Canine Monocytic Ehrlichiosis / Tropical Canine Pancytopenia: A Narrative Review

Georgen G Edana[1], Deepak Chandran[2], Ajin S Kumar[3], Salini Varghese[4]
[2, 3]Veterinary Surgeon, Kerala, India
[1, 4]College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, Kerala – 680651, India.

Abstract- *Canine ehrlichiosis is a tick borne, febrile, debilitating disease of dogs and wild canidae caused by Ehrlichiacanis and transmitted by the brown dog tick Rhipicephalus sanguineus. The infections are known to occur world-wide. Like Chlamydia, Ehrlichia go through three developmental stages namely elementary bodies (individual Ehrlichia organisms), initial bodies (immature organismal inclusions) and morula (mature organismal inclusions. Studies conclusively proved trans-stadial transmission where in larvae, nymphs and adults are able to transmit the disease. The most common pathological finding in an acute E canis infection is the extensive invasion of parenchymal organs and perivascular cuffing by plasma cells particularly lungs, meninges, kidneys and spleen suggesting an immunopathologic etiology. Characteristic clinical signs of the disease include depression, anorexia, weight loss, oculonasal discharge, conjunctivitis, occasional vomiting, lymphadenopathy, occasional fever, corneal opacity, regenerative or non-regenerative anaemia with severe leukopaenia and thrombocytopenia. The most commonly followed methodology for diagnosis of acute ehrlichiosis is by microscopic detection of organism in Giemsa-stained blood smears or buffy coat preparations. Among all therapeutics tested, tetracyclines are the most effective for the treatment of E canis and other ehrlichial infections of dogs. All new dogs should be free of tick infestation and serological status screened before introducing them to E canis free groups.*

Keywords- Ehrlichiosis, Ehrlichiacanis,Rhipicephalus sanguineus, Anaemia Leukopenia, Thrombocytopenia, Tetracyclines.

I. INTRODUCTION

Canine ehrlichiosis is a tick borne, febrile, debilitating disease of dogs and wild canidae caused by *Ehrlichiacanis* transmitted by the brown dog tick *Rhipicephalus sanguineus. E canis* is a Gram-negative, extremely pleomorphic bacterium that has a rippling thin outer membrane. [1, 2]. It was initially discovered in 1935, and in 1963, it was discovered in the United States. The infections are known to occur worldwide. The disease is also known as canine typhus, canine haemorrhagic fever, tracker dog disease, idiopathic haemorrhagic syndrome and tropical canine pancytopaenia. During the warm season, when the vector tick is active, *E canis* infection is most common. When treated with doxycycline or other tetracyclines at acceptable dosages and for adequate durations of time, most dogs recover from the acute and subclinical phases [3, 4]. The brown dog-itch *Rhipicephalus sanguineus*, a tick with a growing global distribution, transmits it [5]. *Dermacentor variabilis* has been proven to transmit *E canis*in an experimental setting [6]. Ehrlichia are obligate intracellular organism which differ from rickettsiae because they replicate in the phagosome of host cell whereas all Rickettsia, with one exception (*C. burnetti*) grow free within the cytoplasm. *Ehrlichiacanis*and other members of the genus Ehrlichia are minute organisms that stain dark blue to purple with Romanowsky stain and with Machiavello method they stain light red and brown-black with silver stain. Studies conclusively proved trans-stadial transmission where in larvae, nymphs and adults are able to transmit the disease. Pathogenesis depends on the strain of organism, breed of dog affected, concomitant diseases and the host defense mechanism. Though it is largely an animal pathogen, it has been documented to have zoonotic potential. After tick encounter, humans can become infected with *E canis* and other species [7]. It is a multisystemic disorder with acute, subclinical, and chronic manifestations. Death is the result of the later type [8]. Clinical and haematological indications of the disease have been described in three phases: acute, subclinical and chronic[9]. A high fever accompanied by depression, lethargy, and anorexia are the non-specific clinical indications of acute illness [10]. The disease is characterized by a wide variety of clinical signs of which depression, lethargy, weight loss, anorexia, pyrexia, lymphadenomegaly, splenomegaly, and bleeding tendencies are the most common.

Major hematologic abnormalities comprise of thrombocytopenia, mild anaemia, and mild leukopenia during the acute stage, mild thrombocytopenia in the subclinical stage, and pancytopenia in the severe chronic stage. The main biochemical abnormalities include hypoalbuminemia, hyperglobulinemia, and hypergammaglobulinemia. Lymphadenomegaly, splenomegaly, and hemorrhagic

tendencies are commonly seen on physical examination, with cutaneous petechiae and ecchymoses, as well as epistaxis. The most common haematological finding is thrombocytopenia. The most common and consistent haematological abnormality of dogs infected with *E Canis* naturally or experimentally is thought to be thrombocytopenia. Mechanisms assumed to be involved in the pathogenesis of thrombocytopenia in the acute phase of the disease include increased platelet consumption due to inflammatory changes in blood vessel endothelium, increased splenic sequestration of platelets, and immunologic destruction or injury resulting in a significantly decreased platelet life span [11, 12]. In this stage, non-regenerative anaemia and a decrease in leukocyte count are possible. At this stage, there are no overt clinical signs and the haematological parameters are normally within the normal range, although the platelet counts may be lower than normal[13]. It is characterised by pancytopenia due to inhibition of bone marrow cells[8]. The only consistent finding among cases of ehrlichiosis was inconsistency and hence diagnosis of ehrlichiosis was difficult since there were no pathognomonic signs. Diagnosis of ehrlichiosis is by microscopic detection of organism in Giemsa-stained blood smears or buffy coat preparations. The morular rosettes take a light blue or lilac tint within the cytoplasm of the monocytes. Tetracyclines are the most effective for the treatment of *E canis* and other ehrlichial infections of dogs. *E canis* causes a potentially fatal disease in dogs that requires rapid and accurate diagnosis to initiate appropriate therapy leading to a favourable prognosis [14].

