Prevalence of Blood parasites in stray and pet Dogs in Hyderabad Area : Comparative sensitivity of different Diagnostic techniqes for the detection of microfilaria

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Abstract

Investigation on the prevalence of blood-parasites in stray and pet dogs in Hyderabad area of Sindh province was carried out during the summer season. A total of 300 blood samples (200 from stray and 100 from pet dogs) were collected and tested for blood parasites. Data was analyzed to determine prevalence of various species of blood parasites to establish the correlation of these infections with age, sex and month. A comparison was also made to evaluate the sensitivity of these techniques for the detection of microfilarae. An overall prevalence of blood-parasites was recorded as 11.66 %; *Dirofilaria immitis*, *Dipetalonema reconditum* and *Babesia canis* being 5.33, 1.33 and 5.00 %, respectively. The prevalence in stray and pet dogs was recorded as 13 and 9 % respectively. The highest percentage of infection was recorded in the month of July (13.3 %). The adult dogs were more commonly affected (14.70%) than pups (7.69%). The percentage of infection was greater in females (18.6%) than males (9.33%). Among the various techniques used, modified Knott's technique was found to be the most sensitive technique for the detection of microfilariae.

Keywords: Dog, blood-parasites, prevalence and diagnostic techniques.

Introduction

The dog (*Canis lupus familiaris*) is a domestic subspecies of the wolf, a mammal of the Canidae family of the order Carnivore. Dogs are not only "man's best friend," but they can also be trained to be man's best helpers in many ways. Guide dogs are trained to guide blind people through their daily activities. Dogs can also be taught to act as "ears" for the deaf or perform specialized police work. Of course they are best at being family companions. Learn how to train a puppy and find out about some of the most popular breeds.

Dogs are susceptible to various diseases, ailments, and poisons, some of which affect humans in the same way, others of which are unique to dogs. Dogs, like all mammals, are also susceptible to heat exhaustion when dealing with high levels of humidity and/or extreme temperatures.

Infectious diseases commonly associated with dogs include rabies (hydrophobia), canine parvovirus, and canine distemper. Congenital diseases of dogs can include a wide range from hip dysplasia and medial patellar luxation to epilepsy and pulmonic stenosis. Canines can get just about anything a human can get (excluding many infections which are species specific) like hypothyroidism, cancer, dental disease, heart disease, etc.

Dogs suffer from a variety of internal and external parasites. They have internal parasites ranging in size from microscopic single-celled Giardia (intestinal protozoa), babesia (intra-erythrocytic protozoa), and trypanosome (inter-erythrocytic protozoa) to grotesquely long tapeworms and filarial worms.

Babesia sp. parasitizes erythrocytes, causing anemia in dogs. Different species exist with varying host specificity. *B. canis* and *B. gibsoni* are two organisms commonly known to infect dogs. Both organisms have *Ixodid* tick vectors and are found throughout Asia, Africa, Europe, the Middle East, and North America, with *B. canis* being more prevalent (Soulsby, 1982).

Each year thousands of dogs become disabled or die from lung, heart or circulatory problems caused by heartworm disease. Heartworm disease in dogs and related canines is caused by a filarial

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nematode (a large thread-like round worm), *Dirofilaria immitis*. The adult worms live in the right side of the heart (right ventricle) and adjacent blood vessels (pulmonary arteries), and because of their location, are commonly called dog heartworms.

The adult worms are thin, almost thread like. Males are 12 to 30 cm long and females are 25 to 31 cm long, and are found in the right ventricle of heart and less often in the right auricle, pulmonary artery and vena cava. The male and female copulate in these sites. The viviparous female releases highly motile microfilarae, which circulate in the blood. They are taken up from cutaneous circulation by certain biting insects (mosquitoes), in whose bodies they undergo stages of development. The infective microfilarae then enter into the tissues of final host through the bite of intermediate host. The filarial larvae undergo further development in muscles, subcutaneous, and adipose tissues of the new host. When they reach a length of about 5 cm, they enter into veins and are carried to right heart. Transplacental infection of the foetal pups with microfilarae may occur. However, these do not develop into adult and disappear after two months (Vegad and Katiar, 2001).

Trypanosomiasis in dogs is not uncommon and is caused by *Trypanosoma evansi*, *T. brucei, T. cruzi* and many other species. These species are transmitted by biting flies such as *Tabanus*, *Stomoxys*, *Haematopota* and *Lyperosia*, etc. The disease is prevalent in South America, North Africa, South West United States and Indian sub-continent (Ristic and Smith 1974 and Soulsby, 1982). The characteristic clinical signs of trypanosomiasis in dogs include fever, anemia, emaciation, mycocarditis and coroneal opacity. In Babesiosis fever, anemia, jaundice and bloodglobinurea are noted symptoms (Urquhart *et al*. 1987).

