

Original Research Article

INVESTIGATION OF CANINE EHRLICHIOSIS IN KHARTOUM STATE, SUDAN

ABSTRACT:

The study was extended for two years and covered different seasons of the years 2020, 2021 and 2022. Dogs in different localities of Khartoum State were investigated for presence of Canine Ehrlichiosis. A total of 599 dogs from different breeds and ages were investigated for the presence of the diseases. According to the results of *E. canis* Ab – Bio Note- Canine Rapid Test for the detection of canine Ehrlichiosis, the prevalence of Canine Ehrlichiosis in Khartoum State was 5.6%. Male and female dogs were susceptible for Ehrlichiosis. Dogs of all ages were susceptible for the disease. Dogs were more susceptible for the infestation during autumn. German shepherd breed was more susceptible for Ehrlichiosis. Most cases of Ehrlichiosis were treatable. This is the first study concerning investigation of Canine Ehrlichiosis in Khartoum State. Vaccination and Deworming programs against different diseases are recommended for dogs in Khartoum State.

Key words: Canine; Khartoum State; Ehrlichiosis; Protozoal infestation; Sudan

1. INTRODUCTION:

Canine Ehrlichiosis is a disease with high incidence among domestic dogs with extensive mortality [1] [2] [3] [4]. It's a tick-borne disease caused by Gram-negative bacteria, of the species *Ehrlichia canis*, mostly transmitted by *Rhipicephalus sanguineus* (brown dog tick) and *E. chaffeensis* and *E. ewingii*, which are predominantly transmitted by *Amblyomma Americanum* [5] [6]. This disease has no predilection for age or sex and compromises the host's organic systems in several different ways, with varying degrees of severity [7] [8] [2]. Systemic ehrlichiosis induces an acute-phase reaction from the infected animal's immune system. This response consists of a complex reaction that triggers an increase in the levels of fibrinogen and other proteins known as acute-phase proteins (APPs), which are believed to act towards restoring homeostasis and removing

Formatted: Font: Italic

Formatted: Font: Italic

Comment [h1]: Rewrite the sentences

the cause of disturbance [9] [10] [11]. APPs are Part of the nonspecific reaction of the innate immune system, which is the organism's first line of defense at the early stages of the disease. This physiological mechanism allows time for the organism to activate other more specific lines of defense, such as cellular and humoral immune responses [11]. Determination of APPs levels could contribute towards assessing the onset of diseases and be helpful in establishing exact diagnoses [11]. Tropical canine pancytopenia was originally described in Algeria in 1935 [12] and soon after in Africa, the Middle East, and the Orient [13]. CME is a multisystemic disease manifesting in acute, subclinical or chronic forms. The acute one is accompanied by fever, anorexia, lymphadenomegaly, epistaxis and poetechie [14]. In the subclinical form, dogs appear healthy despite thrombocytopenia and have the potential to remain persistent carriers [15]. This phase may last for years and some dogs will spontaneously eliminate the pathogen, while others will develop chronic form when bone marrow hypoplasia leads to pancytopenia resulting with bad prognosis for the outcome of the infection [16]. *E. canis* can infect all breeds of dogs but the German shepherd dog appears to be more susceptible, showing the more severe form of disease with a higher morbidity and mortality compared to other breeds [17].

This study was aiming at investigating Canine Ehrlichiosis in Khartoum State, Sudan.

Formatted: Space After: 10 pt, Adjust space between Latin and Asian text, Adjust space between Asian text and numbers, Tab stops: Not at 2.98"

2. MATERIALS AND METHODS:

2.1. Area of the study:

The study [was](#) conducted in Khartoum State during the years 2020, 2021 and 2022.

2.2. Samples:

2.2.1. Source of samples:

In this study which lasted for 2 years, 599 dogs of different ages and breeds were investigated for presence of Canine Ehrlichiosis infestation. **Blood** samples were collected during this investigation from all dogs.

Comment [h2]: Mention the criteria of blood sampling

2.2.2. Breeds of dogs:

The investigated dogs were belonged to German shepherd, Local, Lulu, cross, Perro de Presa Canario, Rottweiler, Royal black, Rood dog, Golden Retriever, Malinois, Griffon, Caucasian, Alabi, American bulldog, Saluki, Balboa and Husky breeds of dogs.

2.2.3. Ages of dogs:

The age of the investigated dogs was ranged between 8- 17 months.