Taxonomic classification

Earlier Ehrlichia belonged to the family Rickettsiaceae and Anaplasma belonged to the family Anaplasmataceae. Now these two generas belong to the familyAnaplasmataceae.Ehrlichia species can be divided into three classes based on the host cells they infect, such as monocytic, granulocytic, and thrombocytic cells. On the basis of 16S rRNA sequence homology and antigenic relationship, three genogroups of Ehrlichia have been delineated: (i) *E canis* genogroup (*E canis*, *Echaffeensis*and *E ewingii*); (ii) *Ephagocytophila* genogroup (*E phagocytophila, E equi*, human granulocytic ehrlichiosis agent (HGE) and *A platys*); and (iii) *E senetsu* genogroup (*E sennetsu*, *E resticii* and *E bovis*) [15].

Epidemiology

As early as 1935, the brown dog tick *Rhipicephalus sanguineus* was considered to be the vector of *E canis.* Studies conclusively proved trans-stadial transmission where in larvae, nymphs and adults are able to transmit the disease. In infected ticks *E canis*multiplies within haemocytes and cells of the salivary gland. Then organism enters digestive tract and infects midgut epithelium. Ticks could transmit *E.canis* for 155 days. Attraction of mononuclear cells to the inflamed site of tick bite may facilitate the infection of blood monocytes. *Amblyommaamericanum* and *Otobiusmegnini* were most recently described as potential vectors Animals in most parts of the world are infected with Ehrlichia species, mainly in tropical and temperate temperatures. Ehrlichia can be found worldwide due to the presence of the tick Rhipicephalus sanguineus. It is still not clear where some of these people are located. For dogs that have been relocated to a non-endemic area, illness signs may not appear for years after tick transmission [16].Wild and domestic canids are currently considered the naturally susceptible to infection. Usually, the infection is sporadic and is of worldwide occurrence. Even though disease occurs in all breeds of dogs. German shepherds are most susceptible. All ages are affected, but males are found to be more affected [5, 13].

Etiology

Ehrlichiosis in dogs is caused by *E. canis*, which is transmitted by the tick *Rhipicepalussanguineus.* It mostly affects monocytes, which are white blood cells. However, Ehrlichiosis in humans caused by *E canis*is reported [17]. For this virus, both domestic and wild dogs serve as reservoir hosts, as well as primary hosts for brown dog ticks. Whenever a rickettsial dog bites a brown dog tick, the tick becomes infected with the virus. Located in the tick's midgut and salivary glands, *E canis* can be transmitted to hosts via the saliva of ticks that contain the infection. This occurs when the tick's larva gets infected and the virus persists in the tick's body for the next two life stages, where it can infect the host while feeding on blood [18].

Morphology

Ehrlichiacanis and other members of the genus Ehrlichia are minute gram-negative cocci that stain dark blue to purple with Romanowsky stain and with Machiavello method they stain light red and brown-black with silver stain. Like Chlamydia, Ehrlichia go through three developmental stages namely elementary bodies (individual Ehrlichia organisms), initial bodies (immature organismal inclusions) and morula (mature organismal inclusions). Elementary bodies are small gram-negative organisms about 0.2-0.5 µm in diameter and difficult to detect by light microscopy [1, 3,7]. Elementary bodies are usually coccoid or ellipsoidal. Elementary bodies enter canine monocytes by phagocytosis. Phagolysosomal fusion does not occur in infected cells and elementary bodies begin to grow and divide within the confines of the phagosome. Replication of organism occurs by

binary fission. At 3 -5 days after infection, small members of tightly packed elementary bodies 1.0-2.5 µm in diameter are observable as pleomorphic inclusions, called as initial bodies. During next 7-12 days, additional growth and replication occurs and initial bodies develop to mature inclusions called mulberry or morula (4-6 µm in diameter). Infected monocytes usually contain several morula each containing several dozen elementary bodies. Morula breaks up into elementary bodies when infected cell ruptures and infectious cycle is repeated. All the three growth stages occur within a membrane-lined vacuole of host origin that separates individual organisms or a group of these from the host cell cytoplasm [11,18].

Life cycle

Rhipicephalus sanguineus is the principal tick that carries Ehrlichia. It has elementary bodies, beginning bodies, and morulae as its developmental stages. Intracellular inclusion bodies (morulae: 2 to 5 m) consisting of roughly 100 elementary bodies (0.2 to 0.5 m) develop from small, elementary bodies [15]. After sticking to the plasma membrane, the single-celled organism enters the host cell and grows into larger morula through binary fission. Cellular exocytosis occurs when the organism breaks the cell membrane or when the cell is ruptured[18].