Thus, the present study was conducted to determine the incidence of blood parasites of dogs in Hyderabad city.

Material and Methods

Experimental animals and site: Blood samples of 300 dogs, regardless of their age, sex and breed were examined to determine the prevalence of blood-parasite. The samples were collected from the pet and stray dogs in Hyderabad area of Sindh province.

Collection of blood samples: From each animal 5 ml blood was collected aseptically with the help of disposable syringe from cephalic vein and EDTA was used as anticoagulant. All the relevant information was recorded on Performa for further analysis.

Examination of blood samples (Pratt, 1997): Following techniques were used for the examination of blood samples for blood-parasites.

Wet blood film technique: A drop of blood was placed on a clean glass slide and a cover slip placed on it allowing the blood to spread as a thin layer of cells which was then examined under microscope to observe motile trypanosomes or microfilariae.

Giemsa stained thin smear technique

Preparation of blood smears: A drop of blood was taken near one end of the clean glass slide and another slide used to prepare the blood smear. The edge of second slide, held at an angle of about 45Âº, was touched with the drop spreading it on either side. Then the slide was moved in forward direction allowing the blood to spread as thin layer on the surface of the slide. The smear was allowed to air dry.

Fixing and Staining: The dried blood smears were fixed in methyl alcohol (absolute) for 5 minutes and allowed to dry. The dry smears were placed in a glass staining jar containing working Giemsa stain for 20 min. After that the smears were taken out and washed with PBS to remove excess stain. The slides were allowed to dry in air and then examined under oil immersion lens of the microscope.

Concentration Techniques (Pratt, 1997)

Modified Knott technique: One ml blood was added into a centrifuge tube containing 9 ml of 2% formalin. The blood was mixed gently by inverting closed tube twice and allowed to stand for 15 min to get complete hemolysis. The mixture was then centrifuged at 1500 rpm for 5 min. The supernatant was discarded and sediment was stained with an equal volume of methylene blue (1:1000). The mixture was put on glass slide and examined for microfilariae.

Haematocrit centrifugation technique: Micro-haematocrit tubes were filled two-third with blood and one end of each tube was sealed. The tubes were centrifuged at 3000 rpm for 5 min. The tubes were then broken 1 mm below the buffy layer. The contents of the tube containing buffy coat and plasma were tapped out onto a glass slide. A cover slip was applied on it and observed under microscope.

Results

The study was conducted to record the prevalence of blood-parasites of dogs in Hyderabad city. Blood-parasites belonging to three genera were encountered namely *Dirofilaria, Dipetalonema* and

Babesia. Affected dogs showed increased body temperature, anemia, hemoglobinurea, emaciation, dullness, impaired appetite, edema, coughing and laboured breathing.

Incidence of blood-parasites was studied over a period of 3 months in Hyderabad city. A total of 300 blood samples of dogs, regardless of their age, sex, and breed were examined for blood-parasite. Out of these 100 were pets and 200 were stray dogs, 170 were adults and130 were pups, 75 were females and 225 were males examined.

Prevalence of blood-parasites was determined by wet blood smear, Giemsa staining, haematocrit centrifugation and modified Knott technique. The over all prevalence of blood-parasites was recorded as 11.66%.

In group A, out of 100 pet dogs, 9 (9%) were found infected with blood-parasites while in group B, 26 (13%) were positive for blood-parasites out of 200 stray dogs (Table-1).

10 (7.69%) out of 130 pups were found infected with blood-parasites. In group B which comprised of adult dogs 25 (14.70%) out of 170 were detected infected with blood-parasites (Table-2). The highest percentage of infection recorded was that of *Dirofilaria immitis*. 16 (5.33%) out of 300 were positive for *Dirofilaria immitis,* 12 (5 %) dogs were found positive for *Babesia canis,* 4 (1.33%) were diagnosed positive for *Dipetalonema reconditum* and no case of *Trypanosoma* was recorded in all of the 300 samples (Table-3).

The findings revealed highest prevalence of blood-parasites in the month of July (13.30%) followed by June (11%) and May (10%) (Table-4). The results indicated that the incidence of blood-parasites was higher in females. A total of 75 bitches were examined. Out of them, 14 (18.6% prevalence) were infected. In male dogs the incidence was recorded as 9.33% as out of 225 samples, 21 were found positive (Table-5)

Comparative sensitivity of different techniques for the detection of microfilariae (Table-6)

The result of the comparative sensitivity of different techniques used for the detection of the microfilaria indicated that modified Knott's technique to be the most sensitive technique (90%) followed by wet blood film technique (65%), Giemsa staining (45%) and haematocrit centrifugation (30%).

