Ehrlichiosis in Dogs

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In dogs, ehrlichiosis is caused by three Ehrlichial species, namely *Ehrlichia canis*, *E. ewingii*, and *E. chaffeensis*; however, *E. canis* is the pathogen that most affects platelets, monocytes, and granulocytes. Globally, *Rhipicephalus sanguineus* is mainly responsible for vectoring the Ehrlichia species; however, *Haemaphysalis longicornis* is also involved in vectoring this species in east Asian countries. This disease causes acute, sub-clinical, and chronic clinical complications. There is no preferable age or sex for ehrlichiosis. The disease can be diagnosed by various methods including microscopy, indirect immunofluorescence test (IFAT), and polymerase chain reaction (PCR).

Ehrlichia canis | Ehrlichia chaffeensis | Ehrlichia ewingii | dog | epidemiology | tick borne diseases

1. Introduction

Ehrlichiosis is induced by a group of emerging rickettsial tick-borne pathogens of public importance that are Gram-negative obligate intracellular bacteria of the genus *Ehrlichia*, family Anaplasmataceae [1]. Ehrlichiosis in dogs is a significant vector-borne bacterial ailment spreading worldwide. It is also recognized as canine rickettsiosis, canine typhus, canine hemorrhagic fever, tropical canine pancytopenia, and tracker dog disease [2]. Numerous species of *Ehrlichia* are famous for infecting a wide range of animals. Among them, *E. canis* is the well-known etiological pathogen of canine ehrlichiosis affecting platelets, monocytes, and granulocytes [3][4]. Although *E. chaffeensis* is considered the main etiological agent of human monocytic ehrlichiosis (HME), it is also reported in canines [5]. A minimum of five different species of ticks (*Amblyomma americanum*, *Haemaphysalis longicornis, Rhipicephalus sanguineus*, *Haemaphysalis yeni,* and *Dermacentor variabilis*) have been identified as vectors transmitting clinical ehrlichiosis in dogs [6]. These disease-transmitting vectors become more potent during summer and spring seasons [7]. *Rhipicephalus sanguineus* (the brown tick of dogs) is generally considered the main vector responsible for canine ehrlichiosis [8][9]. In recent years, ehrlichiosis has been expanded to new regions which were thought to be disease free, such as northern China, temperate regions of the Indian sub-continent, and central and northern states of the USA [10].

Ticks are considered the second most prevalent hematophagous parasites after mosquitoes. Along with causing anemia, they also act as vectors for the transmission of many protozoan, bacterial, and viral diseases [11][12]. In recent years, ecological variations due to global warming, exponential increase in human population, deforestation,

and frequent transport of pet animals from one continent to another have modified and enhanced the transmission patterns of all vector-borne pathogens around the globe [13]. In south and east Asia, regardless of the suitable climatic conditions for vectors and parasites and huge population of stray and pet dogs, scarce knowledge is available related to diagnosis, epidemiology, prevention, and control strategies associated with ehrlichiosis in dogs. However, with the expansion of a region's economy and inculcation of foreign culture, the responsibilities of veterinarians have increased to devise control strategies about canine tick-borne ailments [14]. In less developed areas of east and south Asia, almost 75 percent of dogs are categorized as stray dogs, which further increases the risk of emergence of new tick-borne parasitic zoonoses [15].

2. Epidemiology

Ehrlichiosis is a disease of global importance, but it is more prevalent in sub-tropical and tropical regions. However, due to the chronic nature of the disease in some cases, accurate geographical distribution cannot be determined. The reason may be due to the fact that the clinical signs appear years after the first inoculation of the pathogen from ticks and after the canine species has traveled to non-endemic countries where this specific ailment might not be included in differential diagnostic lists by clinicians and scientists [16]. On the basis of different diagnostic methods, the prevalence in the south and east Asia ranges from 0.0% (South Korea) to 86.9% (India) [17][18]. The prevalence percentage of all *Ehrlichia* spp. related to canines in different countries of south and east Asia is presented in **Table 1**.

Table 1. Prevalence studies on ehrlichiosis in dogs conducted in south and east Asian countries.