PATHOGENESIS

Pathogenesis depends on the strain of organism, breed of dog affected, concomitant diseases and the host defense mechanism. The most common finding in an acute *E canis* infection is the extensive invasion of parenchymal organs and perivascular cuffing by plasma cells particularly lungs, meninges, kidneys and spleen suggesting an immunopathologic etiology. Lymphocytes of infected dogs exert a cytotoxic effect upon autologous monocytes. Leukopaenia along with a highly prominent thrombocytopenia is a pathognomonic haematologic manifestation for canine ehrlichiosis. The clinical findings such as pale mucous membranes, hepatomegaly, lymphadenopathy, splenomegaly, emaciation, increased hair loss and hematological alterations observed in these infected animals have been previously reported in cases of canine ehrlichiosis. Thrombocytopenia, leucopenia, and anemia are most frequently observed in CME. Infected dogs presented mild to moderate hematological changes in acute experimental infection for just a few weeks [4, 9]. The tendency for the hematological parameters to return to normal was evident at the end of the experiment. This result may be a consequence of transient suppression of bone marrow activity due to *E canis* infection. The pathogenesis of thrombocytopenia is due to Anti-platelet antibodies produced in CME. Anemia may explain the observed paleness of mucous membranes and most organs. Lymphadenopathy, splenomegaly, ascites, paleness of mucous membrane, kidney and liver, and discrete pulmonary congestion were observed at the necropsy of inoculated dogs as has been previously reported in cases of canine ehrlichiosis.

There is no peptidoglycan or lipopolysaccharide in the cell walls of ehrlichial organism, which is thought to contribute to its capacity to resist the immunological response of the host. Due to the lack of these two components, the cell wall is less stiff and the cell wall's outside is more dynamic, which helps *E canis* cells evade antibodies in their host organism's body. There are no complicated internal structures that allow *E canis* to synthesise sugar and instead it uses amino acids as its energy source [19]. An incubation period of 8 to 20 days precedes the acute, subclinical, and, in some cases, chronic phases of the disease's aetiology[11]. It is important to note that Ehrlichia do not include pili, thus once they adhere to the host cell's outer membrane, they can enter and cause infection. Encapsulated within the host cell, Ehrlichia create membrane bound compartments (endosomes) that aid in the maintenance of the bacteria's unique cytoplasmic compartments within. To infect mononuclear phagocytic cells, *E canis* must first be transferred to the cells. Monocytes are the most commonly infected cells in the human or canine host. But monocytes are not the only cells that are susceptible to infection. Monocytes, on the other hand, are not the only cells susceptible to infection. ehrlicial infection has also been seen in lumphocytes, promyelocytes, and metamyelocytes in another research. In spite of this, mononuclear phagocytes are believed to be able to retain the productive infection within their cells due to their higher frequency and higher rate of infection [20]. The number of morulae in an infected monocyte is usually between 1 and 2. Because Ehrlichia reproduce within the host's endosomes, they are able to survive. As a result, these infections are able to ensure their own life by reprogramming the host cell's defence systems and mechanisms [21].

Clinical disease

After an incubation period of 10-15 days canine ehrlichiosis begins as an acute febrile disease. The first stage is characterized by fever 40-41.4ºC, depression, anorexia, weight loss, oculonasal discharge, conjunctivitis, occasional vomiting and lymphadenopathy. Oedema of limbs and ataxia may also be present. The acute phase typically last for 2 or 3 weeks. Most dogs survive acute phase followed by a subclinical phase lasting several months. Animals remain infected but are generally asymptomatic and blood values remain subnormal. Characteristic features of TCP are occasional fever, corneal opacity, regenerative or non-

regenerative anaemia with severe leukopaenia and thrombocytopenia [6, 7]. Initially there may be bone marrow hyperplasia followed by hypoplasia due to the exhaustion of cellular elements in the bone marrow. The outcome of the third and the terminal phase depends on the breed of the dog. Beagles, for example, may become chronic carriers. German shepherds usually succumb. Clinical manifestations include fever, anorexia, severe weight loss, marked pancytopaenia, anaemia and peripheral oedema. Ecchymosis and petichiae commonly occur at multiple sites. Unilateral or bilateral epistaxis is common. Death occurs due to extensive mucosal or serosal haemorrhage or due to secondary bacterial infection prompted by the dog's debilitated condition. Increased ESR and prolonged bleeding time is very characteristic. Dogs are also susceptible to infection with *Eequi* and *Eristicii* which is mild and inapparent [1-5].

In its acute, subclinical and chronic phases, CME is a multisystemic disease that affects multiple organ systems. There is no preference for either gender or age. Pathogenicity of *E canis* strains and co-infections with other arthropod-borne pathogens such *Babesia canisvogeli* and *Hepatozooncanis* transmitted by the same vector can impact disease symptoms [22]. Fever, fatigue and anorexia are some of the symptoms of the acute form of the disease. Other symptoms include lymphadenomegaly and hemorrhagic tendencies. Dermal petechiae and ecchymoses, as well as epistaxis, are common manifestations. Lesions of the eye, such as anterior uveitis, chorioretinitis, papilledema, and retinal hemangiomas, are common in patients with MS. As a result, ophthalmological abnormalities, such as anterior uveitis, chorioretinitis and papilledema are common [23]. Blood hyperviscosity can cause subretinal haemorrhage and retinal detachment, resulting in blindness [24]. When meningitis and/or meningeal haemorrhage are present, neurological symptoms might include a wide spectrum of symptoms. At this stage, no clinical indications are visible [25]. The chronic phase is characterised by pale mucous membranes, weakness, bleeding, and considerable weight loss [26].

