

# Occurrence of Canine Borreliosis in Egyptian Dogs: A Public Health Threat

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## Abstract

**Background:** Lyme borreliosis is a worldwide zoonosis caused by the spirochete *Borrelia burgdorferi*, which is transmitted by a tick bite, primarily from *Ixodes scapularis* and *Ixodes pacificus*. In Egypt, few articles reported the existence of Lyme disease in humans and animals. The aim of the current study is to investigate the presence of *B. burgdorferi* in dogs, ticks and contact human cases in Egypt. **Methods and findings:** A total of 100 samples of dogs (70), contact human (15) and ticks (15) were collected and subjected for culture, PCR and/or IgM ELISA. 50/70 (71.4%) of canine sera were sero-reactive, while 3/15 (20%) in human cases were reactive. *ospA* gene was detected in the blood of 13 out of 70 dogs (18.5%). 6/15 (4%) ticks (*Rhipicephalus sanguineus*) that collected from dogs contained the *ospA* gene, while none of human blood samples was positive by PCR. None of the investigated samples give positive results for *B. burgdorferi* by culture. **Conclusions:** This study confirms the occurrence of *B. burgdorferi* infection in Egyptian dogs in which the only identified tick species is *Rhipicephalus sanguineus*.

**Keywords:** Lyme borreliosis; Dogs; Egypt

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## Introduction

Lyme disease, an *Ixodes* spp. tick-associated zoonosis caused by *Borrelia burgdorferi*, causes significant morbidity in humans and dogs [1]. Infected dogs may develop fever, anorexia, fatigue, renal failure and most commonly limb and joint disorders [2]. Several animal species can be infected by *B. burgdorferi* spirochete, including rodents, deer, dogs, cats, cows, horses, reptiles and birds, and since tick species could infest such animals [3], however, dogs are considered the most important reservoir for ticks in the home environment [4]. In Egypt, available data about the incidence and prevalence of Lyme disease are scarce [5]. Few old and recent studies have pointed out the evidence of occurrence of *B. burgdorferi* in animals, ticks and humans [6-8]. The aim of the current study is to detect *B. burgdorferi* in dogs, tick reservoirs and human contacts by isolation of the pathogen in a specific medium, BSK-H, detection of its DNA by PCR and investigate the specific IgM-ELISA in sera of dogs and companion humans.

## Methods

### Sampling

A total of 100 samples of (70 blood samples from stray dogs hosted in animal shelter, 15 blood samples from human contacts

working in the shelter who have accepted to let us draw blood samples from them relying on ethically approved consent forms, 15 hard ticks infesting some dogs from which blood samples were collected) during the period from 2012 till 2013. Dog and human blood were subjected for culture and PCR. Sera from the same samples were separated and preserved at -20° C for serology. Ticks from canine were identified as *Rhipicephalus sanguineus*, and subjected to culture and PCR. All samples were taken prior any antibiotic administration

### Culture

One drop of whole blood or gut contents of the collected ticks was inoculated 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 being examined weekly under the dark-field microscope to observe the characteristic morphology and motility of *B. burgdorferi* indicating the viability of the pathogen [9]. *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.

### DNA extraction from ticks

Briefly, tick specimens were cleaned by sonication for 3-5 minutes 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.

### 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 primer sequence targeted *OspA* gene of *B. burgdorferi* sensu lato was used which was 5'-AATGTTAGCAGCCTTGACGAGAA-3' and 5'-GATCGTACTTGCCGTCTTGT-3' [10]. 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 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. 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 at 309 bp (Figure 1).

### ELISA

Sera from dogs were tested for the presence of IgM class antibodies against *B. burgdorferi* using (DBGM96-Dog EIA *Borrelia* IgM, TestLine, Czech Republic), while human sera were tested using (ab108711-Anti-*Borrelia burgdorferi* IgM Human ELISA Kit, abcam, UK).

