Journal of Applied Veterinary Sciences, 8 (2): 30-34 (April, 2023)
ISSN: Online: 2090-3308, Print: 1687-4072
Journal homepage : https://javs.journals.ekb.eg

Seroprevalence and Evidence of *Borrelia burgdorferi* Infection in Stray Dogs at AS- Sharqia Province, Egypt

Amira. S.H. Hassenin[1]* and Uzema Farid Durrani[2]
[1]Assistant professor, Faculty of Veterinary Medicine, Zagazig University, Egypt
[2]Associate Professor Pet Centre, University of Veterinary and Animal Sciences, Lahore, Pakistan
***Corresponding Author:** Amira. S.H. Hassenin, **E-Mail:** amm_microhass@yahoo.com

ABSTRACT

The stray dog population has no access to veterinary care and most of them are highly infested with ticks. Ticks can transmit various parasitic, viral and bacterial diseases to their host dogs as well as can also lead to various zoonotic infections in humans exposed to them as Lyme disease and skin rashes. Incidence of tick-transmitted bacterial infection Borreliosis is very high in tick infested dogs, especially in hot weather. The current cross-sectional study was carried out for the first time to detect the incidence of borrelia infection in tick-infested dogs in Sharqia province (Zagazig, Egypt) and revealed that female dogs (16.3%) were more heavily infected than male dogs (11.1%), while infected young dogs (20.5%) outnumbered infected adults (6.8%). The local strain of the dog's infection was (53.8%) compared to other imported strains. Controlling ticks is considered most important for the eradication of seasonal and zoonotic diseases and to prevent further human infections. ELISA was used as a chemical test with an immunological relationship for IgM in our cross-sectional studies for various serum samples. IgG titer level detection at different stages of disease and infection. The most common diseases transmitted by tick to human and animals in mixed infection with other Spirochaete, *Anaplasma* and *Ehrlichia* different stages of lifecycle of parasitic infestation and combined with secondary bacterial infection that is makes confusion for diagnosis and detection most common blood -Born parasites and their Zoonotic importance. Epidemiological studies in various research papers mostly do not consider using stereochemical investigation for Lyme diseases infection in human.

Original Article:
DOI:https://dx.doi.org/10.21608/javs.2023.178047.1198
Received : 30 November, 2022.
Accepted :16 February, 2023.
Published in April, 2023.

Keywords: *Borrelia burgdorferi*, Dog, Prevalence, Serum, Tick.

J. Appl. Vet. Sci., 8 (2): 30-34.

INTRODUCTION

Borreliosis is a bacterial disease caused by *Borrelia burgdorferi,* the etiological agent of Lyme disease in humans **(Alice *et al.*, 2021).** Cases of Lyme disease with an unknown form of juvenile arthritis first appeared in Connecticut, USA, in the mid-1970s **(Gray, 2002).** Tick-borne pathogen infection was discovered in new research aspects of molecular biology, veterinary and human medicine, and arthropods with the detection of their ecology and effect on their reservoir tick host **(Bhide, 2004).** Spirochaete was first isolated from the digestive system of a tick species called *Ixodes dammini* in a New York area named Shelter Island **(Burgdorfer and Hayes, 1990).**

Lyme disease is the most prevalent tick-borne zoonotic disease in Europe (nearly more than 62%), North America, and the Far East, such as Japan, Russia and China **(Steere, 1989)**. Borrelia was first ever reported in ticks (**Burgdorfer *et al.*, 1984; Johnson *et al.*, *1982)**, followed by its detection in humans **(Suksawat, 2002)**, rodents **(Praharaj, *et al* ., 2008)** and also in some wild and domestic animals white deer, dogs, rats, mice, chipmunks, grey squirrels, opossums, and raccoons **(Anderson, 1989)**. The emergence of this disease in humans might be linked with the large numbers of reservoir hosts (deer), reforestation of farmlands and presence of ticks **(Barbour, 1998)**. The spirochetes may be pathogenic to both animals and humans **(Gray *et al.*, 2009).**

Clinical signs of *borrelia* infection in dogs include fever, arthritis and a renal disorder but in most of the cases dogs appear symptomatic (**Sykes, 2014**). The significance of Lyme disease as a public and veterinary health problem in addition to the unique association of *Borrelia* and *Ixodes* ticks necessitated

investigations of the relationship of this spirochete with some of its tick vectors and vertebrate hosts in the past **(Lane, 1989).**

MATERIALS AND METHODS

Study design

The purpose of this study was to determine the prevalence of Borrelia burgdorferi species in stray dog populations from various villages in AS-Sharqia Province, as well as the most domesticated dog admitted to the veterinary clinic and animal hospital at the Faculty of Veterinary Medicine, Zagazig University. The study was based on using a serological diagnostic technique (Snap 4Dx). For this study, stray and domesticated dog species were selected as study animals due to their close cohabitation with humans as pet animals. Furthermore, stray dog populations were preferred because they could indicate the presence of disease agents in an area due to the absence of vaccination and/or treatment. The study was conducted from March 2017 to January 2018.