Diagnosis

Diagnosis is done with the help of clinical signs along with other measures like haematological findings, blood smear evaluation, biochemistry, molecular detection etc.

Haematological findings

The complete blood count (CBC) is a vital diagnostic tool for CME. Major haematological findings during the acute period include moderate to severe thrombocytopenia. In order to distinguish between real thrombocytopenia and in vitro pseudo-thrombocytopenia, a blood smear examination of platelet counts is required. Anemia and a mildly diminished white blood cell count accompany the thrombocytopenia in the acute phase. Subclinically, thrombocytopenia may be present even if there are no clinical symptoms. Experimentally infected dogs had platelet levels as low as 42 percent below normal. There may be a minor thrombocytopenia in the subclinical period, even though there are no symptoms. In experimentally infected dogs, platelet counts were lowered by up to 42 percent, with numbers as low as 140,000/L being reported [3]. Thrombocytopenia is frequently severe in the chronic phase, along with anaemia and leukopenia. In the chronic severe type, marked pancytopenia due to bone marrow hypoplasia is a defining feature [26]. Different processes are responsible for the thrombocytopenia in CME, depending on the illness stage. Thrombocytopenia in the acute phase of the disease is thought to be caused by increased platelet consumption due to inflammatory changes in blood vessel endothelium, increased splenic sequestration of platelets, and immunologic destruction or injury resulting in a significantly decreased platelet life span, among other mechanisms [27]. As a result, these infections are able to ensure their own life by reprogramming the host cell's defence systems and mechanisms [21].

Blood smear examination

Using light microscopy, the presence of *E canis*morulae in monocytes in blood smear samples clearly suggests CME. Using electron microscopy, morulae are membrane-bound vacuoles that are typically densely packed with bacteria [28]. Using numerous buffy coat smears raises the odds of discovering *Ecanis*morulae substantially. Platelets, azurophilic lymphocytic nuclei, and phagocytosed material can all be mistaken for ehrlichial inclusions. Canine monocytes may also be infected by other ehrlichial organisms belonging to the Anaplasmatacea family such as *Ehrlichiachaffeensis* or *Neorickettsiaristicii*. Virus-infected dogs may have reactive monocytes, erythrophagocytosis, thrombophagocytosis, nuclear material phagocytosis, and megaplatelets in their blood. In advanced stages, large granular lymphocytosis can occur. Co-infections (eg.*Babesia canis* and *Hepatozooncanis*) that affect disease onset, severity, and treatment outcome must also be evaluated by blood smears [29].

Biochemistry

When dogs are infected with *E canis*, the most common abnormalities detected are hypoalbuminemia, hyperglobulinemia, and hypergammaglobulinemia [11]. Anemia in CME may be caused by peripheral albumin losses

to edematous inflammation fluids due to an increase in blood loss or decreased protein production due to minor liver illness or it may be caused by a minimal-change glomerulopathy, according to the American Society of Nephrology. When the oncotic pressure is low, the drop in albumin concentrations may operate as a compensation mechanism for hyperglobulinemia, preserving the oncotic pressure and keeping blood viscosity from increasing [30]. Typically, the hypergammaglobulinemia in CME is polyclonal in nature. Monoclonal gammopathy is an uncommon condition that can cause hyperviscosity and other symptoms [31]. Dog ehrlichiosis febrile phase is characterised by a rise in Gamma globulin levels that continues into the subclinical and chronic phases of the disease [32]. Gamma globulin concentrations and particular *E canis* antibody titers had a poor association. The lack of association between these two characteristics and the polyclonal gammopathy observed in most ill dogs suggests that *E canis* induces nonspecific antibody production and that anti-*E canis* antibodies are not the predominant source of gamma globules in most sick dogs. Anti-*E canis*antibodies are not the main source of hyper-gammaglobulinemia based on the poor association between these two parameters as well as polyclonal gammopathy observed in most sick dogs, which suggests that *E canis* induces nonspecific antibody production. When antigenic stimulation is extended, this behaviour is known to occur in various illnesses [33]. It is common for dogs to have mild elevations in alanine aminotransferase and alkaline phosphatase during the acute period. During clinical CME, protein concentrations in the acute phase may increase. On average, C-reactive protein (CRP) values rise between 4 and 16 days after pea consumption. Five dogs were inoculated with E. canis and their C-reactive protein (CRP) levels increased between 4 and 16 days and peaked between 15 and 42 days later [34]. All experimentally inoculated dogs with *E canis* showed a 2-to-9-fold increase in CRP and AAG levels 4–6 days post-inoculation *Ecanis* remained in the dogs for a total of 34 days post-exposure, however CRP and AAG concentrations gradually returned to their pre-exposure levels by day 34. Additionally, CRP and AAG were elevated in most naturally unwell canines [35].

Serology

Several serological techniques have been developed for the diagnosis of CME and are regarded as helpful screening and/or diagnostic tools for this disease. A serological 'gold standard' is the indirect immunofluorescence antibody (IFA) test for anti-*E canis* IgG antibodies. *Ehrlichiacanis* exposure is not reliably determined by IgM due to the inconsistency with which IgM antibodies develop during the course of the disease [36]. Serum antibodies to *E canis* have been discovered as early as 7 days after initial infection in dogs infected experimentally, while some dogs do not become seropositive until 28 days following infection. The serology should be performed after 2-3 weeks if ehrlichiosis is highly suspected clinically in a seronegative dog. Titres >1:80 of IgG antibodies were once regarded diagnostic, but current research has shown that titers less than 1:80 should be considered questionable, and serology should be revisited in 2-3 weeks, or PCR and Western immunoblotting may be examined as alternatives. When clinical symptoms and clinicopathological abnormalities compatible with canine ehrlichiosis are discovered, a diagnosis should be made and treatment initiated [37]. It has been discovered that enzyme-linked immunosorbent assays (ELISA) can be used to diagnose the condition in addition to IFA tests. To identify *E canis* IgG antibodies, commercially available dot-ELISA kits can be utilised. To solve this sensitivity issue, it was recommended that the ELISA test be repeated 1–2 weeks following the first antibody testing [38].

