Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/1903
DOI URL: http://dx.doi.org/10.21474/IJAR01/1903
ISSN NO. 2320-5407

REVIEW ARTICLE

CANINE TICK BORNE HAEMOPROTOZOANDISEASES; EPIDEMIOLOGY, PATHOGENESIS AND DIAGNOSTIC APPROACHES– A REVIEW.

AdhikariSahu*, M.R. Panda, B.N. Mohanty and M. Dehur.
C.V.Sc& A.H, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India.

Manuscript Info

Manuscript History

Received: 12 August 2016
Final Accepted: 22 September 2016
Published: October 2016

Key words:-
Haemoprotozoan Diseases, Babesiosis, Tryanosomiasis, Hepatozoonosis

Abstract

Tick borne haemoprotozoan diseases (TBDs) are most common in tropical and subtropical regions across the world because of the high prevalence of tick vectors. Among these, Babesiosis, Hepatozoonosis andTrypanosomiasis are the major TBDs affecting the canine species. Affected dogs usually exhibit clinical manifestations like anorexia, lethargic conditions, anaemia, icterus and often death in severe cases. The haematological profile of the affected dogs shows alterations in blood cell counts like thrombocytopenia, neutrophilia etc. This present review depicts in details the epidemiology, pathogenesis and different diagnostic approaches like microscopic examinations, serological and molecular nucleic acid based tools as the aid for diagnosis.

Introduction:-

Dog is the first pet animal to be domesticated worldwide at about ten to fifteen thousand years ago and considered as the best companion animal of human being because of its well-known qualities like faithfulness, usefulness and intelligence services.India is home to 25 million dog and the dog population can be divided into four categories: family dogs (partially restricted, wholly dependent), community dogs (unrestricted, partially dependent), pet dogs (restricted and supervised), and feral dogs or stray dogs which are unrestricted and independent (Menezes, 2008). The dogs, like other animals are also prone to many infections and diseases. They suffer from bacterial, viral, parasitic diseases etc. Most of the bacterial and viral diseases can be taken care of by administration of vaccines, but question of parasitic diseases remain unsolved. Both stray and pet dogs are attacked by various types of ecto and endoparasites. Stray dogs always act as the source of infection for pet dogs.Among ectoparasites, ticks are the major problems as they suck blood causing anemia, irritation and annoyance to the dogs and most importantly act as vectors for transmitting many fatal diseases. In tropical and subtropical regions,tick-borne diseases of bacterial, viral and most commonly the haemoprotozoan origin are a common feature (Irwin and Jefferies, 2004).The tropical climatic condition (hot and humid environmental condition) of India favors the growth, development and multiplication of ticks (Jadav et al., 2011).Among the tick borne diseases, haemoprotozoan infections are of great importance because of their severe pathogenicity.

Major HaemoprotozoanDiseases of Dogs:-

*Babesia*spp. and *Hepatozoon*spp. cause the major canine tick-borne blood protozoan diseases. However,co-infections of *Babesia*with *Bartonella, Ehrlichia, Hepatozoon, Leishmania*and*Rickettsia* species have also been observed in dogs (O'Dwyer*et al.*, 2001).Canine babesiosis is an important disease of both domestic and wild Canidae across the globe. This is a highly pathogenic disease where members of the genus *Babesia*readily parasitize

Corresponding Author:-AdhikariSahu.
Address:-C.V.Sc& A.H, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha, India.

the red blood cells leading to progressive anemia. Canine *Babesia*can be morphologically categorized into large (*Babesiacanis)*and small forms*(Babesiagibsoni)*. The smaller parasite *B.gibsoni* has been noticed frequently amongst dogs and very commonly*Rhipicephalussanguineus* is considered as the main alleged vector (Senthilkumar et al., 2009).Dogsacquire the infection by ingesting infected ticks containing sporulatedoocyst (Soulsby, 1982). The most common pathogenic symptoms associated with this protozoan infection includesanorexia, weight loss, lethargy,enlargement of spleen, vomition, jaundice,amber to brown discoloration of urine, pale mucous membranes, tachycardiaandtachypnea.

Canine hepatozoonosis is a blood protozoan disease caused by the protozoan parasite, *Hepatozooncanis*which is transmitted by ingestion of infected dog tick named as *R. Sanguineus*and not by the biting of ticks because the infective stages don't reach the salivary glands of the vectors (Baneth et al., 2001). The prevalence of *Hepatozooncanis* in India has been reported earlier by Pawar and Gatne (2005); Lakshmanan and John (2007) and Palanivel et al. (2010).The clinical symptoms of *H. canis* infection vary from subclinical to severe life-threatening conditions (Baneth et al., 2003).*Hepatozooncanis* primarilyaffects the haemolymphatic tissue and blood cellforming organs such as the bone marrow, spleenand lymph nodes. Dogs with severe clinical conditions show signs like fever, loss of appetite, weight loss, hyperglobulinaemia often resulting in hepatitis, anaemia, glomerulonephritis and pneumonia. Co-infection of *H. canis* with other blood parasites such as *Ehrlichia, Leishmania* and parvovirus is a common condition (Baneth and Weigler, 1997).