Country/Region	Sample Source	Causative Agent/Species	Diagnostic Method	Prevalence % (Positive/Total Number of Samples)	Reference
South Asia Pakistan	Blood	*Ehrlichia canis*	PCR	28 (42/151)	[19]
Pakistan	Blood	*Ehrlichia canis*	PCR	24.5 (12/49)	[20]
India	Blood	*Ehrlichia canis*	PCR	8 (12/150)	[21]
India	Blood	*Ehrlichia canis*	PCR	8.40 (70/833)	[22]
India	Ticks Blood	*Ehrlichia canis* *Ehrlichia canis*	PCR PCR	16.1 16.9	[23]
India	Blood	*Ehrlichia canis*	PCR	30 (18/60)	[24]
India	Blood	*Ehrlichia canis*	PCR	41.59 (89/214)	[25]
India	Blood Serum	*Ehrlichia sp.* *Ehrlichia canis*	Microscopy ELISA	14.28 (12/84) 86.90 (73/84)	[17]

Country/Region	Sample Source	Causative Agent/Species	Diagnostic Method	Prevalence % (Positive/Total Number of Samples)	Reference
India	Blood Blood	*Ehrlichia sp.* *Ehrlichia canis*	Microscopy PCR	19.38 (19/98) 50 (49/98)	[26]
India	Blood	*Ehrlichia canis*	PCR	0.39 (3/778)	[27]
India	Serum	*Ehrlichia canis*	ELISA	48.33 (29/60)	[28]
India	Blood	*Ehrlichia canis*	PCR	20.6	[14]
India	Serum Blood	*Ehrlichia canis* *Ehrlichia canis*	ELISA PCR	57.5 (293/510) 8.8 (45/510)	[29]
India	Blood Serum Blood	*Ehrlichia canis* *Ehrlichia canis* *Ehrlichia canis*	Microscopy ELISA PCR	1.33 (3/225) 19.11 (43/225) 5.78 (13/225)	[30]
India	Serum	*Ehrlichia canis*	ELISA	19 (9/48)	[31]
Nepal	Blood	*Ehrlichia canis*	PCR	27.14 (19/70)	[32]
Nepal	Blood	*Ehrlichia spp.*	Microscopy	8 (4/50)	[33]
Bangladesh	Blood	*Anaplasma/ Ehrlichia spp.*	PCR	2.9 (3/68)	[34]
East Asia People's Republic of China (PRC)	Serum	*Ehrlichia canis*	ELISA	1.29 (4/309)	[35]
PRC	Blood Blood	*Ehrlichia canis* *Ehrlichia canis*	PCR PCR	12.12 (36/297) 15.23 (108/709)	[36]
PRC	Blood Ticks	*Ehrlichia canis* *Ehrlichia canis*	PCR PCR	1.4 (15/1114) 4.1 (6/146)	[37]
PRC	Ticks Serum	*Ehrlichia canis* *Ehrlichia canis* *Ehrlichia canis* *Ehrlichia canis*	PCR PCR PCR ELISA	11.03 (50/453) 3.29 (3/91) 13.69 (10/73) 1.33 (7/526)	[38]
PRC	Serum	*Ehrlichia canis*	SNAP test (EISA)	2.17 (13/600)	[39]
PRC	Ticks	*Ehrlichia canis*	PCR	0.97 (3/308)	[40]
PRC	Ticks	*Ehrlichia spp. (E.canis and E.muris like)*	PCR	28.7 (24/849)	[41]

