Original Research Article

SEROLOGICAL INVESTIGATION OF CANINE EHRLICHIOSIS IN KHARTOUM STATE, SUDAN

ABSTRACT

The study was extended for 2 years and covered different seasons of the years 2021 and 2022.The study was aiming at investigating the prevalence of Canine Ehrlichiosis disease among dogs.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 forEhrlichiosiswith the prevalence of 2.8% and 2.8% respectively. Dogs of all ages were susceptible for the disease. Dogs were more susceptible for the infestation duringautumn with the prevalence of 8.8%. According to Chi-2 test, cross breed dogs were more susceptible for Ehrlichiosisin Khartoum State. Eighty-eight-point two percent of Canine Ehrlichiosiscases respond for the treatment trials with Doxycycline. 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 *Ehrlichiacanis*, mostly transmitted by *Rhipicephalussanguineus* (brown dog tick). Other species of Ehrlichia(*E. chaffeensis*and *E. ewingii*) are predominantly transmitted by *AmblyommaAmericanum*[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 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.

2. MATERIALS AND METHODS

Area of the study

The study wasconducted in Khartoum State during the years 2020, 2021 and 2022.

Samples

Source of samples

In this study which lasted for 2 years, 599 dogs of different ages and breeds were investigated for presence of Canine Ehrlichiosisinfestation. Whole blood samples were collected during this investigation from all dogs.

Breeds of dogs

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

Ages of dogs

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

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.

Detection of Canine Ehrlichiosis

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

Purpose: Detection of *E. canis* Antibodies.

Principle: Immunochromatographic assay**.**

Specimen: Whole blood.

Materials provided

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

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 (Figure1).

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

Treatment trial for Canine Ehrlichiosis cases

All positively tested cases were subjected for treatment trials using Doxycycline.

3. RESULTS

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 2 and 3).

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).

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).

The prevalence of canine Ehrlichiosis in summer

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

Prevalence of Canine Ehrlichiosis in different dog's breeds

Among 34 dogs of different breed, the prevalence of Canine Ehrlichiosiswas 47.2% in German shepherd, 29.4% in cross breed, 11.8% in Lulu, 2.9% in local breed, Perro de PresaCanario, Rottweiler and Royal black breeds. According to Chi-2 test, cross breed dogs were more susceptible for Ehrlichiosisin Khartoum State.(Table 5).

Treatment trial for Canine Ehrlichiosis cases

Eighty-eight-point two percent of Canine Ehrlichiosiscases respond for the treatment trialswith Doxycycline.(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 PresaCanario	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 Ehrlichiosisin 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 PresaCanario	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 (3.6%)**	**96 (46.6%)**	**11 (5.2%)**	**93 (44.6%)**	**208 (100%)**

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

Breed	Infected Male	Healthy Male	Infected Female	Healthy Female	Total
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 PresaCanario	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 (2.5%)**	**92 (45.5%)**	**7 (3.5%)**	**98 (48.5%)**	**202 (100%)**

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

Breed	Infected Male	Healthy Male	Infected Female	Healthy Female	Total

German shepherd	1	34	0	43	**78**
Lulu	0	9	3	14	**26**
Cross	2	8	1	8	**19**
Perro de PresaCanario	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 (2.6%)**	**73 (45.3%)**	**7 (4.3%)**	**77 (47.8%)**	**161 (100%)**

Table (5): Chi-2 test for breeds of dogs infected with Canine Ehrlichiosis.

Breed	Infected Male	Healthy Male	Infected Female	Healthy Female
German shepherd	0.8	0.0	0.1	0.1
Local	1.0	0.3	2.6	0.0
Lulu	0.1	0.6	2.3	0.2
Cross	38.9	0.9	0.5	0.6
Rottweiler	0.3	0.0	1.5	0.0
Royal black	0.2	3.1	2.6	3.9
Rood dog	0.2	3.2	0.2	3.8
Golden Retriever	0.2	1.1	0.2	1.5
Saluki	0.1	1.3	0.1	1.0
Griffon	0.1	1.3	0.1	0.8
American bulldog	0.1	0.0	0.1	0.0
Malinois	0.2	0.6	0.2	0.3
Alabi	0.1	0.1	0.1	0.2
Perro de PresaCanario	0.5	0.2	0.4	0.2
Caucasian	0.1	0.4	0.1	0.6
Balboa	0.0	0.6	0.0	0.5
Husky	0.0	0.6	0.0	0.6

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

Fig. (3): Negative *E. canis* test for detection of Canine Ehrlichiosis.