Canine trypanosomosis is a widespread disease of the Western Hemisphere andis endemic in many parts of South and Central America affecting both animals and humans (Barr et al., 1991). This disease is mainly classified into two primary types: the American form (Chagas disease) caused by *Trypanosomacruzi* and the African form (sleeping sickness or surra), caused by *Trypanosomaevansi*. Furthermore, 'surra', a pathological disease condition due to *T.evansi*infection, is most prevalent inthe large ruminants and horses of tropical region. Tabanid flies (*Tabanus* spp.) and *Stomoxys* spp. Of flies are the vectors for mechanical transmission of the infective agent of "Surra". However,by consumption of the carcass of an infected animal, dogs can also acquire the infection.Trypanosomiasisis widespread in Asia, although it appears to affect companion animals rarely (Irwin and Jefferies, 2004).*T.evansi* infection in dogs causes a severe disease characterized by fever, malaise,generalized oedema, corneal opacity, anaemia, hepatomegaly and mortality in severe cases. Canine trypanosomiasis is generally diagnosedby observation of thick or thin blood films, buffy coat smears for presence oftrypomastigotes or in tissues by mouse inoculation. However, examination by microscopy may under-diagnose the disease as in chronic infection the level of parasitemia can be very low (Da Silva et al, 2009). Although, it is a widespread disease of the western hemisphere, several research workers have reported the incidence of *T.evansi* in India (Varshney et al,1998; Dakshinkar and Bhojne 2001, Ramprabhuet al,2001;Chowdhuryet al, 2005 and Sonika et al,2007).

Canine leishmaniasis is caused by blood protozoaof the genus *Leishmania*. Among the *Leishmania* species infecting the human population and reported worldwide, only *Leishmaniatropica* and *Leishmaniadonovani* are supposed to be anthroponotic i.e. it can be transmitted from human to other animals(Ashford, 1996 and Herwaldt, 1999).Leishmaniasis is of two types cutaneous and visceral. Cutaneous leishmaniasis is caused by members of *Leishmaniaaethiopica, L. major, L. tropica, L. mexicana* and *L. braziliensis* whereas visceral leishmaniasis is associated with L. *donovani*, and *L. Infantum*(Barr and Bowman, 2006).In dogs, clinical observation ofLeishmaniasismay take up to 7 years due to its slowpathogenesis. However, many dogs show natural resistant to this parasite and may remain asymptomatic but the reason is not clearly understood(Snappendelet al, 1998). It is believed that only 10% of dogs living in endemic areas actually develop the clinical symptomswhile the majority acts as subclinical carriers (Barr and Bowman, 2006).Cutaneous lesions are exhibited in up to 89% of the infected dogs, which may or may not be associated with overt signs of visceral involvement. However, it should be noted that any animal exhibiting apparent lesions should be assumed to have disseminated leishmaniasis because involvement of the integument generallyobserved at a later stage in disease progression. One of the most reliable findings among dogs infected with *Leishmania* spp. is the presence of hyperproteinaemia due to hyperglobulinaemia, commonly associated with hypoalbuminaemia. Moreover, the deposition of immune complexes into joints and kidneys lead to polyarthritis and glomerulonephritis, respectively (Snappendelet al., 1998).

Ehrlichia is an alpha-proteobacteriaof protozoan family Ehrlichiaceae. In canines, the major species that are capable of producing infection includes *E. canis* (causes tropical canine pancytopaenia), *E. ewingii* (causes canine granulocytic ehrlichiosis), and *E. chaffeensis* causing human monocyticehrlichiosis (Anderson et al, 1991 and Irwin and Jefferies, 2004). The one that most frequentlyinvades dogs and causes the most severe clinical signs

is *Ehrlichiacanis*and is transmitted *by*. The prevalence of *E. canis* depends on the distribution of its vector*Rhipicephalussanguineus* tick, which is commonlyspread in tropical and subtropical regions (Shaw et al, 2001).