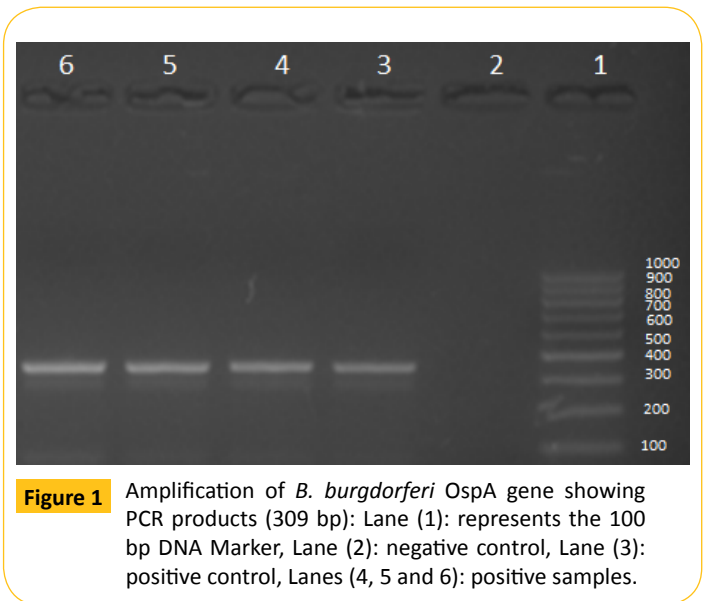
## Results

### Culture result

None of the dogs, ticks or human samples showed recovery for *B. burgdorferi*.

### PCR result

Out of 15 collected ticks from dog origin, 6 were positive for amplification of *ospA* gene (4%), while 13 (including those



**Figure 1** Amplification of *B. burgdorferi* *OspA* gene showing PCR products (309 bp): Lane (1): represents the 100 bp DNA Marker, Lane (2): negative control, Lane (3): positive control, Lanes (4, 5 and 6): positive samples.

infested with positive ticks) out of 70 dogs were positive (18.5%). On the other hand, none of the blood samples collected from persons in close contact to such dogs was positive.

### ELISA result

50 out of 70 canine sera (71.4%) were positive by ELISA, including those 13 which are positive by PCR, while only 3 out of 15 human serum samples (20%) were reactive.

## Discussion

Lyme borreliosis is a worldwide zoonotic disease caused by the spirochete *Borrelia burgdorferi*, which is transmitted by a tick bite, primarily from *Ixodes scapularis* and *Ixodes pacificus*. It is characterized by multi-systemic disorders.

In Egypt, Lyme disease has been recently reported in animals and transmitted by tick species out of family *Ixodidae*, *Rhipicephalus sanguineus* [8]. This finding was confirmed in the current study as we could detect the specific *ospA* gene of *B. burgdorferi* in 4% of the collected ticks.

Herein the current study, *B. burgdorferi* could not be recovered from the given samples when cultured in BSK-H medium. Although culture is the gold standard diagnostic test, isolation of *B. burgdorferi* is labor-intensive, more expensive, and much slower, requiring up to 12 weeks of incubation before being considered negative [7,11] and this makes culturing as a lower feasible method.

Our results showed that 50 out of 70 serum samples (71.4%) of the collected canine sera were sero-reactive and contained anti-borrelia IgM. Anti-borrelia IgM are produced one week post-infection, and remain elevated for two months [12,13]. From those 50 sero-reactive dogs, *B. burgdorferi* DNA was detected in 13 animals and also in 6 *Rhipicephalus sanguineus* ticks that have been infesting such dogs. Moreover, 20% of human contacts were sero-reactive to *B. burgdorferi*.

The current study gives an evident to the existence of *B. burgdorferi* in stray dogs in which *Rhipicephalus sanguineus* is

the only tick species that has been identified so far. Although this kind of ticks has not been considered the main vector for *B. burgdorferi*, the present investigation and a previous one [8] suggest that *Rhipicephalus sanguineus* is incriminated in the epidemiology of *B. burgdorferi* infection.

## Conclusions

This information is useful and may be of epidemiological value in monitoring the circulation of such pathogen, determining

potential zoonotic risk and implementing prevention and intervention measures like vaccination and control programs which are not applied in Egypt.

It is also important to increase awareness about Lyme borreliosis among veterinarians and physicians in Egypt and to strengthen laboratory capacity for its diagnosis in veterinary and infectious hospitals.

**Competing Interests:** Nothing to declare

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