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Research Article

Seroprevalence for Evidence Detection of Borrelia Infection in Dogs

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Abstract

Stray dog received no treatment and or hygienic measures so usually highly infested with Ticks (3). Tick transmitting most common parasitic, viral as well as bacterial diseases that causing human infection apparently called zoonotic diseases (7). dog admitted to clinic and hospital with history of emiction and heavy infested with ticks, most researchers did not expect that causative agent is bacterial diseases and all pay attention that is blood parasite and viral infection. our cross-sectional study considered first study on borrelia detection in dogs at Sharqia province, Egypt.

Keywords: Dog; Borrelia; Tick; Serum; Elisa; Zoontic

Introduction

Borreliosis is a bacterial diseases caused by Borrelia burgdorferi (3) an etiological agent of Lyme disease in man. Lyme disease emerged during the mid-1970s, when an unusual form of juvenile arthritis was observed in Lyme, Connecticut, USA (1) The discovery of the new tick-borne pathogen sparked off a flurry of research in different aspects of veterinary and human medicine, molecular biology, arthropods especially ticks, biology of reservoir hosts and their ecology (1). After the initial isolation of the spirochete from the midgut diverticulum of the tick, *Ixodes dammini* from Shelter Island, New York (2)

Lyme disease is the most prevalent tick-borne zoonotic disease of Europe, North America and the Far-East such as Japan, Russia and China (6). These borrelial organism was first reported in ticks (2,3), subsequently reported in humans (7), rodents (8) and also in some wild and domestic animals (9). Emergence of the disease in humans may be related to the large numbers of reservoir hosts (deer), reforestation of farmlands and presence of ticks (10) . The spirochaetes may be pathogenic to both animals and humans (11).

Clinical signs Infection in dogs is associated with fever, arthritis and renal disorder, but in most cases dogs do not show the clinical signs (12). The significance of Lyme disease as a public and veterinary health problem in addition to the unique association of Borrelia and Ixodid ticks necessitated investigations of the

relationship of this spirochaete with some of its tick vectors and vertebrate hosts in the past (14).

Material & Methods

Our study was designed to determine the prevalence of *Borrelia* species in dogs admitted to clinic and animal hospital at faculty of veterinary medicine , Zaazig university, Egypt in the study area using serological techniques (Snap 4Dx). The dogs in general were chosen due to their close cohabitation with humans as pet animals. Dogs were preferred because they provide an indication of disease agents in an area due to the absence of vaccination and/or treatment. The study was carried out between March 2017 and January 2018.

The units of analysis considered in the study were the individual dogs sampled from different locations in and around Sharqia province. The sample size of the cross-sectional study was calculated using OpenEpi © version 2.3 (OpenEpi, Atlanta, GA, US).

Sample Collection

Out of 117 blood samples73 of dog infested with ticks 55out of them showed signs of infection and 18 of them apparently healthy and 44 samples were collected from dog free from ticks from different locations at Sharkia province for detection of *Borrelia* species. About 4ml of blood collected from the jugular vein were transferred to a labeled plain vacutainer collection tubes. Ticks were also collected from dogs on examination. The ticks were collected from around the ear, neck, back, ventral abdomen, medial thighs and as well as the inter-digital spaces. They were collected with gloved hands, some with forceps. On average, 3-9 ticks were collected per dog and placed into properly labeled and sterile microcentrifuge tubes for further processing. Age, breed and gender of the dogs from which blood and ticks were collected were recorded.

Upon reaching the laboratory, the blood samples were centrifuged at 12,000 rpm for 5 m to obtain the serum. After transferring the serum into properly labeled 2ml microcentrifuge tubes, it was then stored at -80^{0}C pending serological analysis. The collected ticks were placed in plastic vials or tubes in 500-700µl of 95% ethanol and then stored at normal refrigeration temperature for further analysis

Detection of Borrelia Burgdorferi Antibodies in Canine Serum

A total of 117 serum samples collected were tested using ELISA serological kit devices (SNAP 4Dx, IDEXX Laboratory, Westbrook, Maine, USA). The SNAP 4Dx was designed to detect antibodies against Heart worm (Dirofilaria}, Anaplasma, and Ehrlichia apart from antibodies against Borrelia species. The sera stored at -80°C were removed and allowed to thaw then centrifuged at 6000 rpm for thirty seconds to one minute before subjecting to the test. The test devices together with the sera could equilibrate at room temperature for 30 minutes before running the test according to the manufacturer's instructions. The test procedure was as follows; after allowing the sample to equilibrate to temperature of 18-25^{0}c (room temperature), three drops of the sample were then dropped into the new sample tube using the transfer pipette followed by four drops of conjugate (reagent). The sample tube was then capped and mixed thoroughly by inverting three to five times. Then the test device was placed on a horizontal surface and the entire sample tube contents were poured into the sample well allowing it to flow towards the result window reaching the activation circle. When the first color appeared in the activation circle, the activator was pushed firmly until it was flushed with device body. The results were recorded at eight minutes.

Data Analysis

The data generated during this study were recorded and stored in a spreadsheet of Microsoft Excel® 2016 for Windows (Microsoft Corporation). A frequency table was used to calculate the seroprevalence based on age, breed and gender of the stray dogs at the estimated

confidence interval of 95%. The data were then subjected to statistical analysis using statistical package IBM SPSS statistics (version 20.0, SPSS Inc., Chicago, IL, USA). A Chi-square test was used to establish the association among/between proportions of the categories at a significance level of α=0.05.