Isolation

A continuous canine macrophage cell line (DH82) has been used successfully to culture *E canis*. Isolation of *E canis* is used more in research laboratories and less as a diagnostic tool.

Molecular detection

Ehrlichiacanis DNA can be detected and characterised using PCR and sequencing, which are highly sensitive procedures. A false positive result can occur when annealing temperatures are kept relatively low or when impurities or non-specific amplification occur. No target DNA was found in the PCR, but this does not mean that no DNA was present in the sample (false negative result). In 4–10 days after immunisation, it is possible to detect *E canis* DNA in the blood [39]. However, the most popular assays are those using 16S rRNA and p30 as target genes (eg. 16S-ribosomal RNA (rRNA 16S) as well as other target genes such as dsb and virB9). The nested PCR test based on p30 has been proven to be more sensitive than the p30-based assay. In comparison to 16S rRNA, the p30-based nested PCR assay was proved to be more sensitive [40]. To determine ehrlichial elimination, blood and bone marrow samples are more sensitive than spleen samples for PCR [3, 4]. Quantitative PCR is more sensitive than traditional PCR and allows for assessment of bacterial burden. When measuring ehrlichial burden in normally infected dogs as well as experimentally infected dogs [41].

Treatment strategies

Canine Monocytic Ehrichiosis is treated with Doxycycline, a semi-synthetic tetracycline, which is particularly successful in treating acute CME [22]. A dose of 5 mg/kg, orally, twice daily, for at least 28 days is recommended for CME[37], although persistently infected dogs may need a longer course of treatment [13]. As a time-dependent medicine, doxycycline is normally taken twice daily. If you take doxycycline once or twice daily, the drug level is unlikely to fall below MIC threshold of *E canis*because of doxycycline's low MIC value [42]. Anorexia, vomiting, and diarrhoea are all possible side effects of doxycycline medication in dogs.If your dog has anorexia, vomiting, diarrhoea, or rapid spikes in alanine aminotransferase, or alkaline phosphatase activities after therapy, doxycycline may not be for them [43]. As an alternative to doxycycline, rifampicin, an inhibitor of DNA-dependent RNA polymerase's B subunit, has been studied as a potential treatment for CME. According to experiments conducted in vitro, rifampicin was as effective as doxycycline in treating *E canis* and had an identically low minimum inhibitory concentration (MIC), which was 0.03 mg/L[44].

Rifampicin (15 mg/kg, PO, twice daily for seven days) reversed haematological abnormalities and prevented *E canis* DNA from being amplified from blood in two experimentally infected, moderately pancytopenic dogs [45]. When dogs were treated with rifampicin (10 mg/kg, PO, once daily for 21 days), their haematological recovery was faster than when they were not treated, but they only cleared *E canis* in 2/5thof the dogs, as measured by blood PCP [46]. Although Imidocarb dipropionate is no longer recommended for CME unless there is a co-infection with protozoa such as *Babesia canis*, it has not been shown to be beneficial in promoting haematological recovery or eliminating natural or acute experimental infections.However, in vitro studies of *Ecanis* infection showed that the DNA gyrase inhibitor, Enrofloxacin, was inefficient and failed to offer haematological recovery or clearing of acute experimental *E canis* infection[48, 49].

When doxycycline treatment is initiated, dogs with acute CME exhibit rapid clinical improvement within 24–48 hours [4, 37, 46, 50]. In order to temporarily offset the systemic implications of severe anaemia, the reasonable administration of balanced crystalloid solutions and/or the periodic administration of blood-typed, cross-matched packed red blood cells or whole blood transfusions should be considered [51]. Thrombocytopenia recurrence doxycycline treatment failure, re-infection, or concomitant infection with organisms that are partially doxycycline-responsive but not curable (eg. Babesia species and Bartonella species) can be detected 2–4 weeks after the drug is stopped [49]. Two weeks following the start of treatment, haematological examination (including blood smear examination) should be undertaken to assess the hematologic response to treatment.It is therefore necessary to do two weeks of haematological examinations (including blood smear examination) before, throughout, and four weeks after completion of treatment in order to assess hematologic response[46]. PCR is currently the most reliable and economical approach in the clinical environment for proving the clearance of *E canis* infection [4, 46]. A further three-to-four-week therapy should be delivered if PCR findings are still positive, and the dog should be treated with acaricide to avoid re-infection.This medication should be continued for 3–4 weeks. Acaricides should be used to prevent re-infection as well as retesting the dog if PCR findings are still positive. It is possible to use an alternative medicine (such as switching from doxycycline or minocycline to rifampicin) if PCR results remain positive after two treatment rounds, as the infection may not be cleared [52].

