

Epidemiological study for Toxoplasmosis and Leishmaniasis in stray dogs in Diwaniya city/Iraq

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Abstract:

A total of 175 blood sample of stray dogs from urban and rural in Diwaniya city /Iraq were examined for detection of Toxoplasmosis by Latex test and dipstick test to visceral Leishmaniasis , with Mercaptoethanol test to detect acute and chronic infection for both disease. The total rate infection that recorded was 48% positive to Toxoplasmosis in urban and rural , 63% acute and 36.9% chronic to the disease in urban and rural , and from the total 45% was acute in female with 51.6% acute in male .

In conclusion of samples recorded 46.8% positive for Leishmaniasis in urban and rural, 28% Acute and 71.9% chronic for disease . According to the sex 64.4% female acute and 35.5% chronic for disease with 45.9% Male acute and 54% chronic for disease , the mixed infection of Leishmaniasis and toxoplasmosis show 42.6% in urban and 18% in rural .

Key word: Toxoplasmosis & Leishmaniasis in stray dogs.

الخلاصة:

جمعت 175 عينة دم من الكلاب السائبة من مركز المدينة (الحضر) والريف (القرى) لمدينة الديوانية/ العراق وفحصت للتحري عن الإصابة بداء المقوسات باختبار اللاتكس والتحري عن الإصابة بالليشمانيا الحشوية بواسطة اختبار آل dipstick ثم إجراء اختبار الميركيتوايثانول لتحديد الإصابة الحادة والمزمنة لكلا المرضين وحسب الجنس. كانت الإصابة بداء المقوسات 48% من المجموع الكلي للعينات في المدينة والريف ومنها 63% كانت إصابة حادة و 36.9% مزمنة ، ومن المجموع الكلي كانت 45% إصابة حادة في الإناث و 51.6% حادة في الذكور و 54.7% مزمنة في الإناث، 48.3% مزمنة في الذكور .

وكانت نسبة الإصابة للعينات الموجبة 46.8% حالة إصابة الليشمانيا حيث كانت 28% حالة حادة و 71.9% مزمنة في المدينة أما بالنسبة للجنس فكانت 64.4% حالة حادة في الإناث و 35.5% مزمنة والذكور كانت 45.6% حادة و 54% مزمنة. وسجلت الإصابة المختلطة 42.6% في المدينة و 18% في الريف.

Introduction:

Leishmania infantum and *Toxoplasma gondii* are zoonotic protozoan parasites widely distributed in the world (1,2) *Toxoplasma gondii* is worldwide zoonotic protozoan which can infect virtually all mammals and birds ,the definitive hosts of parasite are domestic cats and other felines , the sexual cycle of this parasite occurring

only in these species (3). Humans and animals become infected either by consuming food contaminated with sporulated oocysts or by eating raw meat containing cyst(4,5).Dogs have recently been considered as a potential risk factor for *T. gondii* infection in human due to mechanical transmission of oocysts(6). *L.infantum* causes canine Leishmaniasis

(visceral and cutaneous), in humans it considered the third most opportunistic parasitic disease of human immunodeficiency virus patients HIV (7). Dogs are exposed to wide range of pathogens therefore can harbor the pathogens of zoonotic and veterinary diseases(8). Dogs are important as sentinels of disease agents because they have roamed free, during this period of free roaming, they would have had access to variety food items (rubbish, rodents ect.) that can serve as a source of pathogens, they have been outdoors exposed to vectors of disease such as sand flies or ticks, and they did not receive any preventive or curative treatment, conversely, they had been adopted in proximity to humans and other pet animals or livestock. At that moment they can transmit the pathogens to which they came into contact(9). Recently visceral leishmaniasis and toxoplasmosis are increased between children in Diwaniya governorate therefore our study is to determine the importance of the reservoir host (dog) in contact with human we did this study.

Materials and Methods:

This study was conducted in the beginning of March, 2011 until the end of August, 2011 was done in college of Vet. Med. Al-qadisiya University.

A total of 175 stray dogs blood samples (75 from urban and 100 from rural (villages)) . Dogs were collected randomly by catching and entrapping methods. Blood sample volume taken from dogs are (5ml) from saphenous vein with information of place, sex were recorded. Serum was prepared by centrifugation at 1200 rpm for 15 minute then stored at -20 °c until its used.