The units of analysis considered in the study were the individual dogs sampled from different locations in Sharqia Province, Egypt.

Sample collection

A total of 117 blood samples from dogs were collected randomly. Ticks infested 73 of the 117 dogs whose blood was sampled. Among tick-infested dogs, only 55 dogs showed signs of infection, while 18 dogs appeared healthy. Another 44 samples were collected from dogs free of ticks in different locations in Sharqia province for the detection of *Borrelia* species. About 4 mL of blood was collected from the jugular vein and transferred to a plainly labelled vacutainer collection tube. Ticks were also collected from dogs following a physical examination. Ticks were collected from around the ear, neck, back, ventral abdomen, medial thighs and inter-digital spaces. On average, 3–9 ticks were collected per dog and placed into properly labelled and sterile microcentrifuge tubes for further processing and further studies. Age, breed, and gender of the dogs whose blood and ticks were collected were recorded. This research was done according to the guidelines of the animal use and care committee at Zagazig University, Egypt with approval number ZU-IACUC/2/F/5/2018.

Upon reaching the laboratory, the blood samples were centrifuged at 12,000 rpm for 5 minutes to obtain the serum. After transferring the serum into properly labeled 2 ml microcentrifuge tubes, it was then stored at -80 ℃ pending serological analysis. Ticks were collected and placed in plastic vials or tubes with 500-700 l of 95% ethanol before being stored at room temperature (25 ℃) for further analysis.

Borrelia burgdorferi antibodies

A total of 117 serum samples were collected and tested using ELISA serological kit devices (SNAP 4Dx, USA). Apart from antibodies against Borrelia species, the SNAP 4Dx is designed to detect antibodies against heartworm (Dirofilaria), anaplasma, and Ehrlichia. The serum stored at -80°C were removed and allowed to thaw then centrifuged at 6000 rpm for 30–60 seconds. According to the manufacturer's instructions the devices and the serum could equilibrate at room temperature for 30 minutes before running the test. The test procedure was as follows; after allowing the sample to equilibrate to room temperature (18–25 ℃), 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 3-5 times. Then the test device was placed on a horizontal surface, and the entire contents of the sample tube were poured into the sample well allowing them to flow towards the result window and reach the activation circle.

When the first colour appeared in the activation circle, the activator was firmly pushed until it was flushed with the device body. The results were recorded at 8 minutes (**Praharaj*, et al.*, 2008**; **Gray *et al.*, 2009**). The diagnostic test kit used in this study was designed to detect *Anaplasma phagocytophilum*, *Anaplasma plays*, *Ehrlichia canis*, *Ehrlichia ewingii* and *Dirofilaria immitis* in addition to *Borrelia burgdorferi*, which facilitated the detection of other major tick-borne pathogens in dogs.

Data analysis

The sample size of the cross-sectional study was calculated using OpenEpi© version 2.3 (OpenEpi, Atlanta, GA, US). The information gathered during this study was entered into a Microsoft Excel® 2016 spreadsheet. A frequency table was used to calculate the seroprevalence based on the age, breed, and gender of the stray dogs at an estimated 95% confidence interval. The data were then statistically analyzed using the statistical package IBM SPSS Statistics (version 20). At a significance level of p 0.05, a Chi-square test was used to establish the association among or between proportions of the categories.

RESULTS

Serological diagnosis

Out of a total of 117 dog blood samples tested for antibodies to *Borrelia burgdorferi* serum, 32 (27.4%) samples were found to be positive for antibodies to the *Borrelia* species (Table 1). Based on data analysis for gender, breed and age positive cases included 13 male dogs (11.1%) and 19 female dogs

(16.3%); the number of infected young dogs was 24 (20.5%), while adult dogs were only 8 (6.8%). The local strain of the dog's infection was 53.8%, compared to other imported strains. Statistical analysis showed significant differences among the total sampled population ($p < 0.05$). A total of 73 (62.4%) dogs examined were found to be infested with *Ixodid* ticks of the species *Rhipicephalus sanguineus.*