2.2.4. Sampling Procedure:

A total of 599 blood samples were collected from 599 dogs in different Localities of Khartoum State. Blood samples were collected from cephalic vein using sterile syringes. Blood samples were transported in iceboxes to the Veterinary Laboratory in college of Veterinary Medicine University of Bahri.

2.3. Detection of Canine Ehrlichiosis:

2.3.1. *E. canis* Ab – Bio Note- Canine Rapid Test (Athens, Attica, Greece) [18].

2.3.1.1. Purpose: Detection of *E. canis* Antibodies.

2.3.1.2. Principle: Immunochromatographic assay.

2.3.1.3. Specimen: Whole blood.

2.3.1.4. Materials provided:

1. Ag Rapid *E. canis* test device.
2. Assay diluent bottle.
3. Anticoagulant tube.
4. Disposable capillary tube.

2.3.1.5. Test procedure:

1. The collected blood sample was put into the anticoagulant tube.
2. The cap on the anticoagulant tube was closed and the tube was inverted 5 times to mix the blood and the EDTA.
3. Ten microliters of the whole blood were added to the sample hole by using capillary tube.
4. Two drops of the assay diluent were added into the sample hole on the test device.
5. The result was read (image 1).

Comment [h3]: Check the format of the text body of materials and methods. Write in a running pharase without subhead

Comment [h4]:

Comment [h5]:



Image (1): Rapid Test Kit for detection of Canine Ehrlichiosis.

3. RESULTS:

3.2. Prevalence of Canine Ehrlichiosis in Khartoum State:

Among 599 dogs of different breed, sex and age, 34 (5.6%) were positive for Ehrlichiosis. Male dogs represented 2.8% and females represented 2.8% of the positively tested dogs. The age of the infected dogs ranged in between 8 and 17 months (Table 1 and Figures 1 and 2).

3.2.1. Prevalence of Canine Ehrlichiosis in autumn:

The prevalence of Canine Ehrlichiosis during the autumn was 8.8%. Male dogs represented 3.6% and females represented 5.2% of the positively tested dogs (Table 2).

3.2.2. Prevalence of Canine Ehrlichiosis in winter:

The prevalence of Canine Ehrlichiosis during the winter was 6.0%. Male dogs represented 2.5% and females represented 3.5% of the positively tested dogs (Table 3).

3.2.3. The prevalence of canine Ehrlichiosis in summer:

The prevalence of Canine Ehrlichiosis during the summer was 6.9%. Male dogs represented 2.6% and females represented 4.3% of the positively tested dogs (Table 4).

3.2.4. Prevalence of Canine Ehrlichiosis in different dog's breeds:

Among 34 dogs of different breed, the prevalence of Canine Ehrlichiosis was 47.2% in German shepherd, 29.4% in cross breed, 11.8% in Lulu, 2.9% in local breed, Perro de Presa Canario, Rottweiler and Royal black breeds (Figure 3).

Comment [h6]:
Comment [h7]: Apply the statistical analysis to evaluate the level of significance among various risk factors

Comment [h8]: Make one table of season wise then no need of breeds????

3.2.5. Treatment trial for Canine Ehrlichiosis cases:

Eighty-eight-point two percent of Canine Ehrlichiosis cases respond for the treatment trials (Figure 4).

Table (1): Prevalence of Canine Ehrlichiosis in Khartoum State.

Breed	Infected Male	Healthy Male	Infected Female	Healthy Female	Total
German shepherd	7	158	9	172	346
Local	1	45	0	45	91
Lulu	1	17	3	24	45
Cross	8	15	2	17	42
Perro de Presa Canario	0	10	1	8	19
Rottweiler	0	5	1	5	11
Royal black	0	7	1	0	8
Rood dog	0	0	0	7	7
Golden Retriever	0	1	0	5	6
Malinois	0	4	0	2	6
Griffon	0	4	0	1	5
Caucasian	0	1	0	3	4
Alabi	0	1	0	2	3
American bulldog	0	1	0	1	2
Saluki	0	2	0	0	2
Balboa	0	1	0	0	1
Husky	0	1	0	0	1
Total	17 (2.8%)	273 (45.6%)	17 (2.8%)	292 (48.8%)	599 (100%)

Table (2): Prevalence of Canine Ehrlichiosis in autumn.

