Full Length Article

PREVALENCE OF HAEMOPARASITIC INFECTIONS IN DOGS FROM DIFFERENT REGIONS OF ANDHRA PRADESH

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ABSTRACT

The prevalence of haemoparasites in dogs in different regions of Andhra Pradesh was presented in this study. The overall prevalence of haemoparasitic infection was 35.0 and 48.23 per cent in Rayalaseema and Coastal Andhra regions, respectively. Statistically, there was no significant (P>0.05) relationship between the region and prevalence of haemoparasites in dogs. Infection with single haemoparasite and co-infection with more than one haemoparasites was non-significantly higher (32.62% and 15.60%, respectively) in dogs of Coastal Andhra than in Rayalaseema region (27.50% and 7.50%, respectively). The prevalence of Babesia spp., Ehrlichia canis and Hepatozoon canis was non-significantly (P>0.05) high in Coastal Andhra than in Rayalaseema region. Among three species identified Babesia spp. were the most prevalent species in dogs in two regions. Co-infection with Babesia spp. and E. canis (10.06%; P>0.05) was more frequently observed in dogs in Coastal Andhra region than in dogs of Rayalaseema region (6.25%).

Keywords: Prevalence, haemoparasites, co-infections, Rayalaseema and Costal Andhra Pradesh regions

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INTRODUCTION

Babesiosis, ehrlichiosis and hepatozoonosis are common tick-borne diseases (TBDs) in dogs, which are caused by *Babesia* spp., *Ehrlichia canis* and *Hepatozoon canis*, respectively. Vector-borne diseases, specifically TBDs cause potentially fatal

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diseases in dogs and have been increasingly reported in dogs worldwide. In dogs, co-infection of *Ehrlichia*, *Babesia* and *Hepatozoon* species occur in endemic areas (Shaw *et al,* 2001; Lakshmanan and John, 2007) and could partially explain variations in clinical presentation, pathogenicity, and response to therapy. Concurrent infection with more than one TBD pathogen occurred in 39.0 per cent of cases from different climatic zones of India (Abd Rani *et al.,* 2011). Early detection and accurate identification of pathogen species, either in single or in co-infection, are crucial for the therapeutic and preventive purpose of canine tick-borne diseases (Baneth *et al.,* 2012). Globally approved tests endure to be developed for all TBDs affecting dogs, so far diagnosis primarily depends on clinical signs, blood smear examination and several serological assays (ELISA, immunofluorescence, and immunoblot). A survey was carried out to study the prevalence of haemoparasites in dogs of different regions of Andhra Pradesh which included both Rayalaseema region and costal Andhra regions using microscopy and multiplex PCR.

MATERIALS AND METHODS

The present study was conducted in Coastal Andhra (tropical wet and dry climate or humid climate or Savannah climate) and Rayalaseema (tropical semi-arid or Steppe climate) region of Andhra Pradesh. Based on presence of tick infestation at the time of presentation and/or showing clinical signs in accordance with the haemoprotozoan infection, namely, fever, haemoglobinuria, anaemia, bleeding episodes, jaundice, lameness, neurological signs, paralysis, lethargy, whole blood samples were collected aseptically in EDTA vacutainers/coated vials from the cephalic vein of the 442 dogs (160 from Rayalaseema region and 282 from Costal Andhra Pradesh region) that were presented to the Veterinary Polyclinics and Veterinary Hospitals in Andhra Pradesh(Table 1). Besides these 908 tick samples were collected from different regions of Andhra Pradesh under this survey for a period of one year from July 2019 to June 2020.

Thin blood smears in duplicate from each blood sample were prepared on clean, grease free microslides, air dried and fixed using methanol. The fixed blood smears were stained by Giemsa stain using 1:10 dilution for 30 min (Coles, 1986). The slides were washed under running tap water and were air dried. Stained blood films were examined under x1000 objective lens for haemoparasites.

Ticks were processed in 10% KOH as per the standard procedure (Soulsby, 1982). An Olympus microscope at the x100 magnification was used to identify the ticks at species level considering the morphological characters like hypostome, palpal segments, capitulum, scutum and legs (Walker *et al*., 2014).