1-Toxoplasmosis Latex Test Kit (plasmatic Laboratory products UK).(10) . Latex reagent is a suspension of polystyrene particles sensitized with *Toxoplasma gondii* antigens . when

infected serum mixed with the Latex particles a distinct agglutination pattern (+ve) is observed as a result of the formation of antigen –antibody complexes, in case (-ve) absence of agglutination will be observed. A positive result gave a level of infection greater than 4IU/μl by using serial drop dilution method (1/2---1/8) and agglutination appearance within less of 4 minutes (10).

2-Leishmaniasis :-in Bios international (USA) We used kala azar Detect Rapid Test for the detection of leishmaniasis *L. donovani* antibody in the serum.

result the sensitivity 100 and specificity 93.137 The kala azar detect test for visceral leishmaniasis is a rapid immunochromatographic strip assay for the qualitative detection of antibodies to members of *L. donovani* in the serum, antibodies to a recombinant antigen specific for visceral Leishmaniasis caused by membrane of the *L. donovani* complex . the based of test immunoassay for the antibodies is pre-coated with rK39 on the test line region and chicken anti –protein –A on the control line region, during testing the serum sample reacts with the dye conjugate (protein –A colloidal gold conjugate) which has been pre-coated in the test device. the mixture then migrates upward on the membrane chromatographically by capillary action with recombinant (VL) antigen on the membrane and generates a red line . presence of this red line indicates positive result(11).

3- Mercaptoethanol test (2METest).

a-Mercaptoethanol inactivate the IgM antibodies while indicate learning the IgG antibodies intact (12), this lead to differentiate between acute (absence of agglutination –ve agglutination) and chronic infection (appearance of agglutination +ve agglutination) .

b-Procedure

-100 μl from 2-mercaptoethanol (0.1 molar) were added to 100 μl of serum and mixed together in vortex .

- incubated at water bath in 37 c for 1 hour.
 - 25µl of the mixed serum and 2-Mercaptoethanol were put on test area of card .
 - then 1drope (about 45µl) of well homogenised of *Toxoplasma gondii* and *Leishmania donovani* antigen was added in each test area.
 -A stirring rod , was used to mixed and spread out the reaction mixture to about 1mm from the edge of test area.
 The test card rotated on a flat bed rotator for 5 minutes at 70 rpm.
 -then the results in absence agglutination mean acute infection and presence agglutination mean chronic infection
 -Molaris of mercaptoethanol are found by the equation

$$M = (\% / 100) * 10 / \text{Molecular Weight (MW)}$$

$$= 98 / 100 * 10 / 78.13 = 0.125$$

Results:

From the total of 175 dogs ,84 (84%) were seropositive for *Toxoplasma gondii* antibodies against *T.gondii* were found in all districts sampled. A highest 53(41.3%) in urban dogs samples and lowest 31 (31%) in rural dogs samples.(table 1) .

To differentiated between acute and chronic Toxoplasmosis infection by Mercaptoethanol from total positive number 31(21)were acute Toxoplasmosis and (10) chronic to the disease in rural ,where overall53(32) acute toxoplasmosis and (21) chronic to the disease in urban area .(table2),(Figure2).

From total positive samples (84) of stray dogs in urban and rural 53 female (24) were acute Toxoplasmosis and 29 chronic to the disease , from 31 male positive sample (16) acute and (15) chronic to the disease .(table 3)

To detection Leishmaniasis in stray dogs from 175 , 82 (46.8%) were positive to the disease in urban and rural districts , 22 (29.3%) in urban and 60 (60%) in rural .table (4). By mercaptoethanol(6) 27.2% were acute from positive samples in urban and 16(72.7%) chronic to the disease.In

rural stray dogs samples 17 (28.3%) acute and 43 (71.6%) were chronic to diseases. (Table5),(Figure 2).

In both area urban and rural the total positive samples to Leishmaniasis 82.(45 female 29 64.4% were acute and 16 57.7% chronic to the disease) . while 37 male (17) 45.9% acute and (20) 54% chronic to the Leishmaniasis .(table6).

The mixed infection between toxoplasmosis and Leishmaniasis in urban stray dogs were 32 from 75 ,32 (42.6%) while 18 (18%) mixed infection in rural area , total 50 (28.5%) cases .table (17).

