

Original Article

Ehrlichiosis in Household Dogs and Parasitized Ticks in Kerman- Iran: Preliminary Zoonotic Risk Assessment

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Abstract

Background: Ehrlichiosis is an emerging tick-borne zoonotic disease caused by the family of Anaplasmatacea. Recently, outbreak of human monocytic ehrlichiosis was reported in northern part of Iran. Besides, serological evidence of canine monocytic ehrlichiosis caused by *Ehrlichia canis* was reported from southeastern of Iran but the epidemiology of this disease is almost undetermined in Iran. The present study was designed to use PCR for detection of *Ehrlichia* spp. in tick infested household dogs and determination of risks of disease transmission to dog's owners.

Method: Blood samples were prepared from 100 tick infested household dogs after complete clinical examination. Complete cell blood count was done for each sample. DNA extraction was done and PCR was carried out by a commercial kit afterwards. Regarding to PCR results, blood samples were collected from owners and family members who were exposed to infected and non-infected dogs. A similar method was utilized for DNA extraction and PCR in human samples.

Result: Ehrlichial DNA was detected by PCR in six percent of *Rhipicephalus sanguineus* tick pools and 9% of the examined dogs. No positive sample was detected among the 67 examined human bloods.

Conclusion: Ehrlichiosis could be considered as an emerging canine disease but owning a dog should not be considered a major risk factor for ehrlichiosis in humans. Further serological and molecular studies in different parts of Iran are required to clarify the epidemiology of ehrlichiosis in canine, ticks, and human population.

Keywords: Ehrlichiosis, Dog, Ownership risk, Tick, Iran

Introduction

Humans and dogs are both susceptible to tick-borne diseases. Whenever dogs have tick infestation, they could be considered as reservoirs for human pathogens, as definitive feeding hosts for vector ticks or as mechanical transporters. Borreliosis, ehrlichiosis and anaplasmosis, are the reported emerging zoonotic diseases, which create ownership risks for tick, infested pet dogs (Fritz 2009).

In the order Rickettsiales *Anaplasma phagocytophilum*, *E. canis*, *E. chaffeensis*, *E. ewingii*, *Rickettsia rickettsii* and *R. conorii* are zoonotic tick-borne pathogens, which expose dogs and their owners (Nicholson et al. 2010).

Ehrlichiosis is a life-threatening emerging human tick-borne zoonosis, caused by obligate intracellular Gram-negative bacteria

named Ehrlichiae. The dog brown tick (*R. sanguineus*) is the main vector of disease in infested dogs (Beugnet et al. 2009).

Rhipicephalus sanguineus is the most prevalent tick in dogs, which has a worldwide distribution. This tick is a common vector of many dogs and human pathogens. This tick is well distributed in both urban and rural areas in tropical, subtropical and some temperate regions. *Rhipicephalus sanguineus* not only infests dogs but also is highly adapted to live within human dwellings (Dantas-Torres 2010). Recent studies have demonstrated that ticks exposed to high temperatures attach and feed on humans more rapidly. This observation suggests that the risk of human parasitism by *R. sanguineus* could increase in areas experiencing warmer and/or longer summers, consequently increasing the risk of transmission of zoonotic agents (Guglielmone et al. 2006, Shoorijeh et al. 2008).

The human parasitism by *R. sanguineus* is relatively common in Europe, particularly during the summer. In contrast, the human parasitism is much less common or maybe much less reported in South America and there is no report about Asian countries (Guglielmone et al. 2006).

Kerman is located in southeast of Iran with warm springs and hot summers which makes it a perfect condition for *R. sanguineus* activity. On the other hand, most of pet dogs in this area are kept outdoor as guard dogs predisposing them to ectoparasite infestation in warm seasons.

Since the major route of human infection with *Ehrlichia* spp. is transmission by a vector tick, the presence of infected ticks near humans could be the most important risk factor for human infection (Unver et al. 2001, Dantas-Torres et al. 2006). Dogs play an important role in transporting infected ticks into their surroundings and their owners (Shoorijeh et al. 2008).

In Iran, outbreak of human monocytic ehr-

lichiosis has been reported from northern part (Babamahmoodi 2004). On the other hand, serological evidences of canine monocytic ehrlichiosis were reported in Kerman and Khuzestan Provinces respectively (Akh-tardanesh et al. 2010, Avizeh et al. 2010). Serological studies usually indicates exposure rather than active infection, and might mislead due to serological cross reactions with other closely related organisms, so the present study was designed to use the polymerase chain reaction assay to confirm presence of *Ehrlichia* spp. in tick infested client-owned dogs and their attached ticks and determine the risks of disease transmission to the dogs' owners in Kerman City.