Prevalence of Haemoprotozoan Infection in Dogs in India:-

Several studies by many research workers on investigating prevalence of canine babesiosis in India have been carried out. Nair et al(1979) reported 30.80 percent incidence of canine babesiosis in New Delhi whileLakshmanan and John (2007) in Madras could observe prevalence of *Babesiacanis* and *B.gibsni*4.34% and concurrent infection of *Ehrlichia* spp. and*Hepatozoon* spp.in8.69% of dogs.The status regarding canine babesiosis in India is so far not so comprehensive. In Chennai only 0.1% of dogs were observed to carry*Babesiagibsoni* infectionmicroscopically(Sunder et al, 2004). Other studies recorded 9% and 22% of dogs in Uttar Pradesh (Chaudhari, 2006) and Assam (Chaudhari and Varshney, 2007), respectively. The pathogenicity of *Babesia* shows variations in different parts of India which may be because ofdifferences in the prevailing species and strains. In India, both *B. vogeli* and *B. gibsoni* are co-endemic and the ticks *Rhipicephalussanguineus* and*Haemaphysalislongicornis* are theassumedrespective vectors (Abd Rani et al, 2010).Gadahiet al (2008) investigated on the prevalence of blood-parasites in stray and pet dogs in Hyderabad area and reported an overall prevalence of blood-parasites as 11.66 % and of which *Babesiacanis*was 5%. The prevalence was more in stray dogs (13%) than pet dogs (9%).Senthil Kumar et al (2009) extensively studied on haemoprotozoan infections in Chennai city dogs and found the infection in about 12% of the dogs. Among the positive cases, *Babesiagibsoni*were 84.9%, followed by *Ehrlichiacanis* 6 %, *Hepatazooncan*is 4.8%, *Babesiacanis* 3.9% and *Trypanasomaevansi* 0.4%. Godaraet al (2010) reported the overall prevalence of haemoprotozoan infection in dogs as 16.39% out of which Babesia spp. (13.1%) and *Ehrlichiacanis* (4.9%).Study by Vairamuthu et al, (2014) reported 13.25% prevalence of blood protozoan diseases among dogs treated at Madras Veterinary College Teaching Hospital during 2006 to 2011. *B.gibsoni* infection was highest among the hemoprotozoan infection in dogs accounting 56.65 % followed by *E. canis* (23.21 %) and*H.canis* (11.23 %). It was observed that only 5.54 % of dogs were having *B.canis*infections. Thus, *Babesiosis* alone contributedalmost 62.19 % of the total infection. Laha et al (2014) using molecular diagnostic techniques polymerase chain reaction (PCR) could observe 48.64 % dogs infected with *B. Gibsoni*and54.05 % dogs were *B. canis* positive. Singh et al (2014) in Ludhiana, Punjab could observe an overall prevalence of 7.47% canine babesiosis encompassing 0.93% *B. canis*and 6.54% of *B.gibsoni* using Giemsa-stained peripheral thin blood smears. However, molecular diagnosis revealed 15.42% prevalence of *B. gibsoni* infection. Wadhwaet al (2011) recorded1.15% prevalence of canine babesiosisin Himanchal Pradesh. Das et al (2015) studied the prevalence of canine babesiosis in and around Kolkatta and could find 31.86% prevalence of canine babesiosis out of which 94% was because of *Babesiagibsoni*. In addition, it was found that the incidence of babesiosis was more in females and also more during May and June months.

Sahu et al (2014) recorded 10.54% prevalence ofhaemoprotozoan parasites in Bhubaneswar, Odisha. The four canine blood protozoan parasites found in the area were *Babesiagibsoni*, *Babesiacanis*, *Hepatozooncanis and Trypanosomaevansi* with percentage of incidence 4.81%, 1.66%, 3.33% and 0.74% respectively. Furthermore, the incidence among dogs less than 1year of age was more (17.07%) as compared to dogs above one year (9.37%). Also, the incidence was found more (12.32%) among males as compared to females (8.68%). Highest percentage of incidence was observed in summer (14.55%) followed by rainy (10.06%) and winter (5.92%).Singh et al (1993) reported the incidence of *T.evansi* in 4.68%dogs at Ludhiana, Indiaonly during the rainy and post-rainy seasons. Chowdhuryet al. (2005) conducted a survey on trypanosomosis of dogs in Kolkata and reported 1.72%.Pawar and Gatne (2005) reported 9.64% prevalence of hepatozoonosis in Mumbai.Palanivelet al (2010) recorded 11.76% prevalence of hepatozoonosis in and around Chennai.Jadavet al(2011) reported that prevalence of canine babesiosis basing on morphological criteria by microscopic examination in India ranges from 0.1-22 % in different parts of India. In addition, it was reported that babesiosis, hepatozoonosis and ehrlichiosis are the main arthropod transmitted diseases prevalent in India.Milanjeet et al(2014) by using PCR-based assays targeting a portion of the 16S rRNA gene for detecting *E. canis* could find a prevalence of 2.34% and the prevalence of *E. canis* was higher in the summer than winter season (P = 0.031) and in dogs of age group less than six-month-old as compared to older dogs.

Selvarajet al(2010) studied haemoprotozoan infection in dogs in Chennai and reported 11.74% of dogs were found positive for haemoprotozoan infections.The overall prevalence of *Babesia species* infection was 8.70 %out of which69% cases were due to *Babesiagibsoni*and the 31% were due to *Babesiacanis*.The incidence was most commonly (20%) observed in Non-descript dogs followed by the pet dogs, spitz(12%).Abd Rani et al (2011) investigated the occurrence and distribution of canine tick-borne disease (TBD) on the basis of

microscopicexamination and PCR based study. 2.3% (12 cases) of the total blood smears examined (525 nos.), were positive for *Hepatozooncanis* infection;5.5% casesfor *Babesiavogeli* and0.2%case for *Babesiagibsoni*infection. Concurrent infection with more than one TBD pathogen occurred in 39% of cases.

Haematologicalchanges due to haemoprotozoan infection in dogs:-

Abdullahiet al (1990) reported that acute and sub-acute form of *Babesiacanis* infection was seen in Nigerian dogs (less than four weeks age). Most frequent clinical signs observed in acute babesiosisincludeAnorexia, lethargic condition, fever, anaemia, generalized lymphadenopathy, splenomegaly, hepatomegalyand jaundice. In hyperacute cases neutrophilicleucocytosis is the main clinical manifestation and regenerative anaemiais seen in either of the cases.Bansalet al. (1990) studied on clinicopathological findings of experimental *Babesiacanis* infection on dogs suggesting leukocytosis with neurophilia in acute disease and leukocytosis with lymhocytosis in chronic disease condition. They also found fever, anemia and fall in haematological values among the infected animals. Symptoms were acute in young and chronic in older dogs.