Table 1: Age, breed, and gender-based incidence of *Borrelia burgdorferi* in serum of stray dogs

Variables	Categories	Negative (%)	Positive (%)	p-value
Age	Adult	93.2109)	6.8 (8)	0.145
	Young	79.5 (93)	20.5 (24)	-
Gender	Female	83.7 (98)	16.3 (19)	0.05
	Male	88.8 (104)	11.2 (13)	-
Breed	Native	46.2 (54)	53.8 (63)	0.137
	Imported	53.8 (63)	46.2 (54)	-

Findings of this study revealed sero-prevalence of other pathogens as for *Anaplasma*, 20.2% for *Ehrlichia* and 8% for *Dirofilaria immitis* (Table 2). All the dogs in which *Borrelia burgdorferi* was detected had co-infection with *Anaplasma* and 2 dogs were carrying *Anaplasma* and *Ehrlichia* species and were tick infested, none of them were positive for *Dirofilaria immitis* (Table 2).

Table 2: Seroprevalence of co-infection of *Borrelia burgdorferi* in stray dogs infected with *Anaplasma*, *Ehrlichia* and *Dirofilaria immitis*

Species	+ve	-ve	Seroprevalence (%)	Co-infection *Borrelia* (%)	Dogs' co-infection
Borrelia burgdoferi	85	32	27.4	9	23
Anaplasma	99	18	15.4	1.4 (3)	6
Ehrlichia	106	11	9.4	1.7(5)	8
Dirofilaria immitis	112	5	4.27	-	-

Immune fluorescence Assay test compared with ELISA test for their sensitivity and specificity of Snap 4Dx test were almost 90-100% for another animal Test **(Chandrashekar, *et al.*, 2008)**. This kit is designed to detect the antibodies to the above listed pathogens in dogs and cats. With this ELISA serological test kit (SNAP 4Dx), the seroprevalence of *Borrelia burgdorferi* and other major arthropod-borne pathogens in dogs was determined using serum samples obtained from stray dogs in Zagizag, Sharqia.

DISCUSSION

Our study showed a significant high level of Seroprevalence of *Borrelia 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. This may increase the risk of transmission of some arthropod-borne diseases, especially in local breeds and stray animals **(Lee *et al., 2016; Ge et al.*, 1998).** The high prevalence of *Borrelia* species may possibly related to the fact that the organism is one of the most common pathogens that cause disease in dogs in an area **(Kont *et al.*, 2015; Keysary *et al.*, 1996).** This result is lower compared to other cases of Borelliosis in dogs in Cairo, Egypt **(Rehab, *et al*., 2014).**

While mixed significant seroprevalence of other major canine arthropod-borne pathogens (*Anaplasma*, *Ehrlichia* and *Dirofilaria immitis*) have been reported in dogs in Europe **Burgdorfer and Hayes, 1990)** and in areas where such disease pathogens are endemic (**Shaw, *et al.*, 2001)**. Co-infection with two and even three pathogens was observed in three *Borrelia burgdorferi* positive stray dogs (Table 2). A dog already infected with one pathogen is likely to be more susceptible to infection with another. **Burgdorfer *et al.*, (1982)** found high co-infection rates among canine arthropod-borne haemopathogens such as *Anaplasma* and *Ehrlichia*, which were commonly found in dogs in Asia. The apparent low prevalence (1.6%) of *Borrelia burgdorferi* may be attributed to the limited distribution of this agent in the area **(Bhide, *et al* ., 2004)**. However, the low prevalence of *Borrelia*, a major tick-borne pathogen, was unexpected considering the seroprevalence reported in humans at the University Hospital Zigzag, Sharqia, Egypt **(Tay, *et al.*, 2002).** Additionally, the health implications may actually be higher as some infected individuals go undiagnosed **(Shaw, *et al.*, 2001)**.

Detection of IgG in the serum occurs within 4-6 weeks and persists with high titers for several years but IgM titers develop in the serum after infection With Lyme disease 1-2 weeks **(Lee, *et al* ., 2016 and Magnarelli, *et al.*, 1990)** 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 (**Divers, *et al.*, 2012)**.

The limitations of this study included the inability to determine whether the subject dogs had seroconverted. This cannot be established whether the positives cases are because of just 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 the *Borrelia burgdorferi* strain. Although, seasonal and regional factors may play a greater role and affect young age hosts in terms of infection with *Borrelia* species in most area. Therefore, dog owners should practise ectoparasitic control and be more vigilant when their animals are outdoors especially in an endemic area.