Materials and Methods

Blood samples were randomly prepared from 100 owned tick infested dogs regardless of their age, sex and clinical status between April to October 2011. The animals were referred to the teaching veterinary hospital of Shahid Bahonar University of Kerman. Each animal was fully clinically examined and attached semi- or fully engorged ticks were collected from dogs using a forceps and transferred into the labeled holding tubes containing absolute ethanol individually. Then detailed questionnaire was filled for each animal and owner (or other family members) who were in close contact with pet dogs. Five milliliter of blood were collected from the cephalic vein of dogs which their owner provided verbal consent for attending in the study and divided in two tubes containing EDTA anticoagulant. Two-milliliter aliquot of blood was used for hematological evaluation and remaining three milliliter refrigerated at -18°C for DNA extraction. The same method was used for the owner and family members who accepted to attend in a pathobiology laboratory for blood collection. Complete blood counts were per-

formed by cell counter (Sysmex KX-21N™, USA) for all samples. The presence of hematological disorders such as anemia, leukopenia and thrombocytopenia was recorded in comparison with reference ranges (Tefferi et al. 2005, Willard et al. 2012).

In parasitology laboratory, collected ticks were taken from the absolute ethanol and their identification was carried out by observation with a binocular microscope (40 x magnifications). Ticks were classified into family, genus and species using the taxonomic and morphometric keys (Walker et al. 2003).

At least one to maximum five adult ticks collected from each dog and each species were pooled in sterile Eppendorf tubes and minced using a sterile scalpel blade. Specimens were incubated overnight after adding of 20-microliter proteinase K. Genomic DNA was extracted from specimens by using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer instructions. DNA extraction from dogs and human blood samples was carried out by viral gene-spin kits (VeTeK™, South Korea). Finally, PCR was done for all samples by VeTeK™ EHR Detection Kit (VeTeK™, South Korea) according to company instructions.

Ethics

The project underwent ethical review and was given approval by an institutional animal care and done by appropriately qualified scientific colleagues.

Results

A total of 408 ticks were collected from 100 selected dogs which all were identified as *Rhipicephalus sanguineus* regarding to specific characteristics including red-brown color, elongated body shape, and hexagonal basis capituli. Six of tick pools (6%) and 9% of examined dogs were positive for *Ehrlichia* spp. by PCR (Fig. 1). Three dogs were *Ehrlichia* spp. positive whereas their tick pools were negative. In dog population, infection rate was not significantly related to age (P value=0.627) and sex (P value=0.682) (Table1). No significant changes were seen in owner's complete blood count tests but some hematological alterations were seen in infected dogs. However, there was no significant difference between the infected and non-infected dogs in PCV level (P value=0.242), WBC (P value=0.345), neutrophil (P value=0.643), lymphocyte (P value=0.408), eosinophil (P value=0.27) and monocyte count (P value=0.45) (Table 2).

Blood samples from 36 persons (owner and family members) that were in close contact with infected dogs (group 1) and 31 owners of non-infected dogs (group 2) were PCR negative. Demographic data for the participants are shown in Table 3. All owners were aware about their dog tick infestation but none of the participants reported a tick bite history.

Table 1. *Ehrlichia* spp. infection status among studied dogs regarding to age and sex groups

Parameters	Studied dogs population NO. (%)	PCR-positive dogs NO. (%)
Age >12 month	25 (25)	2(8)
Age 1-3 years	33(33)	3(9.1)
Age >3 years	42(42)	4(9.5)
Sex Male	58(58)	5(8.6)
Sex Female	42(42)	4(9.5)

Table 2. Hematological alteration in studied dogs regarding to *Ehrlichia* spp. infection status

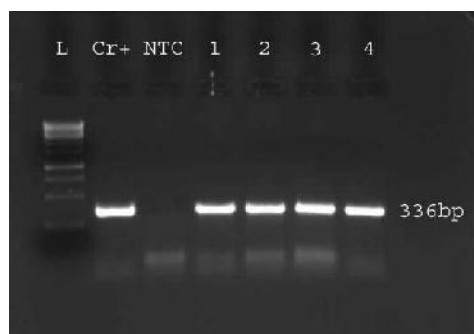
Parameter	Unit	<i>Ehrlichia</i> spp. infection	
		PCR-positive*	PCR-negative*
PCV	%	35.4 ± 3.12	38.5 ± 6.4
WBC	×10 ³ /ml	11.2 ± 3.4	12.4 ± 4.3
Neutrophil	×10 ³ /ml	7.3 ± 2.3	7.6 ± 2.8
Lymphocyte	×10 ³ /ml	4.1 ± 1.1	4.9 ± 3.1
Basophil	×10 ³ /ml	0	0
Eosinophil	×10 ³ /ml	0	0.05 ± 0.01
Monocyte	×10 ³ /ml	0.04 ± 0.01	0.01 ± 0.01

*Data are Mean±SEM

Table 3. Demographic data of owners

Variable	Owners of infected dogs (n=36)	Owners of non-infected dogs (n=31)
Age(mean ± SEM)*	35.4 ± 2.9	42.0 ± 3.6
Sex NO (%)		
Male	23 (63.8)	19 (62)
Female	13 (36.1)	12 (38)
Place of residence No (%)		
Urban	18 (50)	28 (90.3)
Rural	18 (50)	3 (9.7)

* years

**Fig. 1.** Agarose gel electrophoresis for identification of *Ehrlichia* spp. DNA in ticks and dogs blood samples

L: 100 bp DNA ladder, Cr+: positive control (336 bp), NTC: negative control, lanes 1, 2 Positive dogs blood samples, lane 3, 4 positive tick samples.