DNA was isolated from each blood sample (200 µL aliquots of EDTA blood, stored at -20 °C) using a QIAamp DNA Blood Mini Kit (Quiagen ® Kit, Germany, Catalogue

No. 51104), according to the manufacturer's instructions with slight modifications. Yield of the isolated DNAs were determined by UV spectroscopy using a Nanodrop 1000 Spectrophotometer (Thermo scientific, USA) using the convention that one absorbance unit at 260 nm equals 40 µg DNA per mL. The purity of extracted DNA was determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. The samples with acceptable purity (i.e., ratio (A260/A280) of 1.7–1.9 and above) were stored for further analysis. On determination of concentration, samples were diluted for obtaining a final concentration of 100 ng/µL of DNA for further use.

In the present study, the virB9 gene of *Ehrlichia canis*, 16S rRNA gene of *Hepatozoon canis* and 16S rRNA gene of *Babesia* spp. of dogs were amplified at specific loci using following primers as shown in the plate. (Kledmanee *et al*., 2009).

RESULTS AND DISCUSSION

In India, the tropical climate, high population of dogs, and vectors especially, ticks, favour the occurrence of several canine tick-borne pathogens. Early detection and accurate identification of pathogen species, either in single or in co-infection, are crucial for the therapeutic and preventive purpose of these diseases. The present study was carried out to determine the region wise prevalence of common tick-borne pathogens, *Ehrlichia canis*, *Hepatozoon canis*, and *Babesia* spp. in dogs in Andhra Pradesh by microscopy and multiplex PCR utilizing a total of 442 dogs blood samples.

The results revealed higher per cent prevalence of overall haemoparasites including single and co-infections by multiplex PCR when compared to blood smear examination by microscopy (Table 4). Significant variation in the prevalence of single species infection and co-infection with more than one haemoparasites ($X^2 = 13.517$; df=1; P=0.000236) was observed. Ninety samples negative by Giemsa-staining were found positive with at least one haemoparasites by multiplex PCR (Table 3).

The overall prevalence of each haemoparasites, i.e. *Babesia* spp., *E. canis* and *H. canis* infection in dogs by microscopy and multiplex PCR was presented in Table 2 and 4. The prevalence of ehrlichiosis, hepatozoonosis and babesiosis in dogs by microscopy was 8.37, 0.90 and 9.72 per cent respectively. Whereas 11.53, 2.71 and 16.51 per cent of dogs were found to be positive for ehrlichiosis, hepatozoonosis and babesiosis by multiplex PCR. Infection with *Babesia* spp. was found more common in dogs in the study area than *E. canis* and *H. canis* by multiplex PCR as well as by conventional microscopy (Graph).

In total, 908 ticks were collected from infested animals. They were identified as *Riphicephalus* species. The *Riphicephalus* ticks were confirmed as *R. sanguineus* based on short palps and hypostome and hexagonal basis capitulum (Soulsby, 1982). The coxa of the first pair of legs had two spurs. The legs

were successively larger from the anterior to the posterior pair into species based on morphological characteristics. During study no larval and nymphal stages of ticks were identified. Single species of tick infestation was noticed. (Figure).

Hence, it is valuable to identify the tick-borne pathogens in dogs and elucidate the factors that determine their prevalence. The most common tick-borne diseases of dogs are babesiosis, ehrlichiosis and hepatozoonosis (Homer *et al*., 2000; Baneth *et al*., 2003) and are endemic in India (Shaw *et al*., 2001). Information regarding the epidemiology, diagnosis and management of canine vector-borne diseases in India is limited in spite of the combination of favourable climate for parasites and vectors, and large populations of stray dogs. It is a difficult task for veterinarians in treating dogs co-infected with canine TBD pathogens, as it may cause more complex disease conditions and be more challenging to diagnose and treat (Brown *et al*., 2006; Gaunt *et al*., 2010; De Caprariis *et al*., 2011). Baneth *et al.* (2015) confirmed the co-infection of the dog monocytes with *H. canis* and *E. canis* by molecular characterization of the infecting agents and quantitative assessment of co-infected cells in naturally infected dogs suggesting that infection with one pathogen may permit or enhance invasion or prolonged cellular survival of the other.