Country/Region	Sample Source	Causative Agent/Species	Diagnostic Method	Prevalence % (Positive/Total Number of Samples)	Reference
PRC	Blood	*Ehrlichia canis*	ELISA	1.9%	[42]
PRC	Blood	*Ehrlichia canis*	PCR	0.0 (0/162)	[43]
Japan	Serum	*Ehrlichia canis* *Ehrlichia chaffeensis* *Ehrlichia muris*	IFA IFA IFA	18 (27/150) 18.7 (28/150) 11.3 (17/150)	[44]
Japan	Ticks	*Ehrlichia platys*	PCR	9.4 (3/32)	[45]
Japan	Blood	*Ehrlichia platys*	PCR	1.5 (1/67) Yamaguchi 27.6 (24/87) Okinawa	[45]
Japan	Serum	*Ehrlichia muris*	IFA	3.6 (18/499)	[46]
Japan	Blood	*Ehrlichia spp./Anaplasma spp.*	PCR	1.5 (11/722)	[47]
Japan	Blood	*Ehrlichia canis*	PCR+ Electron Microscopy	100 (1/1)	[48]
Japan	Ticks	*Ehrlichia spp*	PCR	0.25 (3/1211)	[49]
Japan	Ticks	*Ehrlichia canis*	PCR	0.0 (0/1211)	[49]
South Korea	Serum Blood	*Ehrlichia canis* *Ehrlichia canis*	SNAP test (ELISA) PCR	4.7 (25/532) 0.0 (0/25)	[50]
South Korea	Serum	*Ehrlichia spp. (E. canis/E. ewingii)* *Ehrlichia chaffeensis*	ELISA/IFA ELISA/IFA	10.3 (228/2215) 2.3 (52/2215)	[51]
South Korea	Serum Serum Blood	*Ehrlichia canis* *Ehrlichia canis* *Ehrlichia canis*	ELISA IFA PCR	0.6 (1/182) 22.5 (41/182) 0.0 (0/182)	[52]
South Korea	Blood	*Ehrlichia canis*	ELISA	6.1 (14/229) Rural dogs 0.0 (0/692) Urban dogs	[53]
South Korea	Serum	*Ehrlichia spp*	ELISA	7.56 (22/291)	[54]

Country/Region	Sample Source	Causative Agent/Species	Diagnostic Method	Prevalence % (Positive/Total Number of Samples)	Reference
	Blood	*Ehrlichia chaffeensis*	PCR	3.09 (9/291)	
South Korea	Blood	*Ehrlichia chaffeensis*	PCR	100 (2/2)	[5]
South Korea	Ticks	*Ehrlichia chaffeensis*	PCR	4.2% (26/611)	[51]
South Korea	Blood [60][61]	*Ehrlichia canis*	(ELISA)	12.3 (29/236)	[55]
South Korea	Ticks	*Ehrlichia canis* [60][61][62] *Ehrlichia chaffeensis*	PCR PCR	0.0 (0/1110) 0.0 (0/1110)	[18]
ROC Taiwan	Blood	*Ehrlichia canis*	ELISA	2 (2/101)	[56]
ROC Taiwan	Blood	*Ehrlichia canis*	ELISA	1.5	[42]
ROC Taiwan	Blood [44][64]	*Ehrlichia canis*	ELISA	9.9 (34/344)	[57]
ROC Taiwan	Blood Blood Ticks	*Ehrlichia canis* *Ehrlichia canis* *Ehrlichia canis*	ELISA PCR PCR	11.4 (20/175) 8.6 (15/175) 1 (3/306)	[58]
Hong Kong SAR, China [65]	Blood	*Ehrlichia canis*	PCR	8 (8/100) stray 6 (6/100) Pet	[59]

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Table 2. Reported tick species carrying ehrlichiosis in south and east Asian countries/regions.

Country/Region.	Tick Vector	Reference
India	*Rhipicephalus sanguineus*, *Rhipicephalus haemaphysaloides*	[23]
People's Republic of China (PRC)	*Rhipicephalus sanguineus* *Haemaphysalis longicornis*, *Rhipicephalus haemaphysaloides* *Haemaphysalis bispinosa*	[37][38][40][41]
Japan	*Rhipicephalus sanguineus* *Haemaphysalis flava*	[67][68]
South Korea	*Haemaphysalis longicornis* *Ixodes nipponensis*	[18][51]
ROC Taiwan	*Haemaphysalis hystricis Haemaphysalis longicornis*	[58]