Prevention and control

Dogs and premises can be kept tick-free in endemic areas by following thorough tick-control measures. Without the use of chemicals, ticks are removed from dogs by hand, with just one gentle touch near where they are attached [53]. By reducing refuge spots like floor cracks, garden debris and overgrown grass we can change the habitat. Indirect immunofluorescence assay should be performed to test new arrivals to kennels that are known to be Ehrlichia negative, and doxycycline should be used to treat any positive dogs prior to being housed with the other dogs. Ticks should also be thoroughly checked for, and the dog should be treated accordingly. Ticks should also be thoroughly checked, and the dogs should be treated with acaricides. There are other tick preventatives such as Frontline and tick collars such as Preventic that you can use, but be sure to follow your veterinarian's instructions on how to use them. Keep your yard's bushes and grass manicured, as ticks love to hang around and reproduce in these spots. The only preventive therapeutic measure for canine ehrlichiosis is administration on a continuous basis Tetracycline at a low dosage of 6.6mg/kg/day. This can be tried in dogs travelling in enzootic area. Routine use of acaricides and control of ticks is recommended. All new dogs should be free of tick infestation and serological status screened before introducing them to *E canis* free groups.Treatment with Doxycycline at 3 mg/kg PO q24h when visiting an endemic location reduces the risk of infection, but may lead to antibiotic resistance [54]. Recovery from a previous infection does not guarantee a long-term cure.

II. CONCLUSION

Canine ehrlichiosis is a tick borne, febrile, debilitating disease of dogs and wild canidae caused by

Ehrlichiacanis transmitted by the brown dog tick *Rhipicephalus sanguineus.* The infections are known to occur worldwide. Ehrlichiosis occurrence in canine usually remains preclinical. The disease was characterized by general debilitation, fever, anorexia, marked pancytopaenia, anaemia and peripheral oedema. Ecchymosis and petichiae commonly occur at multiple sites. Unilateral or bilateral epistaxis is common. The most commonly followed methodology for diagnosis of acute ehrlichiosis is by microscopic detection of organism in Giemsa-stained blood smears or buffy coat preparations. The morular rosettes take a light blue or lilac tint within the cytoplasm of the monocytes. Among all therapeutics tested, tetracyclines are the most effective for the treatment of *E canis* and other ehrlichial infections of dogs.Dogs and premises can be kept tick-free in endemic areas by following thorough tick-control measures. The only preventive therapeutic measure for canine ehrlichiosis is administration on a continuous basis Tetracycline at a low dosage of 6.6mg/kg/day. This can be tried in dogs travelling in enzootic area. Routine use of acaricides and control of ticks is recommended. All new dogs should be free of tick infestation and serological status screened before introducing them to *E canis* free groups.

REFERENCES

[1] Rikihisa Y, PerryBD and Cordes DO. Ultrastructural study of ehrlichial organisms in the large colons of ponies infected with Potomac horse fever. InfectImmun,1985; 49:505–512.

[2] Rikihisa Y, Zhi N, Wormser GP, Wen B, Horowitz HW andHechemy KE. Ultrastructural and antigenic characterization of a granulocytic ehrlichiosis agent directly isolated and stably cultivated from a patient in New York State. J Infect Dis,1997; 175:210–213.

[3] 3)Harrus S, Wane, T, Aizenberg I, Foley JE, Poland AM and Bark H. Amplification of ehrlichial DNA from dogs 34 months after infection with *Ehrlichiacanis*. J Clinical Microbiol, 1998; 36:73–76.

[4] Harrus S, Kenny M, Miara L, Aizenberg I, Waner T and Shaw S. Comparison of simultaneous splenic sample PCR with blood sample PCR for diagnosis and treatment of experimental Ehrlichiacanis infection. Antimicrob. Agents Chemother, 2004; 48: 4488–4490.

[5] Trotz-Williams LA and Trees AJ. Systematic review of the distribution of the major vector-borne parasitic infections in dogs and cats in Europe. Vet Rec,2003; 152: 97-105.

[6] Johnson EM, Ewing SA, Barker RW, Fox JC, Crow DW and Kocan K. Experimental transmission of Ehrlichiacanis (Rickettsiales, Ehrlichieae) by Dermacentor variabilis (Acari: Ixodidae). Vet Parasitol, 1998; 74: 277–288.

[7] Ettinger SJ and Feldman EC: Textbook of Veterinary Internal Me dicine: Diseases of the Dog and Cat, 5 th ed. W.B. Saunders Co., Philadelphia, 2000; 1: 402-406.

[8] Mylonakis ME, Koutinas AF, Breitschwerdt EB, Hegarty BC, Billinis CD, Leontides LS and Kontos VI. Chronic canine ehrlichiosis (Ehrlichiacanis): a retrospective study of 19 natural cases. J Am Anim Hosp Assoc, 2004; 40: 174-184.

[9] Ristic M and Holland CJ. Canine ehrlichiosis, in Rickettsial and Chlamydial Diseases of Domestic Anmals, Z. Woldehiwet and M. Ristic, Editors. Pergamon: Oxford, 1993; pp. 169-186.

[10] Harrus S. and Waner T.Diagnosis of canine monocytotropic ehrlichiosis (*Ehrlichiacanis*): An overview. Vet. J; 2011; 87: 292-296.

[11] Harrus S, Waner T, Weiss DJ, Keysary A and Bark H. Kinetics of serum antiplatelet antibodies in experimental acute canine ehrlichiosis. Vet Immunol Immunopathol. 1996; 51: 13-20.

