abcam, UK). The sensitivity of the test is 93% and the specificity is 98.8%

2.8. Sequence alignments and phylogenetic analysis of the recovered isolate

After purification with a QIAquick PCR purification kit, the nucleotide sequences of *B. burgdorferi* isolate was sequenced using an ABI Prism 310 DNA sequencer (Applied Biosystems, Foster City, CA). Nucleotide Sequence alignment is carried out using BioEdit software and CLC Sequence Viewer 6.3 using CLUSTAL W method which is a program designed by Thompson et al. (1994), CLUSTAL W is a widely used system for aligning any number of homologous nucleotide or protein sequences.

The Phylogenetic relationship was performed using neighbor–joining (N–J) method (CLC Sequence Viewer 6.3, 2009) software. The distance of estimation based on Saitou and Nei (1987) method by using (CLC Sequence Viewer 6.3) software. The distance method employs the number of changes between each pair in a group of sequences to produce a phylogenetic tree of the group. The sequence pairs that have the smallest number of sequence changes between them are termed "neighbors." On a tree, these sequences share a node or common ancestor position and are each joined to that node by a branch. The goal of distance methods is to identify a tree that positions the neighbors correctly and that also has branch lengths which reproduce the original data as closely as possible. Finding the closest neighbors among a group of sequences by the distance method is often the first step in producing a multiple sequence alignment. The distance method and a collection of programs will produce both an alignment and tree of a set of protein sequences (Lebech, 2002). The program CLUSTAL W uses the neighbor–joining distance method as a guide to multiple sequence alignments, the conducted trees were made by CLC Sequence Viewer 6.3 and viewed by MEGA4 software.