4. Transmission Cycle

Brown dog tick (*Rhipicephalus sanguineus)* carries the pathogen from infected dog through blood meal during the acute phase of disease. After the blood meal, *E. canis* resides in the salivary glands and midgut of the carrier tick and it then spreads the pathogen to another healthy dog via its salivary glands during subsequent feeding [69]. Transstadial transmission is well-established for this pathogen in which the larval stage of tick becomes infected with *E. canis,* which can pass the bacteria to the next two stages (nymph and adult) and spread the pathogen during blood meals [70][71]. It has been observed that brown dog tick starts transferring the rickettsial pathogens within three hours of its attachment to a host [71]. If the *Rhipicephalus sanguineus* is transferred to a cold or temperate climate, due to the shifting of hosts, it can still remain active under such man-made protected kennel environments [72][73]. Moreover, enclosures of wild animals, abandoned houses, and kennel environments provide a perfect atmosphere for its reproduction. Under these suitable environmental conditions, only a single female tick was enough to infect and reproduce many subadults [74]. During the chronic or subclinical phase of the disease, the dog seems healthy but still acts as a carrier for this rickettsial pathogen. The only tick that becomes engorged during the acute phase can infect another healthy host. Moreover, this tick can spread the bacteria even after 155 days of its detachment [75]. Many studies have suggested that transovarial spread of the pathogen also occurs in Ixodid ticks and they maintain the bacteria through many generations in nature [76][77]. However, in a recent study, no proof of transovarial spread was found [78].

5. Pathogenicity

Unlike many Gram-negative bacterial pathogens, peptidoglycan and lipopolysaccharide are absent in the cell wall of this bacterium which may help the bacteria in resisting the host's immune response. The cell wall of *E. canis* becomes very flexible due to the absence of these two materials, which in turn facilitates the pathogen in avoiding antibody attack from its host immune system. Other characteristic feature of this rickettsial organism is the lack of complex inner structures, which permits the production of sugars. The main energy source of this bacterium are amino acids [79]. The incubation period of *Ehrlichia* ranges from 8–20 days. This period is sequentially followed by subclinical, acute, or in some cases chronic form.

Pilli are absent in *Ehrlichia* so the outer membrane of this infectious agent helps in the attachment with the host cell. Once the pathogen enters the host cell and starts infection, it forms membrane-bound partitions (endosomes) and maintains its distinctive cytoplasmic shape. The main target of *E. canis* are mononuclear phagocytic cells. Monocytes are the most common cells to be infected both in canine and human hosts. In addition, *Ehrlichia* also attacks the other immune cells such as metamyelocytes, lymphocytes, and promyelocytes. In general, it is assumed that inside the cells only mononuclear phagocytic cells are able to uphold the productive pathogen [80]. On average, a single infected monocyte contains one to two morulae. The endosomal membrane formed by *Ehrlichia* protects the pathogen from the host and it multiplies within this apartment. The exact mechanism of their survival is still unclear but consequently, the pathogen may survive by modulating the host defense system [81]. In one study, researchers identified two paralogous proteins responsible for immune evasion, which may be due to the presence of poly (G-C) tracts in one of the proteins, suggesting that they have a role in facilitating chronic persistent infections and can help in phase deviation [79].

After infecting the monocytes, *E. canis* spreads to the whole lymphatic system including the liver and spleen, where it triggers the abnormal fast growth of cells and the increased size of these organs, described as hyperplasia. Further cell division and replication leads to bacteremia and eventually results in hemolysis. At this stage, severe clinical manifestations, such as high fever, anemia, and thrombocytopenia, can be observed [44]. Dogs suffering from persistent infection develop a more lethal form of chronic disease where the pathogen attacks the bone marrow and destroys the immune system. As a result, other opportunistic infectious agents further aggravate the situation. Severe thrombocytopenia leads to massive hemorrhages and death [79].