Discussion

The parasitic diseases caused by blood-parasites such as *Babesia canis, Trypanosoma evansi, Dirofilaria immitis* and *Dipetalonema reconditum* cause severe infection in dogs throughout the sub-continent and are found all over the world.

The incidence of *Dirofilaria immitis* and *Dipetalonema reconditum* in the present study was recorded as 5.33% and 1.33% (Table-3), respectively, where as the respective prevalence of both of these species was reported as 2.7 and 0% by Durrani *et al.* (1965), 3.06 and 1.3% by Chakrabarti and Chaudhury (1983), 10.9 and 3.6% by Martin and Colin (1985), 5.9 and 0% by Deidrick and Boyce (1986), 3.54 and 4.16% by Bulman *et al* (1989), 23.9 and 5.4% by Magi *et al.* (1989), 12.3 and 2.1% by Perez-Sanchez *et al.* (1989), 53.8 and 0.0% by Hatsushika *et al.* (1992) and 10.7 and 5.5% by Petruschke *et al.* (2001). Our finding fall within in the trend of results obtained by various workers mentioned above.

The prevalence of *Babesia canis* in our study has been recorded as 5% . These results are close to the findings of Collette (2000) who recorded 10% prevalence. However, the prevalence of *Babesia* species has been reported as 26% by Mathewman (1993), 20% by Ulmer *et al* (1993), 40.7 by Onishi *et al.* (1994), and 11.69 by Shakespeare (1995). The possible reason for high prevalence may be attributed to the locality and health status of dogs.

The current study was conducted in summer months (May to July). Although there were no significant differences noted in the prevalence of blood-parasites in different months, however, the lowest prevalence of blood-parasitic infection was recorded in the month of May (10%) with the peak in July 13.13%. Where as Ulmer *et al* (1993) recorded the number of cases of babesiosis from 1979-1992 among the 2000-2500 dogs, over all incidence was 66 cases in July while the highest was 373 in October, Horvath and Papp (1996) reported that the B*abesia canis* infection in 93 dogs, most cases were diagnosed in April, September and October, where as Varshney *et al* (2003) has been reported 60% canine babesiosis in summer months.

The age wise prevalence of blood-parasites did not show statistically significant differences among various age groups of dogs. However, a higher trend of infection was recoded in the dogs of older age groups than in younger dogs. The prevalence was recorded as 7.69% in pups and 14.70% in adults. This is possibly because of a low immunity to fight

blood-parasite infections in old age, these results coincide with those of Perez-Sanchez et al (1989) and Ulmer et al (1993) who have reported similar trend of disease.

In Hyderabad city, there are a reasonable number of stray dogs wandering thought the streets and roads. These dogs usually harbor various parasitic infections due to improper care and unhygienic conditions. Since they did not received any medicine for treatment so these infections usually persisted for a long time. Such dogs act as a reservoir of infections for the pet dogs. Pet dogs should be kept under hygienic environment and should be regularly tested for the presence of any blood parasite infection growing inside the body to avoid some serious consequences.

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TABLES

Table 1: Group-wise prevalence of blood-parasites

Group	Examined	Infected	infection %
A (pet dogs)	100	9	9
B (stray dogs)	200	26	13
Total	300	35	11.66

Table 2: Age-wise prevalence of blood-parasites.

Age	Examined	Infected	Infection %
A (Pups)	130	10	7.69
B (Adult)	170	25	14.70
Total	300	35	11.66

Table 3: Species-wise prevalence of blood-parasites

species	Examined	Infected	Infection %
Dirofilaria immitis	300	16	5.33
Dipetalonema reconditum	300	04	1.33
Babesia canis	300	15	5.00
Trypnosoma evensi	300	00	00

Table 4: Month-wise prevalence of blood-parasite

Month	Examined	Infected	Infection %
May	80	08	10
June	100	11	11
July	120	16	13.30
Total	300	35	11.66

Table 5: Sex wise prevalence of blood-parasites

Sex	Examined	Infected	Infection %
Male	225	21	9.33
Female	75	14	18.6
Total	300	35	11.66

Table 6: Comparative sensitivity of different techniques for the detection of microfilariae

Technique	tested	positive	Sensitivity %
Wet Blood Film	20	13	65
Giemsa Staining	20	9	45
Modified Knott	20	18	90
Haematocrit Centrifugation	20	6	30

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