Discussion

The genus *Ehrlichia* consists of five recognized species, including *E. canis*, *E.*

chaffeensis, *E. ewingii*, *E. muris*, and *E. ruminantium*. Nowadays *Ehrlichia* species are discovered in new areas and new tick species, which emphasize on wider distribution of these agents. Host population, migration, changes in climate and control failure is environmental factors, which have been known to exacerbate the spread of *Ehrlichia* species (Esemu et al. 2011).

Dogs can be infected with different *Ehrlichia* species of which *E. canis*, *E. ewingii*, and *E. chaffeensis* are considered zoonotic. *Ehrlichia canis*, which is the most prevalent species in dogs, has been shown to infect humans in Venezuela whereas *R. sanguineus* was a common ectoparasite on household dogs in both urban and rural areas (Perez et al. 1996, Perez et al. 2006).

Domestic dogs and ring-tailed lemurs are naturally exposed to *E. chaffeensis* and human granulocytic ehrlichiosis which caused

by this organism have been reported in North America, Asia and Europe (Spolidorio et al. 2010).

Ehrlichia canis, *E. chaffeensis* and *E. ewingii* have been recently detected synchronously in dogs and their ticks. Ndip et al. suggest that *R. sanguineus* ticks which are primarily infected with *E. canis*, may get infected with other ehrlichial agents and transmit them to humans (Ndip et al. 2007).

Ehrlichia ewingii and *A. phagocytophilum* which has been identified as pathogens of both dogs and humans are chiefly granulocytotropic (Buller et al. 1999, Ganguly et al. 2008). Serosurvey showed that dogs be routinely guarded for assessing risk for human granulocytic ehrlichiosis in humans in Europe and North America (Cizman et al. 2000, Day 2011).

A. phagocytophilum reported to be prevalent in Ixodex ticks, which infest dogs, so it can easily, transmitted to humans (Nicholson et al. 2010). On the other hand, *A. phagocytophilum* was isolated from Ixodex ticks in northern parts of Iran, which creates risk of human infection in our country (Bashirbod 2004).

The presented data showed that dogs and their ticks can be part of the epidemiological cycle of ehrlichiosis all around the world and surveillance, diagnosis, treatment, and prevention of tick-borne diseases in humans and dogs can yield mutually beneficial information for public and veterinary health.

In this study, three dogs were *Ehrlichia* spp. positive whereas their tick pools were negative. It is not determined that after tick's blood sucking, how long does it take that transmission of ehrlichiosis from infected dogs to naïve ticks occurs. On the other hand, the rickettsemia levels to which ticks are exposed during feeding may also impacts on the proportion of infected ticks, so non-infected ticks may not have enough time to

achieve the infection from their infected hosts (Johnson et al. 1998).

Prevalence of *E. canis* in different tick species collected from dogs from Ardebil in North West of Iran was reported (16.66%) by Khazeni et al. (2013) while nested PCR detected ehrlichial DNA in 63.82% of *R. Sanguineus* ticks and these results warrant studying on vector competence of ticks for the ehrlichiosis agents.

All collected ticks in our study were *R. sanguineus* but results of present study suggest that owning an *Ehrlichia* infected dog should not be considered a major risk factor for human ehrlichiosis in Kerman. This finding must be interpreted cautiously due to the widespread distribution of *R. sanguineus* in hot seasons on owned dogs and increased risk of human parasitism by *R. sanguineus* in tropical areas like as Kerman.

Population at risk for ehrlichiosis are the elderly, immunosuppressed and infants patients and as all of the examined human populations in this study were healthy adults, the absence of association between dogs and human infections must be interpreted conservatively. Low incidence of disease in dogs' population and small population of dog's owners who attend in our study are other confinement factors in this study.

In conclusion, result of present study confirm the presence of ehrlichiosis as an emerging infectious disease in canine population and their collected ticks in Iran, but further research is needed to reveal the importance of pet dogs and their ticks in the cycle of human ehrlichiosis in our country.

Conclusion

Based on the result of the present study, ehrlichiosis could be considered as an emerging canine disease but owning a dog should not be considered a major risk factor for ehrlichiosis in humans. Although our data sug-

gest that ownership of dogs, is not associated with increased risk of ehrlichiosis in human medicine but this finding must be interpreted cautiously due to the widespread distribution of *R. sanguinus* in hot seasons on owned dogs. Further serological and molecular studies in different parts of Iran are required to clarify the epidemiology of ehrlichiosis in canine, ticks, and human population.

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