6. Clinical Signs

Clinical presentation due to ehrlichial infection can be varied and depends on many factors, such as the status of the immune system of the dog, virulence of the strain, and existence of co-infections with other tick/flea-borne diseases. Among all other members of the Anaplasmataceae family, *E. canis* appears to cause more intense clinical abnormalities [82][83][84]. In dogs, three ehrlichial species, namely *E. canis, E. ewingii,* and *E. chaffeensis,* can cause clinical disease [1][3]. The principal host cell targets for *E. canis* and *E. chaffeensis* are agranulocytes, while *E. ewingii* mainly targets the granulocytic white blood cells [2]. These pathogens can induce both clinical and subclinical complications. Clinical signs induced by ehrlichial species are often non-specific and overlapping. The disease can be acute or mild; however, in many cases, the animal becomes a carrier for an extended period of time without presenting any clinical manifestations. Typically, the incubation period for all ehrlichial species ranges from one to three weeks, and results in three possible disease presentations which may be categorized as acute, chronic, and subclinical [85]. The acute phase may last for 2 to 4 weeks and if the animal survives, the signs vanish even without chemotherapeutic treatment. However, some dogs become subclinical carriers after improvement and may become an important source of infection for months and years. In this phase, the animal apparently looks normal and healthy and does not present any clinically visible signs but upon hematological testing, mild thrombocytopenia can be detected [86]. Not all but some subclinically infected dogs may proceed to the chronic stage, which is the most fatal form of the disease, and which cannot be differentiated from the acute phase in clinical settings because most of the clinical manifestations are non-specific. The chronic form is also known as the myelosuppressive form in which it is difficult to distinguish it from acute bone marrow aspiration and complete blood count tests are necessary. Alternatively, hypoplasia of bone marrow and severe pancytopenia will confirm the presence of the chronic phase [87]. The possible factors that cause some dogs to enter the chronic phase are still unclear.

In naturally infected dogs, the common clinical findings are fever, pale mucosa due to anemia, lymphadenomegaly, bleeding disorders, hepatomegaly, lethargy, petechial and ecchymotic hemorrhages, vasculitis, and extended bleeding period during estrus [88][89][90]. Other less common signs of ehrlichiosis have also been defined and include diarrhea, exercise intolerance, neonatal death or abortion, vomiting, and mucopurulent or serous nasal and ocular discharge. Some old studies have mentioned polyarthritis and lameness as a sign of canine ehrlichiosis [91], but it is believed that this manifestation only appears in cases of co-infections. On physical examination, you may observe tick infestation particularly during the acute phase. In addition, other signs like ataxia, vestibular

dysfunction and seizures, and chronic or myelosuppressive form also reveal stomatitis, scrotal or hind limb edema, jaundice, glossitis, and pyoderma [92]. Bleeding tendencies are also more frequent and severe in the chronic form of CME [90].

7. Clinical Pathology

Hematological abnormalities are variable and overlapping. However, severe drop in platelet count or thrombocytopenia is the principal abnormality observed in canine ehrlichiosis. This hematological finding is consistent in almost 80% of the animals, irrespective of the stage of the ailment. However, normal platelet count may not be the only reason to rule out ehrlichiosis [93][94]. In a retrospective study, decreases in total red blood cell count, pack cell volume (PCV), and platelet count while noticeable increases in basophil count were observed. Moreover, blood urea nitrogen (BUN) and creatinine levels were more elevated than normal [95]. Anemia is mostly non-regenerative along with lymphopenia, monocytosis, thrombocytopathy, hyperproteinemia, hypergammaglobulinemia, hypoalbuminemia, and hyperglobulinemia, which are some additional irregularities [96][97][98]. Values of neutrophils are inconsistent and both neutrophilia and neutropenia have been detected based on the phase of the severity. In the chronic form, aplastic pancytopenia, granular lymphocytosis, mild elevation in liver enzymes, and renal azotemia were found. In regions where this disease is endemic, CME should be the top differential in dogs having persistent lymphocytosis [99][100].

Histopathological and gross abnormalities in experimentally infected dogs include edema of the subcutaneous layer, ascites, anemia, jaundice, and emaciation. Cuffing of the lymphatic fluid in the cerebellum and brain is occasionally seen. Lungs of infected animals display vasculitis and interstitial pneumonia. Additionally, a flabby heart and whole heart dilatation can also be found. Grossly, the most frequent signs include apparent splenomegaly, multifocal lymph node necrosis, and widespread lymadenopathy [101].

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