Breed	Infected Male	Healthy Male	Infected Female	Healthy Female	Total
German shepherd	4	61	4	66	135
Local	0	15	0	11	26
Lulu	0	5	3	1	9
Rottweiler	0	4	0	5	9
Saluki	0	1	0	5	6
Cross	2	2	0	2	6

Griffon	0	4	0	0	4
Malinois	0	3	1	0	4
Alabi	0	1	0	2	3
Perro de Presa Canario	0	0	2	0	2
Caucasian	1	0	1	0	2
Balboa	1	0	0	0	1
American bulldog	0	0	0	1	1
Total	8	96	11	93	208
	(3.6%)	(46.6%)	(5.2%)	(44.6%)	(100%)

Table (3): Prevalence of Canine Ehrlichiosis in winter.

Breed	Infected	Healthy	Infected	Healthy	Total
	Male	Male	Female	Female	
German shepherd	2	63	5	63	133
Local	0	17	1	10	28
Cross	2	7	0	8	17
Lulu	1	3	0	6	10
Rood dog	0	0	0	6	6
Perro de Presa Canario	0	0	0	2	2
Caucasian	0	0	0	2	2
Saluki	0	1	0	0	1
Griffon	0	0	0	1	1
Golden Retriever	0	0	1	0	1
Malinois	0	1	0	0	1
Total	5	92	7	98	202
	(2.5%)	(45.5%)	(3.5%)	(48.5%)	(100%)

Table (4): Prevalence of Canine Ehrlichiosis in summer.

Breed	Infected	Healthy	Infected	Healthy	Total
	Male	Male	Female	Female	
German shepherd	1	34	0	43	78
Lulu	0	9	3	14	26
Cross	2	8	1	8	19
Perro de Presa Canario	0	9	0	5	14
Royal black	1	6	1	0	8

Golden Retriever	0	0	1	5	6
Local	0	3	0	0	3
Rottweiler	0	1	1	0	2
American bulldog	0	2	0	0	2
Rood dog	0	0	0	1	1
Malinois	0	0	0	1	1
Husky	0	1	0	0	1
Total	4	73	7	77	161
	(2.6%)	(45.3%)	(4.3%)	(47.8%)	(100%)



Fig. (1): Positive *E. canis* test for detection of Canine Ehrlichiosis.



Fig. (2): Negative *E. canis* test for detection of Canine Ehrlichiosis.

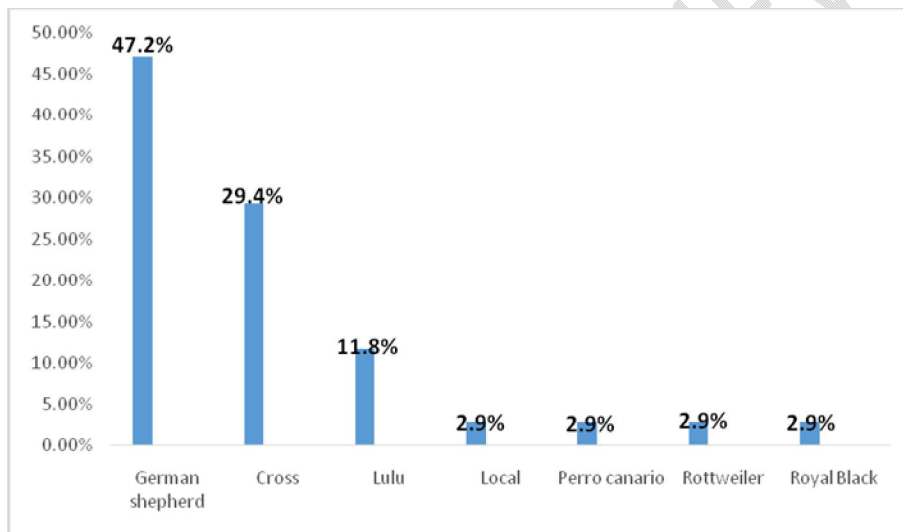


Fig. (3): Prevalence of Canine Ehrlichiosis in different dog's breeds.