Barr et al(1991) inoculated North American *Trypanosome cruzi* isolates in 19 pure bred beagle dogs of various ages. During acute stage of disease, there was severe myocarditis, generalized lymphadenopathy, lymphocytosis observed in all dogs between 14-27 days post inoculation. Conrad *et al.* (1991) reported that *Babesia gibsoni* caused severe autoimmune hemolytic anemia in dogs from southern California. Anorexia, lethargy, anemia, lymphadenopathy including thrombocytopenia were observed as the most common clinical signs found in *B. gibsoni* infection.Baneth and Weigler (1997) found significant increase in body temperature, decrease in total RBC count, hemoglobin concentration, platelet count, hematocrit, and total neutrophil count in *Hepatozooncanis* positive dogs.Salakijet al (1999) reported that most of the dogs, found positive for haemoprotozoan infection exihibit anemia, lymphopenia, hypoproteinemia, leukopenia and eosinopenia whereas the different characteristics like neutropenia in babesiosis and monocytosis in ehrlichiosis were also seen. Dakshinkar and Bhojne (2001) revealed the haematobiochemical changes in chronic trypanosomosis in Nagpur suggesting neutrophil 70%, Eosinophil 3%, Monocytes 4%, TLC 10000/mm^3 and hemoglobin 8.4% etc.

Ramprabhuet al(2001)gave a case report on blood profile of a 3½ years old mongrel male dog infected with concurrent babesiosis and hepatozoonosis. In the blood profile, neutrophilia with monocytosis were noted. Hemoglobin was 4.5 g/dl and PCV was 8%.Ewing and Panciera (2003) found that haematological changes in American canine hepatozoonosis includes persistent neutrophilia as a consistent feature, leukocyte counts greater than 200,000/ml, radiographs revealed extensive periosteal bone proliferation (mostly proximal bones).Matsuuet al (2004) investigated the incidence of *Babesiagibsoni* in dogs between April 2002 and March 2003 in Japan and reported platelet counts of infected dogs were below normal and their PCVs were at various levels. There was thrombocytopenia or anemia in subclinical infection.

Priyaet al(2004) examined a four year old German Sephered dog in Thrissur with *Hepatozooncanis* and reported changes in haemoglobin, PCV, WBCs etc.Boozer and Macintire (2005) reported that *Babesiagibsoni* is a tick-borne protozoan blood parasite that causes hemolytic anemia, thrombocytopenia, lethargy, and splenomegaly.Furlanello et al(2005) examined dogs suffering from babesiosis and reported hemolytic anemia in 70% of dogs and non-hemolytic anemia, in 30% dogs; 69% of dogs showed leucopenia and 74% neutropenia,leukocytosis, mostly due to mature neutrophilia. Activated lymphocytes were noted in 61% of dogs and in all dogs, thrombocytopenia was observed.Pawar and Gatne (2005) in Mumbai, India revealed normocytic nomochromic (64.28%), microcytic hypo chromic (21.42%) and macrocytic normochromic anemia in dogs showing *Hepatozooncanis* infection.

Fogliaet al(2006) examined clinicopathological findings of tick-transmitted diseases in dogs. Most common haematological abnormalities found in canine babesiosis were anemia and thrombocytopenia. Porchetetal(2007) carried out a descriptive epidemiological study on canine babesiosis and suggested that haematological findings in affected dogs include anemia, and thrombocytopenia. Lee *et al.* (2009) reported the hematological examination in *Babesiagibsoni* infection as severe hemolytic anemia and thrombocytopenia in the infected dogs, though the blood smears of 29 infected dogs showed very low levels of parasitemia.Sonikaet al (2007) examined a German sephered dog with the history of bilateral blindness, intermittent fever, dullness, anemia, conjunctivitis, sexual excitement and staggering gait and diagnosed to have *Trypanosomaevansi* infection.Rashid et al (2008) reported certain hematological parameters in a male bull dog of two years age at Lahore, Pakistan infected with *Trypanosoma* spp. as decrease in hemoglobin (Hb) concentration and packed cell volume (PCV) whereasincreased erythrocytes sedimentation rate (ESR) in the infected dogs. Shigueruet al (2008) while examining dogs for babesiosis, reported

the presence of anemia (PCV < 25%), thrombocytopenia (Platelet count < 150000/mm3), leukopenia (WBC count < 5000/mm3) or a combination of two or three of these alterations.Solano-Gallegoet al (2008) reported the main haematological findings of *Babesiacanis* as thrombocytopenia, mild to moderate normocytic-normochromic non-regenerative anemia, hemolysis and neutropenia.