The prevalence of haemoparasites was non-significantly (P>0.05) high in dogs in Coastal Andhra region (48.23 %) compared to that of prevalence in Rayalaseema (35.0 %) region though there is a difference in climatic conditions of two regions in spite of high humidity in Coastal Andhra, which favours the propagation of tick vectors and indirectly tick-borne diseases. Shah et al. (2017) observed no significant (P>0.05) relation with respect to location and haemoparasitic diseases of dogs, from district Peshawar and Charsadda, Pakistan. Similarly, dogs infected with single pathogens were common than that of co-infections with several combinations of two pathogens in dogs from Luanda, Angola (Cardoso *et al*., 2016), Doha, Qatar (Alho *et al*., 2017) and Nigeria (Happi *et al*., 2018).

Dogs living in rural area were 2.3 (OR=2.296; P<0.001) times less likely to be infected than dogs in urban area as dogs in urban area remain as reservoirs for pathogens. Domestic dogs are especially abundant in urban areas of some developing countries where they can be excellent reservoirs for pathogens, since they usually live-in large populations, and are regularly allowed to roam freely, facilitating contact between infected and susceptible hosts (Daniels and Bekoff, 1989, WHO/WSPA, 1990). Contrary, in rural areas, where dog densities and population size are generally low (Acosta-Jamett *et al*., 2010), highly virulent pathogens cannot be continued and the infection die unless new infections are introduced from neighbouring areas (Swinton *et al*., 2002). The prevalence of haemoparasites in dogs was shown to be positively correlated with the presence of ticks on the animal. Dogs without tick infestation were 66 (OR=0.015; P<0.001) times less likely to be infected with haemoparasitic infections than dogs with tick infestation.

Parasite/ Primer	Sequence (5'-3')	Length (bases)	Product size (bp)
E. canis Ehr1401F	CCATAAGCATAGCTGATAACCCTGTTACAA	30	380
Ehr1780R	TGGATAATAAAACCGTACTATGTATGCTAG	30	
Babesia spp. Ba103F	CCAATCCTGACACAGGGAGGTAGTGACA	28	619
Ba721R	CCCCAGAACCCAAAGACTTTGATTTCTCTCAAG	33	
H. canis Hep001F	CCTGGCTATACATGAGCAAAATCTCAACTT	30	737
Hep737R	CCAACTGTCCCTATCAATCATTAAAGC	27	

Table 1. The number of samples collected from different places was as follows

S. No.	District	Number of blood samples collected	Number of tick samples collected
Rayalaseema region (n= 160)			
1	Anantapur	20	44
2	Chittoor	52	104
3	Y. S. R. Kadapa	43	86
4	Kurnool	45	91
Coastal region (n = 282)			
5	Nellore	41	84
6	Guntur	42	85
7	Krishna	35	73
8	Prakasam	24	45
9	Visakhapatnam	42	85
10	Vizianagaram	24	56
11	West Godavari	27	55
12	East Godavari	23	47
13	Srikakulam	24	53
Grand total		**442**	**908**

Table 2. Prevalence of single and co- infection of *Ehrlichia canis*, *Hepatozoon canis* and *Babesia* spp. in dogs by microscopy and multiplex PCR

Parasite	Microscopy (n=442)	Multiplex PCR (n=442)
Single infection		
Babesia spp.	43(9.72)	73(16.51)
Ehrlichia canis	37(8.37)	51(11.54)
Hepatozoon canis	4(0.90)	12 (2.71)
Total	**84 (19.00)**	**136(30.77)**
Co-infection		
Babesia spp.+ *E. canis* + *H. canis*	0	8(1.81)
Babesia spp.+ *E. canis*	7(1.58)	41 (9.28)
Babesia spp.+ *H. canis*	2(0.45)	3(0.68)
E. canis+ *H. canis*	0	4(0.90)
Total	**9(2.04)**	**56(12.67)**
Overall infection	**93(21.04)**	**192(43.44)****

Table 3. Overall prevalence of ehrlichiosis, hepatozoonosis and babesiosis in dogs in different regions of Andhra Pradesh