Discussion:

T.gondii in dogs is world widely distributed with different prevalence rate ranging in different countries (13-16).Our result found serum antibody to *T. gondii* were found in 84 (48%) out of the 175 dogs samples ,these percentage higher than reported in Austria26% (16) and lower than reported in dogs Rondonia , Brazil (76.4%) (14).Our results agreed with the study of stray dogs in Istanbul 51.3%, these variation in prevalence due to compare data of studies which used different serological test , sample volumes and type of live population dogs near with cats .the highest positive samples from strays dogs in urban 41.3% due to found the cats near the environment dogs which gave exposed to an environment contaminated with *T. gondii* oocytes.

Acute Toxoplasmosis in urban is more than in rural due to presence indicates dynamic of the disease and high risk of contact with environment which had oocyst and feed infected food with protozoan(17).

Chronic stage found in this study were means that not only the parasite is circulating in the environment producing constant contact with the cats , but there are period of reaction with or without excretion of oocyst depending on its immune system .Chronically infected hosts with tissue cyst .these may be rupture and

release bradyzoites to the circulation(18) which are eventually destroyed by the immunocompetent host or reactivate the infection in immunosuppressed animal (19). Diagnosis of Toxoplasma infection is conventionally made by direct demonstration or isolation of parasite from biopsy or autopsy material but such techniques are unsuitable for use in large – scale surveys.therefore immunoserological tests specific for host antibodies have been developed and a variety of tests have been described (20, 21).

Numerous epidemiologic studies of canine toxoplasmosis have been reported in most areas of world .Canine toxoplasmosis and Leishmaniasis in stray dogs have been reported by different methods (22,23)were recorded the infection by seroepidemiology in stray dogs in Ankara ,they found 72 (62.06%) of *T. gondii* and 3(2.58%) for *L. infantum*.

In Iraq few study about strays dogs and its important as a constitute potential risk for public health .We aimed to determine the prevalence of Leishmaniasis and Toxoplasmosis for the first study in stray dogs in Diwaniya governorate.

The prevalence of dogs exposed to *T. gondii* increased with age, suggesting acquisition of infection rather than congenital transmission of *T. gondii* in the canine population which is in accordance with reports by others (24,25).its interpreted that older dogs have more chance to feed on food rodents or have contact with the surrounding environment that can be contaminated by *T. gondii* oocyst .

Leishmaniasis was one of the most important and common disease in Iraq, our result show from 175 samples 82 positive

to the disease 22 (29.3%) in urban and 60 (60%)in rural, the result agreements with study of Oscar et al(9) which found 56.3 % of dogs visceral Leishmaniasis in Major Islands ,Spain . The study difference in result which found in Baghdad and suburbs(26) .

They found 3.7% in Baghdad and 6.6% in suburbs (in stray and houses)dogs . These variation may be the high number of dogs from the house dogs well in our study all the sample from the stray dogs and the large number from rural , the sample size and duration with areas of both studies are different in Iraq. The elevated prevalence of chronic infections mean the prolonged incubation periods of disease or the dogs exposure to the vector (sand fly).

The higher chronic Disease in rural than urban because the vector (sand fly) increase distribution in rural than urban .

Visceral Leishmaniasis endemic disease in Iraq and recently the disease become hygiene problem (27).

There are no statistical difference $P > 0.5$ between sex with acute and chronic disease.

These result support the importance of the role of stray dogs in parasite transmission in rural and urban . which the asymptomatic infected dogs can be a source of infection to the vector ,although symptomatic dogs are more effective reservoirs (28).the mixed infection from visceral leishmaniasis and *T. gondii* infection confirms that Diwaniya city/Iraq is an endemic area with *L. donovani* and wide spread presence of *T. gondii* and we need more elaborate study to establish complicity of dogs as reservoir host for Leishmaniasis and Toxoplasmosis in this endemic area.

Districts	Number of samples of stray dogs	Positive for toxoplasmosis No.	%
Urban	75	53	70.6
Rural	100	31	31
Total	175	84	48

Table (1): show the number of positive samples of stray dogs to toxoplasmosis .

Districts	No+	Acute Toxoplasmosis %		Chronic Toxoplasmosis NO+ %	
Urban	53	32	60	21	39.6
Rural	31	21	67.7	10	32.2
Total	84	53	63	31	36.9

Table(2) : show the acute and chronic Toxoplasmosis in urban and rural from positive sample of stray dogs.