Gunaseelan*et al.* (2009) studied the hematobiochemicalchanges in canine trypanosomiasis in Chennai, India by examining a six year old non-descript male dog and reported that affected dog had haemoglobin 9 g%, WBC 3200/mm^3 and RBCs 4.32 milion/mm^3 of blood.Pasaet al (2009) studied clinicopathological signs of hepatozoon infection in 10 dogs and reported neutrophilia in 7 dogs, monocytosis in 2 dogs, eosinophilia and lymphopenia in 1 dog each and thrombocytopenia in 9 dogs.DeCaprariis et al (2011) reported that lymphocytosis was frequently detected among dogs infected with *Babesiavogeli* or co-infected with *Anaplasmaplatys* and *B. vogeli.* There was a significant association ($p<0.01$) between tick infestation and *A. platys* or *B. vogeli.*Karunakaran*et al*. (2011) reported the haematological changes in a German shephered dog infected with *B.gibsoni* from whole blood which revealed haemoglobin value of 3 g%, PCV 20 % and total RBC count 1.1 millions/mm^3.

Diagnostic Approaches for Canine Haemoprotozoan Diseases:-

Routine diagnosis of canine babesiosis is often difficult through blood smear examination due to its lower incidence rate and lower sensitivity in chronic as well as sub-clinical phase of infection (Terkawi et al., 2011). However, presence of vectors (ticks), symptoms along with a quantitative buffy coat smear stained with acridine orange (Levine et al., 1989) may improve the diagnostic procedure in low parasitemic cases. However, for hepatozoonosisand Trypanosomiasis, diagnosis by microscopic examination of Giemsa stained thin blood smear or buffy coat smears will solve the purpose due to higher parasitemic conditions. Among the serological tests immunofluroscent antibody test for antibodies in the serum that reacts with *Babesia* organisms can be performed as an aid for diagnosis.

However, difficulties in differentiation of species and sub species due to cross reactive antibodies as well as least detectable antibodies in some infected animals, particularly young dogs, are the major hindrance. This can be overcome by molecular (PCR based) diagnosis for presence of the *Babesia*DNA in biological samples. To maximize the utility of PCR based diagnosis, blood sample should be collected before initiation of chemotherapy and should be done with stringent quality control measures in experience diagnostic laboratories. Among the molecular diagnostic techniques, conventional PCR, PCR with RFLP, RAPD-PCR and Multiplex PCR are some of the easy to use and more sensitive tools that can analyze large number of samples in clinical and environmental samples(Dey and Singh, 2009). Recently a rapid simple and more sensitive technique called Loop mediated Isothermal Amplification (LAMP) has been developed (Notomi et al, 2000) and by the use of additional loop primers to increase efficiency and rapidity (Nagamine et al, 2002). LAMP technique associated with fluorescent dyes like Syber Green allows visual detection of amplified products (Poon et al, 2006) and measurement of turbidity (Mori et al, 2001). Unlike PCR, this technique doesn't need extraction of DNA as well as use of a thermocycler as it can be carried out at a temperature range of 60-65 0 C(Lieu et al, 2008; Muller et al, 2010; Salih et al, 2008, 2012).

Conclusion:-

Haemoprotozoan parasitic infection in livestock including the canine species is often dependent on the presence of the associated vectors that are most prevalent in tropical and subtropical countries. Therefore controlling these infections is often difficult in some geographic locations. However routine examination of blood smears, frequent serological and molecular nucleic acid based tests to diagnose these diseases along with appropriate chemotherapy may reduce the chances of infection among the pet dogs.