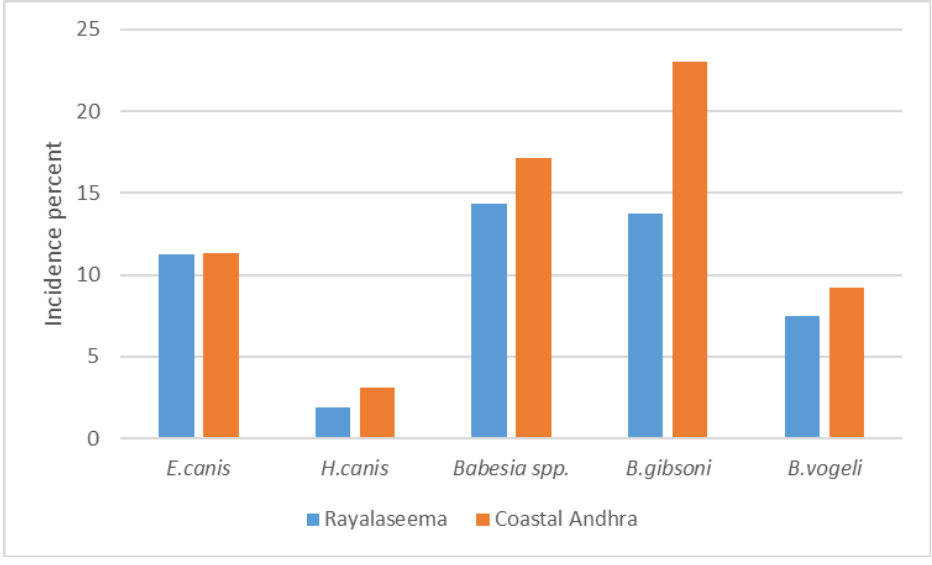
Parasite infection	Region	
Single infection (Microscopy and Multiplex PCR)	**Rayalaseema (n=160)**	**Costal Andhra (n=282)**
Ehrlichia canis	18(11.25)	33(11.30)
Hepatozoon canis	3(1.87)	9(3.08)
Babesia spp.	23(14.37)	50(17.12)
Total	**44(27.5)**	**92(32.62)**
B.gibsoni	22(13.75)	65(23.05)
B. vogeli	12(7.5)	26(9.22)
Co-infection		
E. canis+ *H. canis*+ *Babesia* spp.	2(1.25)	6(2.05)
Babesia spp.+ *E. canis*	10(6.25)	31(10.06)
Babesia spp.+*H.canis*	0	3(1.02)
E.canis+*H.canis*	0	4(1.37)
Total	**12(7.5)**	**44(15.60)**
Overall infection	**56(35.0)**	**136(48.23)**

P>0.05, chi – square test. Figures in parenthesis indicate percent.

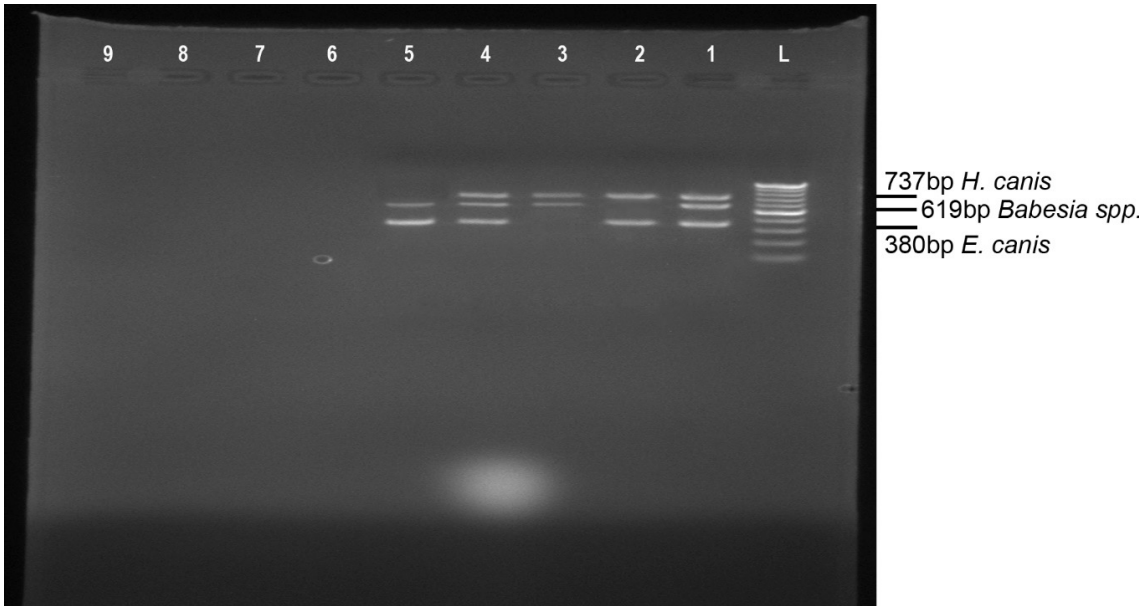
Table 4. Overall prevalence of ehrlichiosis, hepatozoonosis and babesiosis infection in dogs by microscopy and multiplex PCR