[12] 12)Waner T, LeykinI, Shinitsky M, Sharabani E, Buch H, Keysary A, Bark H and Harrus S. Detection of platelet-bound antibodies in beagle dogs after artificial infection with *Ehrlichiacanis.* Vet Immunol Immunopathol. 2000; 77: 145-150.

[13] Harrus S, Waner T, Aizenberg I, Foley JE, PolandAM. and Bark H. Amplification of ehrlichial DNA from dogs 34 months after infection with *Ehrlichiacanis*. J Clin Microbiol. 1998; 36: 73-76.

[14] Waner T, Harrus S, BarkH, Bogin E, Avidar Y and Keysary, A. Characterization of the subclinical phase of canine ehrlichiosis in experimentally infected beagle dogs. Vet Parasitol1997; 69: 307-317.

[15] Kruss H, Weber M, Appel B, Enders HD, Isenberg HG, Schiefer W,Slenczka A and ZahnerH. Zoonoses: Infectious Diseases Transmissible from Animals to Humans. 3 rd edition. Washington. D.C ASM Press., 2003; pp 456.

[16] Neer TM. Canine monocytic and granulocytic ehrlichiosis. In: Greene C.E, ed. Infectious Diseases of the Dog and Cat, 2nd ed. Philadelphia, PA: WB Saunders., 1998; pp139–147.

[17] Perez M, Bodor M, Zhang C, Xiong Q and Rikihisa Y. "Human I nfection with *Ehrlichia Canis* Accompanied by Clinical Signs in Venezuela". Annals New York Acad Sci. 2006; 1078: 110–117.

[18] Bowman D, Susan E, Lorentzen L, Shields J, Sullivan P and Carlin EP. Prevalence and geographic distribution of *Dirofilariaimmitis*, *Borrelia burgdorferi*, *Ehrlichiacanis*, and *Anaplasmaphagocytophilum* in dogs in the United

States: Results of a national clinic based serologic survey. Vet Parasitol. 2009; 160: 138–148.

[19] Mavromatis K, Doyle CK, Lykidis A, Ivanova N, Francino MP, Chain P, Shin M, Malfatti S, Larimer F, Copeland A, Detter JC, Richardson PM, Yu XJ, Walker DH, McBride JW and Kyrpides NC. The Genome of the Obligately Intracellular Bacterium *Ehrlichiacanis* Reveals Themes of Complex Membrane Structure and Immune Evasion Strategies. J Bacteriol. 2006; 188(11): 4015–4023.

[20] Paddock C and Childs E. *Ehrlichiachaffeensis*: a Prototypical Emerging Pathogen. Clinical Microbiol Rev. 2003; pp 37–64

[21] Ismail N, Karen C and Jere W. Human Ehrlichiosis and Anaplasmosis. Clinics in Laboratory Medicine, 30: pp. 261-292.

[22] Gal A, Harrus S, Arcoh I, Lavy E, Aizenberg I, Mekuzas-Yisaschar Y andBaneth G. Coinfection with multiple tick-borne and intestinal parasites in a 6-weekold dog. Canadian Vet J.2010; 48: 619–622.

[23] Komnenou AA, Mylonakis ME, Kouti V, Tendoma L, Leontides L, Skountzou E, Dessiris A, Koutinas AF andOfri R. Ocular manifestations of natural canine monocytic ehrlichiosis (*Ehrlichiacanis*): a retrospective study of 90 cases. Vet.Ophthalmol.2007; 10: 137–142.

[24] Harrus S, Ofri R, Aizenberg I andWaner T. Acute blindness associated with monoclonal gammopathy induced by *Ehrlichiacanis* infection. Vet Parasitol. 1998; 78: 155–160.

[25] Waner T, Harrus S, BarkH, Bogin E, Avidar Y and Keysary A. 1997. Characterization of the subclinical phase of canine ehrlichiosis in experimentally infected beagle dogs. VetParasitol. 69: 307–317.

[26] Harrus S, Waner T and Bark H. Canine monocytic ehrlichiosis update. Compendium for Continuing Education for the Practicing Veterinarian, 1997; 19: 431–444.

[27] Kakoma I, Carson CA, Ristic M, Stephenson EM, Hildebrandt PK and Huxsll DL. Platelet migration inhibition as an indicator of immunologically mediated target cell injury in canine ehrlichiosis. Infect Immun. 1978; 20: 242-247.

[28] Hildebrandt PK, Conroy JD, McKee AE, Nyindo MB andHuxsoll DL.Ultrastructure of Ehrlichiacanis. Infect Immun.1973; 7: 265–271.

[29] Gal A, Harrus S, ArcohI, Lavy E, Aizenberg I, Mekuzas-Yisaschar Y andBaneth G. Coinfection with multiple tick-borne and intestinal parasites in a 6-week-old dog. Canadian Vet J. 2007; 48: 619–622.

[30] Woody BJ and Hoskins JD.Ehrlichial diseases of dogs. Vet. Clin. N. Am. Small Anim. Pract, 1991;21: 75–98.

[31] Perille AL and Matus RE. Canine ehrlichiosis in six dogs with persistently increased antibody titers. J Vet Int Med. 1991; 5: 195–198.

[32] Ristic M and Holland CJ. Canine ehrlichiosis. In Rickettsial and chlamydial diseases of domestic animals. Eds. Woldehiwet Z., Ristic M. (Pergamon Press, Oxford, United Kingdom). 1993; pp 169–186.