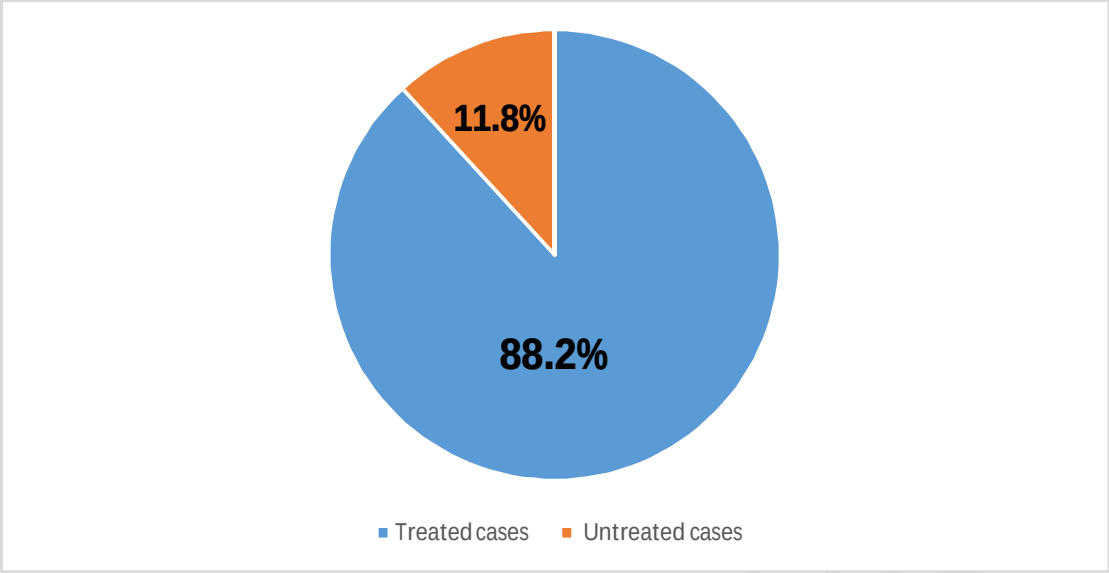

Fig. (4): Treated cases of Canine Ehrlichiosis.

4. DISCUSSION

Ehrlichiosis is a tick-borne disease of dogs usually caused by the rickettsial agent Ehrlichiacanis. EhrlichiaCanis 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 Khaled *et al.*[19] in Egypt and 3.7% by [20] in Brazil. Higher prevalence (18.8%) was reported by Dahmani*et al.*[21] in Senegal, (7.0%) by Adamu*et al.* [22] in Nigeria, (75.2%) by Dhliwayo*et al.*[23] in Zimbabwe, (10.0%) by Almazan*et al.*[24] in Mexico and (14.7%) bySelim*et al.*[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 that was in the same line with some epidemiological studies by Muhammad *et al.*[27], Elisa Brandao [28] and Bowman *et al.* [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 Gray *et al.*[30]. In the present study 88.2% of Canine Ehrlichiosiscases respond for the treatment trials. The survival rate in Europe was reported to be 50% by Angel Sainz*et al.*[31], in Switzerland 86.6% by Kevin Horeckа*et al.* [32] and in UAS 75% by Kathryn *et al.*[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. Cross breedswere 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 done during theinfection and sanitization of fomite, food and water dishes.