Nature of infection	Pathogen species detected in dogs by microscopy			Pathogen species detected in dogs by multiplex PCR		
	***Babesia* spp.**	***E. canis***	***H. canis***	***Babesia* spp.**	***E. canis***	***H. canis***
Single infection	43(9.72)	37(8.37)	4(0.90)	73(16.51)	51(11.53)	12(2.71)
Co-infection with						
Babesia spp.	-	7(1.58)	2(0.45)	-	41(9.27)	3(0.67)
E. canis	7(1.58)	-	-	41(9.28)	-	4(0.90)
H. canis	2(0.45)	-	-	3(0.68)	4(0.90)	-
Babesia spp.+ *E. canis*	-	-	-	-	-	8(1.81)
Babesia spp.+ *H. canis*	-	-	-	-	8(1.81)	-
E. canis.+ *H. canis*	-	-	-	8(1.81)	-	-
Total	**52(11.76)**	**41(9.28)**	**6(1.36)**	**125(28.28)**	**104(23.53)**	**27(6.1)**

Figures in parenthesis indicate percent.



Graph: Region-wise prevalence of haemoparasites in Andhra Pradesh



L: 100bp DNA ladder, 1&4: Co-infection with three spp.; 2: Co-infection with *E. canis* and *H. canis*; 3: Co-infection with *E. canis* and *Babesia* spp., 5: Co-infection with *Babesia* spp. and *E. canis.*

Fig. 2. Picture of *Riphicephalus sanguineus*

CONCLUSION

The survey results demonstrated remarkable variations in the endemicity patterns of canine tick-borne pathogens (ehrlichiosis, hepatozoonosis and babesiosis) in Andhra Pradesh, India. The results of the present study indicated that the multiplex PCR method exhibited much higher sensitivity and reliability for diagnosis of blood parasites in dogs and intern showed significantly higher prevalence rates in Costal Andhra Pradesh region when compared to the Rayalaseema region.

REFERENCES

Abd Rani, P.A.M., Irwin, P.J., Coleman, G.T., Gatne, M. and Traub, R.J. (2011). A survey of canine tick- borne diseases in India. *Parasites and Vectors*, **4**(141): 1 - 7.

Acosta-Jamett, G., Cleaveland, S., Cunningham, A. and Bronsvoort, M. (2010). Demography of domestic dogs in rural and urban areas in coquimbo region of Chile and its implication for diseases transmission. *Preventive Veterinary Medicine*, **94:** 272 – 281.

Alho, A.M., Lima, C. and Latrofa, M.S. (2017). Molecular detection of vector-borne pathogens in dogs and cats from Qatar. *Parasites and Vectors*. **10:** 298

Baneth, G., Mathew, J.S., Shkap, V., Macintire, D.K., Barta, J.R. and Ewing, S.A. (2003). Canine hepatozoonosis: two Disease syndromes caused by separate Hepatozoon spp. *Trends Parasitology,* **19**(1): 27 - 31.

Baneth, G., Bourdeau, P., Bourdoiseau, G., Bowman, D., Breitschwerdt, E. and Capelli, G. (2012). Vector-borne diseases-constant challenge for practicing veterinarians: recommendations from the CVBD World forum. *Parasites and Vectors.* **5:** 55.

Baneth, G., Harrus, S., Gal, A. and Aroch, I. (2015). Canine vector-borne co-infections: *Ehrlichia canis* and *Hepatozoon canis* in the same host monocytes. *Veterinary Parasitology,* **208** (1-2): 30 - 34.

Brown, A.L., Shiel, R.E. and Irwin, P.J. (2006). Clinical, haematological, cytokine and acute phase protein changes during experimental *Babesia gibsoni* infection of beagle puppies. *Experimental Parasitology***, 157:** 185 - 196.