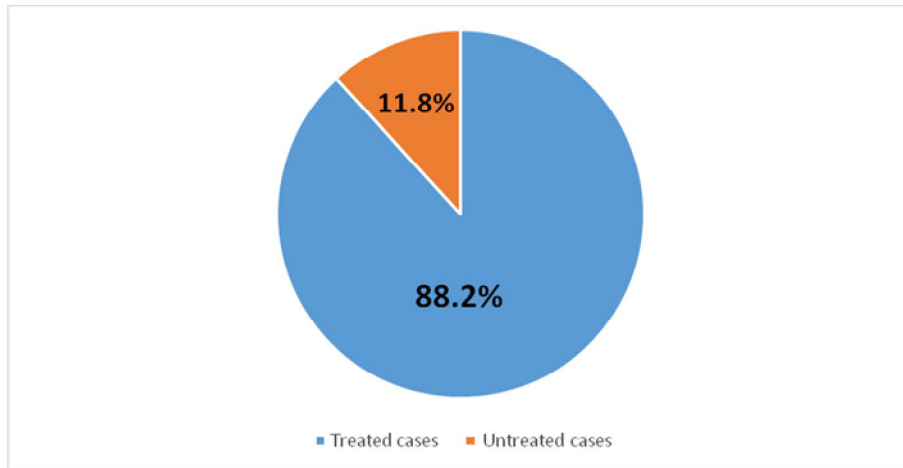


Fig. (4): Treated cases of Canine Ehrlichiosis.

Comment [h9]: Treatment was not mentioned anywhere in the materials and methods

4. DISCUSSION:

Comment [h10]: Rephrase the discussion in a scientific manner

Ehrlichiosis is a tick-borne disease of dogs usually caused by the rickettsial agent *Ehrlichia canis*. *Ehrlichia ~~Canis~~ canis* is the pathogen of animals. In this study the prevalence of Canine Ehrlichiosis in Khartoum State was 5.6%. Lower prevalence of Canine Ehrlichiosis (2.8%), was reported by [19] in Egypt and 3.7% by [20] in Brazil. Higher prevalence 18.8% was reported by Dahmani [21] in Senegal, 7.0% [22] in Nigeria, 75.2% by [23] in Zimbabwe, 10.0% by [24] in Mexico and 14.7% by [25] in Egypt. In this study the prevalence of Canine Ehrlichiosis in male and female dogs was 2.8% and that was the opposite of some published literature which detected increased seroreactivity in males, which was explained by a higher chance of contact with tick species than females because of behavioral features [26] [27]. In this study the age of the infected dogs ranged in between 8 and 17 months and was in the same line with some epidemiological studies by [27] [28] [29] which showed that seropositivity rates were higher in older dogs and that may be due to the higher likelihood of exposure to the *Ehrlichia* pathogen as the dog becomes older. In the present study the prevalence of Canine Ehrlichiosis in winter was 6.0%, 6.9% in summer and 8.8% autumn. The prevalence was high in autumn the season in which the tick is active according to [30]. In the present study 88.2% of Canine Ehrlichiosis cases respond for the treatment trials. The

Formatted: Font: Not Bold, Italic, Check spelling and grammar

Formatted: Font: Not Bold, Italic, Check spelling and grammar

Formatted: Font: Not Bold, Italic, Check spelling and grammar

Formatted: Font: Italic

survival rate in Europe was reported to be 50% by [31], in Switzerland 86.6% by [32] and in UAS 75% by [33].

5. CONCLUSION AND RECOMMENDATIONS:

The prevalence of canine Ehrlichiosis in Khartoum State was 5.6%. Male and female dogs had the same risk of infection with the disease. All the ages had the same chance for the infestation. Dogs were more susceptible for the infestation in autumn season. German shepherd breed was more susceptible for the disease. Most cases were treatable. All dogs must be vaccinated against different diseases and complete the vaccine schedules. Diseased dogs must be treated early for good prognosis. Diseased dogs must be separated from healthy dogs to prevent spread of infection. Hygiene must be doing during infection and sanitizing of fomite, food and water dishes.

REFERENCES:

- [1] Dagnone, A. S.; Morais, H. A. S.; Vidotto, M. C.; Jojima, F. S. and Vidotto, O. (2003). Ehrlichiosis in anemic, thrombocytopenic, or tick-infested dogs from a hospital population in South Brazil. *Vet. Parasitol.* 117(4):285-290.
- [2] Nakaghi, A. C. H.; Machado, R. Z.; Tinucci-Costa, M.; Andre', M. R. and Baldani, CD. (2008). Canine ehrlichiosis: clinical, hematological, serological and molecular aspects. *Cienc Rural.*, 38(3): 766-770.
- [3] Faria, J. L. M.; Dagnone, A. S.; Munhoz, T. D.; Joao, C. F.; Pereira.; W. A. B.; and Machado, R. Z. et al. (2010). Ehrlichia canis morulae and DNA detection in whole blood and spleen aspiration samples. *Rev. Bras. Parasitol. Vet.*, 19(2): 98-102.
- [4] Vieira, R. F. C.; Biondo, A. W.; Guimaraes, A. M. S.; Santos, A. P.; Santos, R. P.; and Dutra, L. H. et al. (2011). Ehrlichiosis in Brazil. *Rev. Bras. Parasitol. Vet.*, 20(1): 1-12.
- [5] Anziani, O. S.; Ewing, S. A. and Barker, RW. (1990). Experimental transmission of a granulocytic form of the tribe Ehrlichieae by Dermacentor variabilis and Amblyomma americanum to dogs. *Am. J. Vet. Res.*, 51:929-931.
- [6] Little, S. E.; O'Connor, T. P.; Hempstead, J.; Saucier, J.; Reichard, MV.; Meinkoth, K.; Meinkoth, JH.; Andrews, B.; Ullom, S.; Ewing, SA. and Chandrashekar, R. (2010).