REFERENCES

[1] Dagnone AS, Morais HAS, Vidotto MC, Jojima FS, Vidotto O. Ehrlichiosis in anemic, thrombocytopenic, or tick-infested dogs from a hospital population in South Brazil. Vet. Parasitol.2003;117(4):285-290.

[2] Nakaghi ACH, Machado RZ,Tinucci-Costa M, Andre MR, Baldani CD. Canine ehrlichiosis: clinical, hematological, serological and molecular aspects. CiencRural.,2008;38(3): 766-770.

[3] Faria JLM, Dagnone AS, Munhoz TD, Joao CF, Pereira WAB, Machado RZ *etal*. *Ehrlichiacanis*morulae and DNA detection in whole blood and spleen aspiration samples. Rev. Bras. Parasitol. Vet.2010;19(2): 98-102.

[4] Vieira RFC, Biondo AW, Guimaraes AMS, Santos AP, Santos RP, Dutra LH *etal*. Ehrlichiosis in Brazil. Rev. Bras. Parasitol. Vet.2011;20(1): 1-12.

[5] Anziani OS, Ewing SA, Barker RW. Experimental transmission of a granulocytic form of the tribe Ehrlichieae by Dermacentorvariabilis and Amblyommaamericanum to dogs. Am. J. Vet. Res. 1990;51:929-931.

[6] Little SE, O'Connor TP, Hempstead J, Saucier J, Reichard MV, Meinkoth K, Meinkoth JH, Andrews B, Ullom S, Ewing SA, Chandrashekar R.

*Ehrlichiaewingii*infection and exposure rates in dogs from the southcentral United State. Vet Prasitol.2010;172: 355-360.

[7] Troy G C, Forrester S. Canine ehrlichiosis. In: Green CE (Ed), Infectious diseases of the dog and cat. Philadelphia: W.B. Sanders. Co., 1990;404-418.

[8] CastroMB,Machado RZ, Tomaz de Aquino LPC, Alessi AC, Costa MT. Experimental acute canine monocyticehrlichiosis: clinicopathological and immunopathologicalfindungs.Vet.Parasitol. 2004;5;119(1):73-86.

[9] Trautwein C,Boker K, Manns MP. Hepatocyte and immune system: acute phase reaction as a contribution to early defence mechanisms. *Gut.*1994;35(9): 1163-1166. P. Mid*:* 7525420.

[10] Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999;340(6): 448-454.

[11] Ceron LL, Eckersall PD, Martinez-Subiela S. Acute phase proteins in dogs and cats: current Knowledge and future perspectives. Vet. Clin. pathol.2005;34(2): 85-99.

[12] Dahmani M, Davoust B, Tahir D, Fenollar F, Mediannikov O. Molecular investigation and phylogeny of Anaplasmataceae species infecting domestic animals and ticks in Corsica, France. Parasites and Vectors. 2017;(10):302.

[13] Ewing S A. Canine ehrlichiosis. Adv. Vet. Sci. Comp. Med.,1969;(13):331-353

[14] Neer TM Harrus S. Canine monocytotropicehrlichiosis and neorickettiosis (*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. St. Louis, Missouri: Saunders Elsevier. 2006; 203-216.

[15] Waner T,Harrus S, Bark H, Bogin E, Avidar Y, Keysary A. Subclinical canine ehrlichiosis (ehrlichiacanis) in experimentally infected beagle dogs. Vet. Parasitol. 1997;69 (3-4): 307-317.

[16] Troy GC, Forrester SD. Canine ehrlichiosis. In: Green CE (Ed), Infectious diseases of the dog and cat. Philadelphia: W.B. Sanders. Co., 1990;404-418.

[17] Nyindo M, Huxsoll D L, Ristic M, Kakoma I, Brown JL, Carson CA, Stephenson EH. Cell-mediated and humoral immune responses of German Shepherd Dogs and Beagles to experimental infection with Ehrlichiacanis. *American Journal of Veterinary Research.*,1980;(41): 250-254.