Cardoso, L., Oliveria, A.C., Graneda, S., Nachum – Biala, Y., Gilad, M., Lopes, A. P., Sousa, S. R., Vilhena, H. and Baneth, G. (2016). Molecular investigation of tick–borne pathogens in dogs from Luanda, Angola. *Parasites and Vectors*, **9:** 252.

Coles, E.H. (1986).Veterinary clinical pathology 4thedition. WB Saunders Company, London,

46 - 47pp.

Daniels, T.J. and Bekoff, M. (1989). Population and social biology of free ranging dogs, *Canis familiaris. Journal of Mammalogy*, **70:** 754 – 762.

De Caprariis, D., Dantas-Torres, F., Capelli, G., Mencke, N., Stanneck, D. and Breitschwerdt, E.B. (2011). Evolution of clinical, haematological, and biochemical findings in young dogs naturally infected by vector-borne pathogens. *Veterinary Microbiology*, **149**(1-2): 206 - 212.

Gaunt, S.D., Beall, M.J., Stillman, B.A., Lorentzen, L., Diniz, P.P.V.P., Chandrashekar, R. and Breitschwerdt, E.B. (2010). Experimental infection and co-infection of dogs with *Anaplasma platys* and *Ehrlichia canis*: hematologic, serologic and molecular findings. *Parasites and Vectors,* **3:** 33.

Happi, A.N., Toepp, A.J., Ugwu, C.A., Petersen, C.A. and Sykes, J.E. (2018). Detection and identification of blood-borne infections in dogs in Nigeriaus Detection, microscopy and the polymerase chain reaction. *Veterinary Parasitology: Regional Studies and Reports*, **11:** 55 - 60.

Homer, M.J., Aguilar-Delfin, I., Telford, S.R. III., Krause, P.J. and. (2000). Persing, D.H: Babesiosis. *Clinical Microbiology Review*, **13:** 451 - 469.

Kledmanee, K., Suwanpakdee, S., Krajangwong, S., Chatsiriwech, J., Suksai, P., Suwannachat, P., Sariya, L, Buddhirongawatr, R., Charoonrut, P. and Chaichoun, K. (2009). Development of multiplex polymerase chain reaction for the detection of *Ehrlichia canis*, *Babesia* spp. and *Hepatozoon canis* in canine blood. *South Asian Journal of Tropical Medicine and Public,* **40**(1): 35 - 39.

Lakshmanan, B., John, L., Gomathinayagam, S. and Dhinakarraj, G. (2007). Molecular detection of *Ehrlichia canis* from blood of naturally infected dogs in India. *Veterinarski Archive* **83**(4): 353 - 354.

Shaw, E.S., Michael, J.D., Birtles, R.J. and Edward, B.B. (2001). Tick-borne diseases of dogs. *Trends Parasitology*. **17**(2): 74 - 80.

Shah, S.S.A., Khan, M.I., Rafiullah, Khan, M.A., Khan, H. Ali, A. and Ali, M.I. (2017). Tick-born diseases-possible threat to humans-dog interspecies bond. *Advances in Animal and Veterinary Sciences*, **5**(3): 115 - 119.

Soulsby, E. J. L. (1982). *Helminths, Arthropod and Protozoa of Domesticated Animals*, 7th ed. Bailliere Tindal and Cassell Ltd., London UK. p. 35 - 740.

Swinton, J., Woolhouse, M. E. J., Begon, M.E., Dobson, A.P., Ferroglio, E., Grenfell, B.T., Guberti, V., Hails, R.S., Heesterbeek, J.A.P., Lavazza, A., Roberts, M.G., White, P.J. and

Wilson, K. (2002). Micro parasite transmission and persistence. *The ecology of wild life diseases*. *Oxford University Press*. pp. 83 - 101.

Walker A R, Bouattour A, Camicas J L, Estrada-Peria A, Horak I G, Latif, A. A., Pegram, R.G. Preston, P.M. (2014). Ticks of domestic animals in Africa: a guide to identification of species, Edinbergh. *Bioscience Reports*, 3 - 210.

WHO/ WSPA, (1990). Guidelines for dog population management. *World Health Organization, Geneva*. World Society for the protection of Animals, London. pp 9 - 13.