Sex	No+	Acute Toxoplasmosis %		Chronic Toxoplasmosis %	
Female	53	24	45	29	54.7
Male	31	16	51.6	15	48.3
Total	84	40	47.6	44	52.3

Table(3): Type of the disease (Acute , Chronic) according to the sex

Districts	No. samples	Positive to Leishmaniasis	%
Urban	75	22	29.3
Rural	100	60	60
Total	175	82	46.8

Table(4):show the number of positive samples to Leishmaniasis.

Districts	No+	Acute Leishmaniasis		Chronic Leishmaniasis	
		No.	%	No.	%
Urban	22	6	27	16	72.7
Rural	60	17	28.3	43	71.6
Total	82	23	28	59	71.9

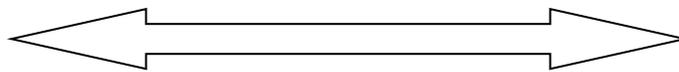
Table(5) show the Acute and Chronic samples for stray dogs to Leishmaniasis.

Sex	No+	Acute		Chronic	
		No.	%	No.	%
Female	45	29	64.4	16	35.5
Male	37	17	45.9	20	54
Total	82	46	56	36	43.9

Table(6): show the type of disease acute , chronic Leishmaniasis according to sex.

Samples	No.	Mixed infection Leishmaniasis and Toxoplasmosis	%
Urban	75	32	42.6
Rural	100	18	18
Total	175	50	28.5

Table (7): Show the mixed infection Leishmaniasis and Toxoplasmosis in stray dogs



Fig(1) Diagnostic results from three serum samples using the rapid rK39 immunochromatographic dipstick test for diagnosing visceral leishmaniasis. The single band represents negative control, while the two results with a double band reflect positive diagnoses for patients with visceral leishmaniasis.

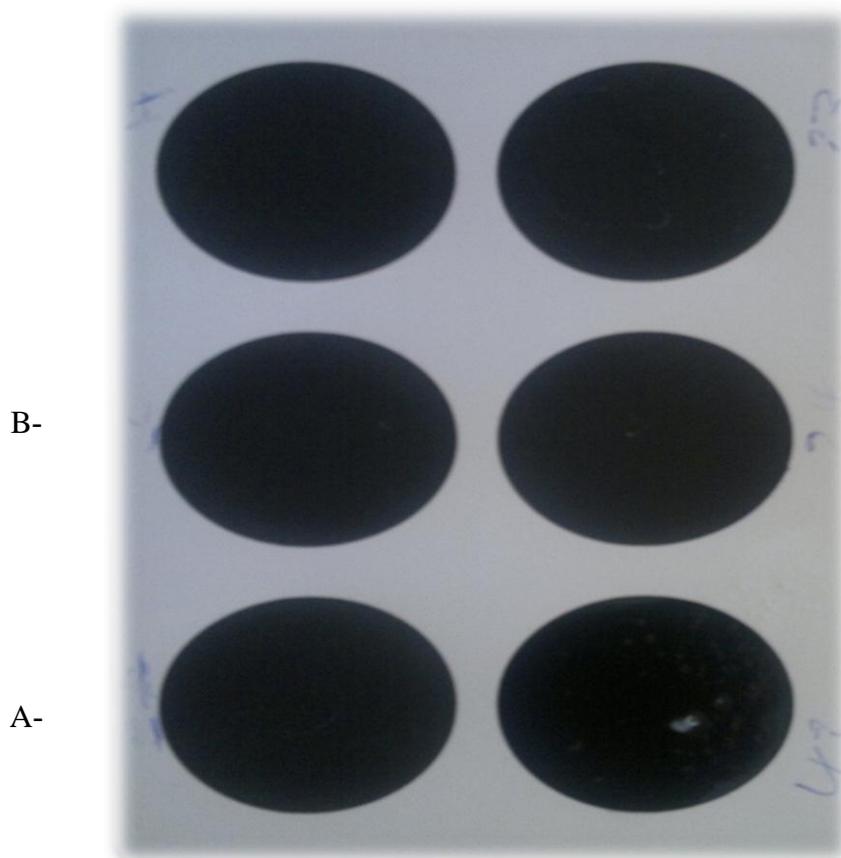


Fig.(2);show the results of mercaptoethanol test.

A-The agglutination mean chronic infections.

B-Absence agglutination mean acute infections

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