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

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

[20] Tanikawa A, Labruna MB, Costa A, Aguiar DM, JustinianoSV, Mendes RS, Melo, ALT, Alves CJ, Azevedo SS.*Ehrlichiacanis*in dogs in a semiarid region of NortheasternBrazil: Serology, molecular detection and associated factors.Res. Vet. Sci.,2013;(94): 474-477.

[21] Dahmani M, Davoust B, Sambou M, Bassene H, ScandolaP, Ameur T, Raoult D, Fenollar F, Mediannikov O. Molecular investigation and phylogeny of speciesof the Anaplasmataceae infecting animals and ticksin Senegal.*Parasit. Vectors*. 2019;(12): 495.

[22] Adamu M, Troskie M, Oshadu DO,Malatji DP, PenzhornBL, Matjila PT. Occurrence of tick-transmittedpathogens in dogs in Jos, Plateau State, Nigeria. Parasit.Vectors. 2014;(7): 119. 3305-3307.

[23] Dhliwayo S, Chihambakwe B, Taonezvi K, Chikerema SM, Tivapasi MT, Pfukenyi DM. Seroprevalence of canine ehrlichiosis and microscopic screening for canine babesiosis in dogs in Harare, Zimbabwe, Vet. Med. Int., 2019;(10).1155. 4130210- 7.

[24] Almazan C, Gonzalez-Alvarez VH, de Mera IGF, Cabezas-CruzA, Rodriguez-Martinez R, de la Fuentec J. Molecularidentification and characterization of *Anaplasmaplatys*and*Ehrlichiacanis*in dogs in Mexico. Ticks Tick-borne Dis.2016;(7):276-283.

[25] SelimAbdelfattah, Said Ahmed Shimaa, GalilaElsayed. Epidemiological and molecular diagnosis of *Ehrlichiacanis*infection among dogs. *Benha.*Vet. Med. J.,2019;(37): 169-171.

[26] Angelou A, Gelasakis AI, Verde N, Pantchev N, Schaper R, Chandrashekar R, Papadopoulos E. Prevalence and Risk Factors for Selected Canine Vector-Borne Diseases in Greece. Parasites. Vectors. 2019;(12), 283.

[27] Muhammad Umair Aziz,SabirHussain,Baolin Song,HammadNayyarGhauri,JehanZeb, Olivier Andre Sparagano.Ehrlichiosis in Dogs: A

Comprehensive Review about the Pathogen and Its Vectors with Emphasis on South and East Asian Countries. Vet. Sci.2023;10 (1): 21.

[28] Elisa BrandãoGuedes *etal*. Canine ehrlichiosis: prevalence and epidemiology in northeast Brazil Paula. Rev. Bras. Parasitol. Vet.2015;24(2):115-21.

[29] Bowman D, Little SE, Lorentzen L, Shields J, Sullivan MP, Carlin EP. Prevalence and Geographic Distribution of DirofilariaImmitis, BorreliaBurgdorferi, EhrlichiaCanis, and AnaplasmaPhagocytophilum in Dogs in the United States: Results of a National Clinic-Based Serologic Survey. Vet. Parasitol.2009;160, 138–148.

[30] Gray J, Dantas-Torres F, Estrada-Pena A, Levin M. Systematics and ecology of the brown dog tick, Rhipicephalussanguineus. Ticks Tick Borne Dis.2013;4(3):171–80.

[31] ÁngelSainz,Xavier Roura,Guadalupe Miró,Agustín Estrada-Peña,Barbara Kohn,Shimon Harrus, Laia Solano-Gallego. Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe.Parasit. Vectors.2015;(8): 75.

[32]Kevin Horecka,Steve PorterE, Susan Amirian, Ellen Jefferson. A decade of Treatment of Canine Parvovirus in an Animal Shelter: A Retrospective Study. Animals(Basel).2020;10(6): 939.

[33]Kathryn JSarpong,Jennifer MLukowski, Cassandra G Knapp. Evaluation of mortality rate and predictors of outcome in dogs receiving outpatient treatment for parvoviral enteritis. *J. Am. Vet. Med. Assoc.*,2017;251(9):1035-1041.