Comment [h11]: As per the standard format of the journals

- Ehrlichia ewingii infection and exposure rates in dogs from the southcentral United State. *Vet Parasitol.* 172: 355-360.
- [7] Troy, G. C.; and Forrester, S. D. (1990). Canine ehrlichiosis. In: Green CE (Ed), Infectious diseases of the dog and cat. *Philadelphia: W.B. Sanders. Co.*, (PP.404-418).
- [8] Castro, M. B.; Machado, R. Z.; Tomaz de Aquino, L. P. C.; Alessi, A. C. and Costa, M. T. (2004). Experimental acute canine monocytic ehrlichiosis: clinicopathological and immunopathological findings. *Vet Parasitol.* 5;119(1):73-86.
- [9] Trautwein, C.; Boker, K.; and Manns, M. P. (1994). Hepatocyte and immune system: acute phase reaction as a contribution to early defence mechanisms. *Gut.* 35(9): 1163-1166. *P. Mid:* 7525420.
- [10] Gabay, C. and Kushner, I. (1999). Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med.* 340(6): 448-454.
- [11] Ceron, L. L.; Eckersall, P. D. and Martinez-Subiela, S. (2005). Acute phase proteins in dogs and cats: current Knowledge and future perspectives. *Vet. Clin. pathol.* 34(2): 85-99.
- [12] Dahmani, M.; Davoust, B.; Tahir, D.; Fenollar, F. and Mediannikov, O. (2017). Molecular investigation and phylogeny of Anaplasmataceae species infecting domestic animals and ticks in Corsica, France. *Parasites and Vectors.* 10:302.
- [13] Ewing, S. A. (1969). Canine ehrlichiosis. *Adv. Vet. Sci. Comp. Med.*, 13:331-353
- [14] Neer, T. M. and Harrus, S. (2006). Canine monocytotropic ehrlichiosis and neorickettsiosis (*E. canis*, *E. chaffeensis*, *E. ruminantium*, *N. sennetsu*, and *N. risticii* infections). In: Greene CE (Ed), Infectious diseases of the dog and cat. 3rd ed. (pp. 203-216). *St. Louis, Missouri: Saunders Elsevier.*
- [15] Waner, T.; Harrus, S.; Bark, H.; Bogin, E.; Avidar, Y.; and Keysary, A. (1997). Subclinical canine ehrlichiosis (*ehrlichia canis*) in experimentally infected beagle dogs. *Vet. Parasitol.* 69 (3-4): 307-317.
- [16] Troy, G. C.; and Forrester, S. D. (1990). Canine ehrlichiosis. In: Green CE (Ed), Infectious diseases of the dog and cat. *Philadelphia: W.B. Sanders. Co.*, (PP.404-418).
- [17] Nyindo, M.; Huxsoll, D. L.; Ristic, M.; Kakoma, I.; Brown, J. L.; Carson, C.A. and Stephenson, E. H. (1980). Cell-mediated and humoral immune responses of German

Shepherd Dogs and Beagles to experimental infection with Ehrlichia canis. *American Journal of Veterinary Research.*, 41, 250-254.

[18] Macieira, D.; MessIck, J.; Cerguera, A.; Freire, I.; Linhares, G. and Almeida, N. *et al.* (2005). Prevalence of *Ehrlichia canis* infection in thrombocytopenic dogs from Rio de Janeiro, Brazil. *Vet. Clin. Pathol.* 34: 44-48.

[19] Khaled Mohamed El-Dakhly; Magdy M. Tawfik; Amany Samir Aboshinaf; Lilian N. Mahrous and Waleed M. Arafa. (2021). Detection of Anaplasmosis and Ehrlichiosis in Blood of Owned Dogs in Alexandria, Northern Egypt. *Adv. Anim. Vet. Sci.*, 9(9): 1383-1389.