Seroolgical Detection of Borrelia Burgdorferi Antibodies and Other Haemopathogens in Stray Dogs in Sharqia Province

32 (27.4%)from 117 dog samples tested for antibodies to Borrelia burgdorferi sera were found to be positive for antibodies to the Borrelia species (Table 1). Based on data analysis for gender, breed and age detected 13 males (11.1%) and 19 female (16.3%) were positive; mostly young infected 24 (20.5%) while 8 adults (6.8%)and mostly native breeds 63(53.8%)infected while other strain and imported 54(46.2%). That explain significant changes (Table 1). Among the total sampled population, 73 (62.4%) of the dogs examined were found to be infested with Ixodid ticks of the species Rhipicephalus sanguineus.

The kit for test with wide ability to detect Anaplasma phagocytophilum, Anaplasma platys, Ehrlichia canis, Ehrlichia ewingii and Dirofilaria immitis in addition to Borrelia burgdorferi, allowing us to also detect and report other major tick-borne pathogens of dogs. The results showed a seroprevalence of 25.5% for Anaplasma, 20.2% for Ehrlichia and 8% for Dirofilaria immitis (Table 2). All the dogs from which Borrelia burgdorferi was detected have co-infection with Anaplasma and two had Anaplasma and Ehrlichia species and were tick infested, none of them were positive for Dirofilaria immitis (Table 2).

The limitations of the study cannot tell whether the dogs may not have seroconverted yet, cannot distinguish whether the positives are as a result of exposure or active infection and sometime cross reactivity may occur with either member of the spirochaete family or we cannot specify which strain of Borrelia is present. It may be Borrelia but not necessarily be B. burgdorferi strain.

Table (1): Age succepitability, breed and gender of Borrelia burgdorferi in sera of dogs

S/N	Variables	Categories	Negative (%)	Positive (%)	Chi-square
1	Age	Adult	84(109)	6.8. (8)	0.145
		Young	79.5 (93)	20.5 (24)	-
2	Gender	Female	83.7 (98)	16.3 (19)	0.05
		Male	88.8 (104)	11.1 (13)	-
3	Breed	Native	64 (54)	53.8 (63)	0.137
		Imported	53.8 (63)	46.2 (54)	-

Table (2): Seroprevalence of co-infection of Borrelia burgdorferi in dog with Anaplasma, Ehrlichia and Dirofilaria immitis.

S/N	Species	Negative	Positive	Seroprevalence (%)	Co-infection *Borrelia* (%)	Dogs co-infection
1	*Borrelia burgdoferi*	85	32	27.4	9	23
2	*Anaplasma*	99	18	15.4	1.4 (3)	6
3	*Ehrlichia*	106	11	9.4	1.7(5)	8
4	*Dirofilaria immitis*	112	5	4.27	-	-

The sensitivity and specificity of Snap 4Dx test were almost 95-100% even when testing other animals as compared with Immuno fluorescence Assay (20). Though this device is designed to detect the antibodies to the above listed pathogens in dogs and cats. With this ELISA serological test kit (SNAP 4Dx), we were able to detect and determine the seroprevalence of Borrelia burgdorferi and other major arthropod-borne pathogens of dogs from serum samples obtained from dogs at Zagazig , sahrqia The high level of seroprevalence of B. burgdorferi in stray dogs in zagazig, despite a report of the pathogen in humans and the nature of the humidity ,warm weather that are conducive for the persistence and propagation of arthropod vectors in the area. Thus may increase the risk of transmission of some arthropod-borne diseases especially when in local breeds or stray animals (17). The high prevalence of Borrelia species may possibly related to the fact that the organism is the most common pathogens that cause disease in dogs in the area. While mixed significan seroprevalence of other major canine arthropod-borne pathogens (Anaplasma, Ehrlichia and Dirofilaria immitis) have been reported in dogs in sharqia (21) and in areas where such disease pathogens are endemic (22). Co-infection with two and even three pathogens was observed in both the three positive B. burgdorferi stray dogs (Table 2). This may be a dog already infected with one pathogen is likely to be more susceptible to infection with another. Konto et al. (21) found high co-infection rates among canine arthropod-borne haemopathogens like Anaplasma and Ehrlichia, which was commonly found in dogs in sharqia province. The apparent low prevalence (1.6%) of B. burgdorferi may be attributed to the limited distribution of this agent in the area (23). However, the low prevalence of Borrelia, a major tick-borne pathogen was unexpected considering the seroprevalence reported in humans at university Hospital (18). Additionally, the health implication may actually be higher as some infected individuals go undiagnosed (22). In Lyme disease, infected individual required more than 1-2 weeks for IgM to appear upon infection and 4-6 weeks for IgG to develop and persist to several years (17). That may be the reason why Borrelia species were detected among older dogs, as older stray dogs may be expected to have higher prevalence of disease than their younger counterparts due to the likelihood of contact to reservoir host or exposure to tick vector, as antibodies to this organism can persist for a longer period in infected individual (24). Although, seasonal and regional factors may play a greater role and affected young age in terms of infection with Borrelia species in most area. Therefore, pet owners should practice ectoparasitic control and be more vigilant when their animals are outdoors especially in an endemic area.

Conclusion

Dog at Sharqia province were infested with significant level of Borrelia burgdorferi 27.4% (32/117). With high level in young age than adult. May indicate diseases may have role in transplacental transmission especially related with high level in female than male. Awareness of arthropod-borne pathogens in dogs warrants instituting control measures not only against the pathogens but also the stray dog population that harbor these pathogens in villages . Furthermore, a more extensive prevalence study should be carried out with a larger sample size in order to determine the geographical distribution of this pathogen and its vector in the study area. Further study needed for better understanding of the epidemiology of the disease.

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