[33] Tizard I. An introduction to veterinary immunology (W. B. Saunders Company, Philadelphia, Pa), 2nd ed. 1982; pp 336–342.

[34] Shimada T, Ishida Y, Shimizu M, NomuraM, Kawato K, Iguchi K andJinbo T. Monitoring C-reactive protein in beagle dogs experimentally inoculated with *Ehrlichiacanis*. Vet Res Commun. 2002; 26: 171–177.

[35] Rikihisa Y, Yamamoto S, Kwak I, Iqbal Z, Kociba G, Mott J andChichanasiriwithaya W. C-reactive protein and alpha 1-acid glycoprotein levels in dogs infected with *Ehrlichiacanis*. J Clinical Microbiol. 1994; 32: 912–917.

[36] McBride JW, Corstvet RE, Gaunt SD, Boudreaux C, Guedry T and Walker DH. Kinetics of antibody response to *Ehrlichiacanis* immunoreactive proteins. Infect Immun. 2003; 71: 2516–2524.

[37] Neer TM, Breitschwerdt EB, Greene RT and Lappin MR. Consensus statement on ehrlichial disease of small animals from the Infectious Disease Study Group of the ACVIM. J Vet Intern Med. 2002; 16: 309-315.

[38] Harrus S, Alleman AR, Bark H, Mahan SM andWaner T. Comparison of three enzyme-linked immunosorbant assays with the indirect immunofluorescent antibody test for the diagnosis of canine infection with Ehrlichiacanis. Vet Microbiol.2002; 86: 361–368.

[39] Iqbal Z, Chaichanasiriwithaya W andRikihisa Y. Comparison of PCR with other tests for early diagnosis of canine ehrlichiosis. J Clinical Microbiol. 1994; 32: 1658–1662.

[40] Stich RW, Rikihisa Y, Ewing SA, Needham GR, Grover DL andJittapalapong S. Detection of Ehrlichiacanis in canine carrier blood and in individual experimentally infected ticks with a p30-based PCR assay. J Clinical Microbiol. 2002; 40: 540–546.

[41] Baneth G, HarrusS, Ohnona FS and Schlesinger, Y., 2009. Longitudinal quantification of Ehrlichiacanis in experimental infection with comparison to natural infection. Vet Microbiol. 2009; 136: 321–325.

[42] Ruiz SM, Olvera LG, ChacónSdel C, Estrada DV. Pharmacokinetics of an oral extended-release formulation of doxycycline hyclate containing acrylic acid and polymethacrylate in dogs. Am J Vet Res. 2015; 76: 367–372.

[43] Schulz BS, Hupfauer S, Ammerm H, Sauter-Louism C andHartmannm K. Suspected side effects of doxycycline

use in dogs-a retrospective study of 386 cases. Vet Rec. 2011; 169:229p.

[44] Brouqui P andRaoult D. Susceptibilities of Ehrlichiae to antibiotics, Antimicrobial Agents and Intracellular Pathogens. First edn. CRC Press, Boca Raton, FL, USA. 1993; pp179–199

[45] Schaefer JJ, Kahn J, Needham GR, Rikihisa Y, Ewing SA and Stich RW. Antibiotic clearance of Ehrlichiacanis from dogs infected by intravenous inoculation of carrier blood. Ann. N. Y. Acad. Sci. 2008; 1149: 263–269.

[46] Theodorou K, Mylonakis ME, Siarkou VI, Leontides L, Koutinas AF, Koutinas CK, Kritsepi-Konstantinou M, Batzias G, Flouraki E. andEyal O. Efficacy of rifampicin in the treatment of experimental acute canine monocytic ehrlichiosis. J AntimicrobChemother. 2013; 68: 1619–1626.

[47] Sainz A, Roura X, Miro G, Estrada-Pena A, Kohn B, Harrus S andSolano-Gallego L. Guideline for veterinary practitioners on canine ehrlichiosis and anaplasmosis in Europe. Parasit Vectors. 2015; 8: 75.

[48] Kontos VI andAthanasiou LV. Use of enrofloxacin in the treatment of acute canine ehrlichiosis. Canine Pract. 1998; 23: 10–14.

[49] Neer TM, Eddlestone SM, Gaunt SD andCorstvet RE.Efficacy of enrofloxacin for the treatment of experimentally induced *Ehrlichiacanis* infection. J Vet Int Med. 1999; 13: 501–504.

[50] Breitschwerdt EB, Hegarty BC and Hancock SI. 1998. Doxycycline hyclate treatment of experimental canine ehrlichiosis followed by challenge inoculation with two *Ehrlichiacanis* strains. Antimicrob Agents Chemother. 1998; 42: 362–368.

[51] Shipov A, Klement E, Reuveni-Tager L, Waner T andHarrus, S. Prognostic indicators for canine monocytic ehrlichiosis. Vet Parasitol. 2008; 153: 131–138.

[52] Neer TM, Breitschwerdt EB, Greene RT and Lappin MR. Consensus statement on ehrlichial disease of small animals from the infectious disease study group of the ACVIM: American College of Veterinary Internal Medicine. J Vet Intern Med. 2002; 16: 309–315.

[53] Blagburn BL and Dryden MW. Biology, treatment and control of flea and tick infestations. Vet Clinic Small Ani.2009; 39: 1173-1200.

[54] Goodman JL Ehrlichiosis, ticks, dogs, and doxycycline. N Engl J Med. 2009; 341: 195–197.