[20] Tanikawa, A.; Labruna, M. B.; Costa, A.; Aguiar, D. M.; Justiniano, S. V.; Mendes, R. S.; Melo, A. L. T.; Alves, C. J.; and Azevedo, S. S. (2013). *Ehrlichia canis* in dogs in a semiarid region of Northeastern Brazil: Serology, molecular detection and associated factors. *Res. Vet. Sci.*, 94: 474-477.

[21] Dahmani, M.; Davoust, B.; Sambou, M.; Bassene, H.; Scandola, P.; Ameer, T.; Raoult, D.; Fenollar, F. and Mediannikov, O. (2019). Molecular investigation and phylogeny of species of the Anaplasmataceae infecting animals and ticks in Senegal. *Parasit. Vectors.* 12: 495.

[22] Adamu, M.; Troskie, M.; Oshadu, D. O.; Malatji, D. P.; Penzhorn, B. L. and Matjila, P. T. (2014). Occurrence of tick-transmitted pathogens in dogs in Jos, Plateau State, Nigeria. *Parasit. Vectors.* 7: 119. 3305-3307.

[23] Dhliwayo, S.; Chihambakwe, B.; Taonezvi, K.; Chikerema, S. M.; Tivapasi, M. T. and Pfukenyi, DM. (2019). Seroprevalence of canine ehrlichiosis and microscopic screening for canine babesiosis in dogs in Harare, Zimbabwe, *Vet. Med. Int.*, 10.1155. 4130210-7.

[24] Almazan, C.; Gonzalez-Alvarez, V. H.; de Mera, I. G. F.; Cabezas-Cruz, A.; Rodriguez-Martinez, R. and de la Fuente, J. (2016). Molecular identification and characterization of *Anaplasma platys* and *Ehrlichia canis* in dogs in Mexico. *Ticks Tick-borne Dis.* 7: 276-283.

[25] Selim Abdelfattah1, Said Ahmed Shima and Galila, Elsayed. (2019). Epidemiological and molecular diagnosis of *Ehrlichia canis* infection among dogs. *Benha. Vet. Med. J.*, 37: 169-171.

- [26] Angelou, A.; Gelasakis, A.I.; Verde, N.; Pantchev, N.; Schaper, R.; Chandrashekar, R. and Papadopoulos, E. (2019). Prevalence and Risk Factors for Selected Canine Vector-Borne Diseases in Greece. *Parasites. Vectors.* 12, 283.
- [27] Muhammad Umair Aziz; Sabir Hussain; Baolin Song; Hammad Nayyar Ghauri; Jehan Zeb and Olivier Andre Sparagano. (2023). Ehrlichiosis in Dogs: A Comprehensive Review about the Pathogen and Its Vectors with Emphasis on South and East Asian Countries. *Vet. Sci.* 10 (1): 21.
- [28] Elisa Brandão Guedes.; *et al.* (2015). Canine ehrlichiosis: prevalence and epidemiology in northeast Brazil Paula. *Rev. Bras. Parasitol. Vet.* 24(2):115-21.
- [29] Bowman, D.; Little, S. E.; Lorentzen, L.; Shields, J.; Sullivan, M. P.; and Carlin, E. P. (2009). Prevalence and Geographic Distribution of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* in Dogs in the United States: Results of a National Clinic-Based Serologic Survey. *Vet. Parasitol.* 160, 138–148.
- [30] Gray, J.; Dantas-Torres, F.; Estrada-Pena, A.; Levin, M. (2013). Systematics and ecology of the brown dog tick, *Rhipicephalus sanguineus*. *Ticks Tick Borne Dis.* 4(3):171–80.
- [31] Ángel Sainz; Xavier Roura; Guadalupe Miró; Agustín Estrada-Peña; Barbara Kohn; Shimon Harrus and Laia Solano-Gallego. (2015). Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. *Parasit. Vectors.* 8: 75.
- [32] Kevin Horecka; Steve Porter, E.; Susan Amirian and Ellen Jefferson. (2020). A decade of Treatment of Canine Parvovirus in an Animal Shelter: A Retrospective Study. *Animals (Basel)*.10(6): 939.
- [33] Kathryn J.; Sarpong; Jennifer M. Lukowski and Cassandra G. Knapp. (2017). Evaluation of mortality rate and predictors of outcome in dogs receiving outpatient treatment for parvoviral enteritis. *J. Am. Vet. Med. Assoc.*, 251(9):1035-1041.