CONCLUSION

Dogs in Sharqia province were found to be infested with a significant level of *Borrelia burgdorferi* 27.4% (32/117) for the first time. Infection incidence was higher in young dogs than adults which may be an indication that transplacental transmission may be involved in infection spread especially related to higher levels in females than males. Awareness of arthropod-borne pathogens in dogs warrants instituting control measures not only against the pathogens but also the stray dog population that harbours these pathogens in villages. Furthermore, a more extensive prevalence study with a larger sample size should be carried out to determine the geographical distribution of this pathogen and its vector in the study area.

ACKNOWLEDGMENT

Author thanks to staff, scientists, and veterinary technician at Faculty of Veterinary Medicine, Zagazig University for their help and great effort to make this research success.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the research data and tools used with this study.

REFERENCES

ALICE, R., SOLÈNE, P.D., CÉLINE, C., PIERRE, T., CAROLE, E., 2021. Borreliosis and relapsing fever. *Rev Prat*. 2021 Dec;71(10):1113-1117. PMID: 35147372

ANDERSON, J.F. 1989. Epizootiology of borrelia in ixodes tick vectors and reservoir hosts. Review on Infectious Disease, 11(6): S1451-S1459. DOI: https://www.doi.org/10.1093/clinids/11.Supplement_6.S1451

BARBOUR, A. G. 1998. Fall and rise of Lyme disease and other *Ixodes* tick-borne infections in North America and Europe. British Medical Bulletin, 54: 647-658. DOI: 10.1093/oxfordjournals.bmb.a011717

BHIDE, M., CURLIK, J., TRAVNICEK, M., and LAZAR, P., 2004. Protein A/G dependent ELISA a promising diagnostic tool in Lyme disease seroprevalence in game animals and hunting dogs. Comparative immunology, microbiology and infectious diseases, 27**:** 191-199. DOI: 10.1016/j.cimid.2003.10.001

BURGDORFER, W., and HAYES, S.F., 1990. Vector/spirochete relationships in louseborne and tick-borne borrelioses with emphasis on Lyme disease. In: K.F. Harris (Editor), Advances in Disease Vector Research, Vol. VI. Springer, New York, NY. pp. 127-150. Available at: https://link.springer.com/chapter/10.1007/978-1-4612-3292-6_5

BURGDORFER, W., BARBOUR, A.G., HAYES, S.F., BENACH, J.L., GRUNWALDt, E., and DAVIS, J.P., 1982. Lyme disease-a tick-borne spirochetosis? Science, 216 (4552)**:** 1317-1319. DOI: https://www.doi.org/10.1126/science.7043737

CHANDRASHEKAR, R., DANILUK, D., MOFFITT, S., LORENTZEN, L., and WILLIAMS, J., 2008. Serologic Diagnosis of Equine Borreliosis: Evaluation of an In-Clinic Enzyme-Linked Immunosorbent Assay (SNAP 4Dx). International Journal of Applied Research in Veterinary Medicine, 6**:** 145-150. DOI: 10.1177/104063870802000309

DIVERS, T., GRICE, A., MOHAMMED, H., GLASER, A., and WAGNER, B., 2012. Changes in *Borrelia burgdorferi* ELISA antibody over time in both antibiotic treated and untreated horses. Acta Veterinaria Hungarica, 60**:** 421-429. DOI: 10.1556/AVet.2012.036

GRAY, J.S., KAHL, O., LANE, R.S., and STANEK, G., 2002. Lyme borreliosis: Biology, epidemiology, and control. CABI international., New York, p.347. Available at: https://b2n.ir/g86052

GE, Y., LI, C., CORUM, L., SLAUGHTER, C. A., and CHARON, N. W., 1998. Structure and expression of the FlaA periplasmic flagellar protein of *Borrelia burgdorferi*. Journal of bacteriology, 180**:** 2418-2425. DOI: 10.1128/JB.180.9.2418-2425.1998

GRAY, J., DAUTEL, H., ESTRADA-PEÑA, A., KAHL, O., and LINDGREN, E., 2009. Effects of climate change on ticks and tick-borne diseases in Europe. Interdisciplinary perspectives on infectious diseases . DOI: 10.1155/2009/593232

JOHNSON, R.C., SCHMID, G..P, HYDE, F.W., STEIGERWALT, A.G., and BRENNER, D.I., 1984. *Borrelia burgdorferi* sp. nov.: Etiologic agent of Lyme disease. International journal of systematic Bacteriology, 34(4): 496-497. DOI: https://www.doi.org/10.1099/00207713-34-4-496