References:-

1. AbdRani,P.A.M.,Irwin,P.J.,Coleman,G.T.,Gatne,M.andTraub,R.J.(2011).AsurveyofcanineticknbornediseasesinIndia.Parasites&Vectors.4:141-148.
2. Abdullahi, S.U., Mohammed, A.A.,Trimnell, A.R.,Sannusi, A. andAlafiatayo, R. (1990).Clinicalandhaematologicalfindingsin70naturallyoccurringcasesof caninebabesiosis.J.SmallAni.Practice.,31(3):145-147.
3. Anderson BE, Dawson JE, Jones DC, WilsonK.H.(1991)Ehrlichiachaffeensis, a new species associated with human ehrlichiosis. J ClinMicrobiol. 29(12):2838-42.
4. Ashford RW Leishmaniasis reservoirs and their significance in control – Review ClinDermatol. 1996 Sep-Oct; 14(5):523-32.
5. Baneth G, Mathew JS, Shkap V, Macintire DK, Barta JR, Ewing SA Canine hepatozoonosis: two disease syndromes caused by separate *Hepatozoon* spp. Trends Parasitol. 2003 Jan; 19(1):27-31.
6. Baneth G, Samish M, Alekseev E, Aroch I, Shkap Transmission of *Hepatozooncanis* to dogs by naturally-fed or percutaneously-injected *Rhipicephalussanguineus* ticks. V J Parasitol. 2001 Jun; 87(3):606-11.
7. Baneth G, Weigler B Retrospective case-control study of hepatozoonosis in dogs in Israel. J Vet Intern Med. 1997 Nov-Dec; 11(6):365-70.
8. Baneth, G. andWeigler, B. (1997).Retrospective case-control study of hepatozoonosisindogsinIsrael.*J*.Vet.Intern.Med.,11(6):365-70.
9. Bansal,S.R.,Kharole,M.U.andBanerjee,D.P.(1990).ClinicopathologicalStudiesinExperimental*Babesiacanis*infectioninDogs.*J*.vet.parasitol.,4(1):21-25.
10. Barr, S.C., Bowman, D.D. (2006). The 5-Minute Veterinary Consult Clinical Companion: Canine and Feline Infectious Diseases and Parasitology. Iowa, Blackwell Publishing
11. Barr, S.C.,Gossett, K.A. andKlei, T.R. (1991). Clinical, clinicopathologic, andparasitologicobservations of trypanosomiasis in dogs infected with North American *Trypanosomacruzi*isolates. Am.J.Vet.Res.,52(6):954-960.
12. Chaudhuri, S., Varshney JP(2007) Clinical management of babesiosis in dogs with homeopathic *Crotalushorridus* 200C. Homeopathy.96(2):90-4.
13. Chaudhuri, S. MVSC Thesis. Indian Veterinary Research Institute; 2006. Studies on clinico-therapeutic aspects of babesiosis in dogs.
14. Chowdhury,P., Biswas, U., Guha,C. and Jana, P.S. (2005). Prevalence of canine trypanosomosisin and around Kolkata city.IndianVet.J.82(7):797-798.
15. Conrad, P., Thomford, J. Yamane, I., Whiting, J., Bosma, L., Uno, T., Holshuh, H.J. and Shelly, S. (1991). Hemolyticanemia caused by *Babesiagibsoni* infection in dogs. J. Am. Vet. Med. Assoc., 199(5):601-605.
16. Da Silva, A.S., Costa, M.M., Wolkmer, P., Zanette, R.A, Faccio, L., Gressler, L.T., Dorneles. T.E., Santurio JM, Lopes ST, Monteiro SG. (2009).*Trypanosomaevansi*: hematologic changes in experimentally infected cats. ExpParasitol.123(1):31-4.
17. Dakshinkar, N.P. and Bhojne, G.R. (2001). RefractorychronicTrypanosomosis.IndianVet.J.,78(8):721-722.
18. Das, M.K., Baidya, S., Mahato, A., Pandit, S., Ghosh, J.D., Chaudhuri, S and Das, M. (2015) Incidence of canine babesiosis in and around Kolkata, West Bengal, India. *Explor*Anim Med Res,5(1): 102-107.
19. De Caprariis, D., Dantas-Torres, F., Capelli, G., Mencke, N., Stanneck, D., Breitschwerdt, E.B. and Otranto, D. (2011). Evolution of clinical, haematologicaland biochemical findings in young dogs naturally infected by vector-borne pathogens. Vet.Microbiol.,149(1-2):206-212.
20. Dey A, Singh S (2009). Progress of science from microscopy to microarrays (Part 1): Diagnosis of parasitic diseases. J. Lab. Phys. 1: 2-6.
21. Ewing,S.A.andPanciera,R.J.(2003).AmericanCanineHepatozoonosis.ClinicalMicrobiologyReviews,16(4):688–697.
22. Foglia, M.V., Cappiello, S. And Oliva, G. (2006).Tick-transmitteddiseasesindogs:clinicopathological findings. Parassitologia.48 (1-2):135-136.
23. Gadahi,J.A.,Arijo,A.G.,Abubakar,M.,Javaid,S.B.andArshed,M.J.(2008).Prevalenceofbloodparasitesin stray and pet dogs in Hyderabad Area: Comparative sensitivity of different diagnostic techniques for the detection of microfilaria.VeterinaryWorld.1(8):229-232.
24. Godara, R., Sharma, R.L., Sharma, C.S. and Sharma, D.K. (2010). Parasitic infections in dogs in semi-arid Jaipur (Rajasthan).J.Vet.Parasitol.,24(1):83-86.
25. Gunaseelan,L.,SenthilKumar,K.,Selvaraj,P.andKathiresan,D.(2009).Haemato- biochemical changes in a case of canine trypanosomiasis.TamilnaduJ.Vet.andAni.Sci.,5(3):122-123.
26. Herwaldt, B.L.Leishmaniasis Lancet. 1999 Oct 2; 354(9185):1191-9.

27. Irwin PJ, Jefferies *R* Arthropod-transmitted diseases of companion animals in Southeast Asia. Trends Parasitol. 2004 Jan; 20(1):27-34.

28. Irwin P.J., Jefferies, R. (2004). Arthropod transmitted diseases of companion animals in Southeast Asia. TrendsParasitol.20:27–34.
29. Jadhav, R.K., Kumari, R.R., Jameel, A.J. and Kumar, P. (2011). Emergence of arthropod transmitted infections in kennel dogs. Vet.World,4(11):522-528.
30. Karunakaran, S., Pillai, U.N. and Sasidharan, H.P. (2011). *Babesiagibsoni*infection in a German shepherd dog. VeterinaryWorld.4(6):269-270.
31. Laha, R., Bhattacharjee, K., Sarmah P. C., Das M., Goswami A., Sarma D., and Sen, A. (2014) *Babesia* infection in naturally exposed pet dogs from a north-eastern state (Assam) of India: detection by microscopy and polymerase chain reaction. J Parasit Dis .Dec; 38(4): 389–393.
32. Lakshmanan, B. and John, L. (2007).Clinical findings and concurrent infections in canine ehrlichiosis.Indian Vet. J., 84(8):863-864.
33. Lee,M.J., Yu, D.H., Yoon, J.S., Li, Y.H., Lee, J.H., Chae, J.S. and Park,J. (2009). Epidemiologic and clinical surveys indogs infected with *Babesiagibsoni*in South Korea. Vector Borne Zoonotic D*is.*, 9(6):681-686.
34. Levine, RA, Wardlaw SC, Patton CL (1989). Detection of haematoparasites using quantitative buffy coat analysis tubes. Parasitol. Today 5:132-33.
35. Liu, Z, Hou J, Bakheit MA, Salih DA, Luo J, Yin H, Ahmed JS, Seitzer U (2008). Development of loop-mediated isothermal amplification (LAMP) assay for rapid diagnosis of ovine theileriosis in China. Parasitol. Res. 103:1407-1412.
36. Matsuu, A.,Kawabe, A.,Koshida, Y.,Ikadai, H.,Okano, S.,Higuchi, S. (2004).Incidenceofcanine*Babesiagib*soniinfectionandsubclinicalinfectionamongTosadogsinAomoriPrefecture,Japan.J.Vet.Med.Sci.,66(8):893-897.
37. Menezes, R. Rabies in India. Can Med Assoc J. 2008;178:564. doi: 10.1503/cmaj.071488.
38. Milanjeet, Singh H., Singh N.K., Singh N.D., Singh C. and S.S. Rath (2014). Molecular prevalence and risk factors for the occurrence of canine monocyticehrlichiosis. *VeterinarniMedicina*, 59(3): 129–136.
39. Mori, Y., Nagamine, K., Tomita, N., Notomi, T. (2001). Detection of loop mediated isothermal amplification reaction by turbidity derived from magnesium pyrophosphate formation. Biochem. Biophys. Res. Commun. 289:150-154.
40. Muller, H., Aysu, N, Liu, Z., Salih, D.A., Karagenc, T., Beyer, D., Kullmann, B., Ahmed, J.S. andSeitzer, U. (2010). Development of a Loop-mediated Isothermal Amplification (LAMP) Assay for Rapid Diagnosis of *Babesiacanis* infections. Transbound. Emerg. Dis. 57:63-65.
41. Nagamine, K., Hase, T., Notomi, T. (2002). Accelerated reaction by loop mediated isothermal amplification using loop primers. Mol. Cell. Probes 16:223-229
42. Nair, R.P.N., Pal, M. and Dube, G.D. (1979). A prelimnary report on the occurrence and treatment of *Babesia*infection infection in dogs. Ind.J.Ani.Res.,13(2):95-97.
43. Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N. andHase, T. (2000). Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 28:E63.
44. O'Dwyer, L.H., Massard, C.L., Pereira de Souza, J.C. (2001). *Hepatozooncanis* infection associated with dog ticks of rural areas of Rio de Janeiro State, Brazil. Vet Parasitol., 94(3):143-150.
45. Palanivel, K.M., Vijayalingam, T.A. and Selvasubramanian, S. (2010). Perspective studies on canine hepatozoonosis in Chennai. Tamilnadu J. Veterinary & Animal Sciences., 6(6):291-293.
46. Pasa,S.,Kiral,F.,Karagenc, T.Atasoy,A.andSeyrek,K.(2009).Description of dogsnaturally infected with *Hepatozooncanis*in the Aegean region of Turkey. Turk. J.Vet.Anim.Sci.33(4):289-295.
47. Pawar, S.D. and Gatne, M.L. (2005).Prevalence of canine hepatozoonosis inMumbai. Indian Vet. J., 82(5):554-555.
48. Poon, L.L., Wong, B.W., Ma, E.H., Chan, K.H., Chow, L.M., Abeyewickreme, W., Tangpukdee, N., Yuen, K.Y., Guan, Y., Looareesuwan S andPeiris, J.S. (2006). Sensitive and inexpensive molecular test for *falciparummalaria* : detecting *Plasmodium falciparum* DNA directly from heat-treated blood by loop-mediated isothermal amplification. Clin. Chem. 52:303-306.
49. Porchet, M.J., Sager,H., Muggli, L., Oppliger, A., Müller, N. And Frey,C. (2007). A descriptive epidemiological study on canine babesiosisin the Lake Geneva region. *SchweizArchTierheilkd.*149(10):457-465.
50. Priya,P., Mathew, B., Vijaykumar, K. And Saseendranath, M.R.(2004). A case report of canine Hepatozoonosis.IndianVet.J.,81(2):200-201.