KONTO, M., TUKUR, S., WATANABE, M., ABD-RANI, P., SHARMA, R., and FONG, L., 2015. Molecular prevalence of tick-borne hemopathogens of stray dogs in Klang Valley, Malaysia. PCVC6 and 27VAM. DOI: 10.3390/pathogens10050561

KEYSARY, A., WANER, T., ROSNER, M., WARNER, C. K., DAWSON, J. E., ZASS, BIGGIE, R. K. L., and HARRUS, S., 1996. The first isolation, *in vitro* propagation, and genetic characterization of Ehrlichia canis in Israel. Veterinary parasitology, 62**,** 331-340. DOI: https://doi.org/10.1016/0304-4017(95)00866-7

LEE, S.-H., YUN, S.-H., CHOI, E., PARK, Y.S.,. LEE, S.E., CHO, G.J., KWON, O.D., and KWAK, D., 2016. Serological Detection of *Borrelia burgdorferi* among Horses in Korea. The Korean Journal of Parasitology, 54**:** 97-101. DOI: 10.3347/kjp.2016.54.1.97

LANE, R., PIESMAN, J., and BURGDORFER, W., 1989. Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. Annual review of entomology, 36**:** 587-609. DOI: 10.1146/annurev.en.36.010191.003103

MAGNARELLI, L. A., ANDERSON, J. F., and SCHREIER, A.B., 1990. Persistance of antibodies to *Borrelia burgdorferi* in dogs in New York and Connecticut. Journal of American Veterinary Medical Association, 196**:** 1064-1068. Available at: https://pubmed.ncbi.nlm.nih.gov/2329074/

MINISTRY OF FEDERAL TERRITORIES, 2015. Greater Kuala Lumpur. In *Greater Kuala Lumpur/Klang Valley Unit Policy Planning Division*, editon. MFT. Availabe at: http.//app.kwpkb.gov.my/greaterklkv/overview/).

PRAHARAJ, A., JETLEY S., and KALGHATGI A., 2008. Seroprevalence of *Borrelia burgdorferi* in North Eastern India. Medical Journal of Armed Forces India, 64(1): 26-28.
DOI: 10.1016/S0377-1237(08)80140-2

REHAB A E., EL-ENBAAWY, M.I., AHMED SAMIR, 2014. Lyme borreliosis: A neglected zoonosis in Egypt. J. Actatropica1., 40:188-92. Doi: 10.1016/.2014.09.005.

SHAW, S. E., DAY, M. J., BIRTLES, R. J., and BREITSCHWERDT, E. B., 2001. Tick-borne infectious diseases of dogs. Trends in parasitology, 17**,** 74-80. DOI: 10.1016/s1471-4922(00)01856-0

STEERE, A.C. 1989. Lyme disease. New England Journal of Medicine, 321: 586-596. Available at: https://www.nejm.org/doi/full/10.1056/nejm198908313210906

SUKSAWAT, J., XUEJIE, Y., HANCOCK, S. I., HEGARTY, B. C., NILKUMHANG, P., and E. BREITSCHWERDT, B., 2001. Serologic and Molecular Evidence of Coinfection with Multiple Vector-Borne Pathogens in Dogs from Thailand. Journal of Veterinary Internal Medicine, 15**:** 453-462. DOI: 10.1892/0891-6640(2001)015<0453:sameoc>2.3.co;2

SYKES, J. E. 2014. *Canine and feline infectious diseases.*: Elsevier.BookAvailable at: https://www.elsevier.com › books

SHAW, S. E., J., DAY, M., BIRTLES, R. J., and BREITSCHWERDT, E. B., 2001. Tick-borne infectious diseases of dogs. Trends in parasitology, 17**:** 74-80. DOI: https://doi.org/10.1016/s1471-4922(00)01856-0

TAY, S., KAMALANATHAN, M., and ROHANI, M., 2002. *Borrelia burgdorferi* (strain B. afzelii) antibodies) among Malaysian blood donors and patients. Southeast Asian Journal of Tropical Medicine and Public Health, 33**:** 787-793

How to cite this article:

Amira. S.H. Hassenin and Uzema Farid Durrani, 2023. Seroprevalence and Evidence of *Borrelia burgdorferi* Infection in Stray Dogs at AS- Sharqia Province, Egypt. Journal of Applied Veterinary Sciences, 8 (2): 30 - 34. DOI: **https://dx.doi.org/10.21608/javs.2023.178047.1198**