51. PuteriAzaziahMegatAbd Rani, Peter J Irwin, MukuleshGatne, Glen T Coleman, and Rebecca J Traub Canine vector-borne diseases in India: a review of the literature and identification of existing knowledge gaps.Parasit Vectors. 2010; 3: 28.
52. Ramprabhu,R.,Prathaban,S.,Nambi,A.P.andDhanapalan,P.(2001).Concurrent ehrlichiosis, babesiosisandhepatozoonosisinpup- a case report. Indian Vet.J.,21(1):54.
53. Rashid,A.,Rasheed,K.andHussain,A.(2008).TrypanosomiasisinDog;ACaseReport.IranianJ.Arthropod-BorneDis.,2(2):48-51.
54. Sahu, A., Mohanty, B., Panda, M. (2014). Incidence of haemoprotozoan parasites in dogs in and around Bhubaneswar.Indian vet.J., 91(07): 93-95
55. Salakij,C.,Salakij,J.,Rochanapat,N.,Suthunmapinunta,P.(1999).Hematologicalcharacteristicsofbloodparasiteinfecteddogs.*KasetsartJ.NaturalSciences*.33(4):589-600.
56. Salih, D.A, Liu, Z., Bakheit, M.A., Ali, A.M., El Hussein, A.M, Unger, H, Viljoen G, Seitzer U, Ahmed JS (2008). Development and evaluation of a loop-mediated isothermal amplification method for diagnosis of tropical theileriosis. Transbound. Emerg. Dis. 55:238–243
57. Salih, D.A., Ali, A.M., Liu, Z., Bakheit, M.A, Taha, K.M., E.L, Imam, A.H., Kullmann, B., El Hussein, A.M., Ahmed, J.S., Seitzer, U. (2012). Development of a loop-mediated isothermal amplification method for detection of *Theilerialestoquardi*. Parasitol. Res. 110:533-538.
58. Selvaraj,P.,Kumar,K.S.,Vairamuthu,S.S.P.andSrinivasan,S.R.(2010).*Babesiagibsoni*–AnemergingchallengeincaninepediatricpracticeinChennai.TamilnaduJ.Vet.&Ani.Sci.,6(3):122-124.
59. Senthil Kumar, K., Vairamuthu, S. and Kathiresan1, D. (2009). Prevalence of haemoprotozoans in canines in chennai city. Tamilnadu J. Vet. &Ani. Sci.,5(3):104-108.
60. Shaw, S.E., Day M.J., Birtles RJ, Breitschwerdt EB (2001) Tick-borne infectious diseases of dogs. *Trends Parasitol*. 17(2):74-80.
61. Shigueru,J.,Garcia,J.L.,Vidotto,M.C.,Balarin,M.R.,Fabretti,A.K.,GasspariniM.R.,Coelho,A.L.andVidotto, O.(2008).Occurrenceandmolecular characterizationof*Babesia*speciesina caninehospitalpopulationinthe Londrina Region, ParanaState,Brazil. RevBrasParasitolVet.,17(1):277-283.
62. Singh A., Singh H., Singh N. K., Singh N. D. andRath S. S. (2014) Canine Babesiosis in Northwestern India: Molecular Detection and Assessment of Risk Factors. Biomed Res Int. 2014; 2014: 741785.
63. Singh, B., Kalra, I.S., Gupta, M.P. and Nauriyal,D.C. (1993). *Trypanosomaevan*siinfection indogs: seasonal prevalence andchemotherapy. Vet.Parasitol.,50(1-2):137-141.
64. Slappendel, R.J., Ferrer, L. Greene CE: Infectious Diseases of the Dog and Cat. Philadelphia: WB Saunders Co; 1998. pp. 450–458.
65. Solano-Gallego, L.,Trotta, M.,Carli,E.,Carcy,B.,Caldlin, M.andFurlanello, T. (2008). *Babesiacaniscanis*and *Babesiacanisvogeli*clinicopathologicalfindings andDNA detection bymeans ofPCR-RFLP inblood from Italian dogs.VetParasitol.,157(3-4):211-21.
66. Sonika, J., Sharma, G. and Juyal, P.D. (2007). Trypanosomiasisin a German sepheredDog-Acasereport.J.Vet.Parasitol.21(1):81-82.
67. Soulsby, E.J.L. (1982). Helminth, Arthropod and Protozoa of Domesticated Animals.7th Ed. Bailliere, Tindall and Cassel Ltd, London.
68. Sundar, N., Balachandran, C., Senthivelan, A. (2004). Incidence of *Babesiagibsoni* infection in dogs in Tamil Nadu. J Vet Parasitol.18(1):79–80.
69. Terkawi, M.A., Thekisoe, O.M., Katsande, C., Latiff, A.A, Mans, B.J., Matthee, O., Mkize, N., Mabogoane, N., Marais F, Yokoyama N, Xuan X, Igarashi, I. (2011). Serological survey of *Babesiabovis* and *Babesiabigemina* in cattle in South Africa. Vet. Parasitol. 182:337-342.
70. Vairamuthu,S. Ranju,R. S. Bhaskaran Ravi Latha, B. Dhivya, and C. Balachandran (2014) A six year (2006–2011) retrospective study of hemoprotozoan parasites affecting dogs in Chennai, Tamil Nadu, India. J Parasit Dis .38(2): 193–195.
71. Varshney, J.P., Varshney, V.P. and Dwivedi, S.K. (1998). Clinico-endocrinologicalfindings in clinical trypanosomiasis in dog. J.Vet.Parasitol.,12(2):143-144.