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A Preliminary Survey of Sheep and Goat Reservoirs for Visceral *Leishmaniasis* in Ismailia and Sharqia Governorates, Egypt

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Abstract: Leishmaniasis is of a veterinary and zoonotic importance. This study asserted the sheep and goat carriers of Visceral Leishmaniasis in Ismailia and Sharqia Governorate, Egypt. Screening of domestic animals, sheep and goats was carried out to detect antibodies against Leishmania donovani and to see the involvement of animal reservoirs. A total of 513domestic animals were included in the study. Serum samples were collected and surveyed by indirect hemagglutination assay (IHAT). The overall seroprevalence of sheep and goats Leishmaniasis infection in Ismailia and Sharqia Governorate, Egypt was 7.6 % (39/513).Seroprevalence of sheep and goat Leishmaniasis in Sharqia Governorate was 7.1 % (15/210) while in Ismailia Governorate was 7.9% (24/303). The highest titer in sheep and goat leishmanisis was 1/512 with percentage of 20.5%. The overall prevalence rate of Leishmaniasis infection in Ismailia and Sharqia Governorate was significantly higher ($P<0.05$) in old age and females than young age and males. The study was thrown light on the possibility of varied species of domestic animals to harbor the parasite and hence play a central role in the Leishmaniasis transmission in Ismailia and Sharqia Governorate, Egypt. Consequently, this may hurdle our clarification of disease dynamics.

Key words: Epidemiology · Animals · Parasite · Survey · Serology

INTRODUCTION

Leishmaniasis, visceral form is one of the most important parasitic diseases [1]. In eighteenth century beginning, Visceral *Leishmaniasis* appeared as serious disease. Severe form of Visceral *Leishmaniasis* is fatal specially the untreated cases [2]. Phlebotomine sand flies studying in the Sinai peninsula, Egypt is very important because it acts as vector of Phlebotomus fever virus and two species of Leishmania. Faunal exchange of sand flies in Egypt and surrounding areas was unclear dispersal routes between zoogeographic regions of mountains of the Red Sea coast, Nile valley and Sinai [3]. In contemporary years, high prevalence of leishmaniasis has been reported in North Africa [4] and in Europe [5] due to activity of human being (wandering, decampment, civilization, etc.), or a gradual increase in the overall temperature of the earth's atmosphere [6]. Leishmanin skin sensitivity test survey was applied in various villages of the Nile Delta, Egypt and positive cases were in Al Sharqia Governorate and 17 % of cases was in Faquus area in both sex [7]. Domestic animals act as a source of infection to leishmaniasis. *Leishmania donovani* DNA was detected in blood of domestic animals [8]. Positive samples of ancient Egyptian and Nubian mummies that originate from around 4000 B.C to *Leishmania donovani* DNA was detected [9]. Antibodies of *Leishmania* species were detected in 10% stray dogs in Egypt by commercial immunochromatographic strip assays based on recombinant antigen K39 [10]. Confirmatory diagnosis of Visceral leishmaniasis is depended on the detection of Leishmania spp. in biopsy from bone marrow, lymph nodes, liver and spleen. This technique is highly specific but accident occasionally occurs due to bleeding [11]. Seroprevalence of Leishmaniasis are widely done by serological tests including ELISA, direct agglutination and indirect fluorescence but these tests cross reacted with other Leishmania spp. in endemic area [12]. In North West Ethiopia seroprevalence of L. donovani in domestic animals samples by (DAT) Modified Direct Agglutination Test was 30.5% [13].

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The aim of the present study was to detect the seroprevalence of sheep and goat visceral Leishmaniasis in in Ismailia and Sharqia Governorates, Egypt by indirect hemagglutination test (IHA).

MATERIAL AND METHODS

The study was applied in Sharqia and Ismailia Governorates. Sharqia Governorate is the 3rd most populous of the governorates of Egypt. Located in the northern part of the country, its capital is the city of Zagazig. Ismailia is a city in north-eastern Egypt. Ismailia is situated on the west bank of the Suez Canal, it is the capital of the Ismailia Governorate.

Veterinary Medicine of Zagazig university ethical board gave permission to conduct the study within the institutional research mandate as stipulated by the National Ethical Board.

The Number of animal serum samples were 513 (180 goat and 333 sheep) were collected from Ismailia and Sharqia Governorates. In Ismailia Governorate, 303 animal serum samples (183 sheep and 120 goats) were collected from different farm localities. InSharqia Governorate, 210 animal serum samples (150 sheep and 60 goats) were collected from clinic hospital of veterinary medicine college, Zagazig University. The samples were collected at summer season 2015.

Animal blood samples were collected in a clean, dry tube and left to clot in a slanting position at room temperature for about 3 hours. Samples were transported to the department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Zagazig University. Blood was centrifuged at 600 g for 10 minutes. Only clear non hemolysed sera were transferred carefully by automatic pipette into clean and dry eppendorf tube. Then all sera were kept in deep freeze at –10°C until used for serological diagnosis.The collected sera were examined for detection of specific antibodies to Leishmania donovani using the indirect hemagglutination assay (IHAT) with a commercially available kit (Cellognost Leishman, Siemens Healthcare Diagnostic Products GmbH, 35041 Marburg/Germany) according to the manufacturer's instructions. Sera were added to 96-well non-irradiated V bottomed polystyrene (Greiner) micro titeration plates and dilution starting from 1:16 tell 1:16384.Leishmaniasis IHA reagent were dispensed into the all wells of rows 2–12. The plates were shaken with a micro titeration plates shaker for 15 to 20 seconds and then incubated at +15 to +25 °C for 2 to 24 hours without shaking. Serum dilution of 1:32 and higher provide diagnostically useful titers. Mid-range serum titers lie between 1:256 and 1:2048. Positive and negative controls. The data were analysed According to The t-test and one-way Analysis of Variance (ANOVA) analysis. Differences were considered significant when $P \leq 0.05$.

Fig. 1: Ismailia and Sharqiagovernorates map

RESULTS

The overall seroprevalence of sheep and goats Leishmanias is infection in Ismailia and SHARQIA Governorate, Egypt was 7.6%(39/513) Table (1). Seroprevalence of Leishmanias is in Sharqia Governorate was 7.1 % (15/210) while in Ismailia Governorate was 7.9% (24/303). In SHARQIA Governorate, the seroprevalence rate of Leishmaniasis in sheep and goats was 9.3% and 1.7% respectively while in Ismailia Governorate the seroprevalence rate of Leishmaniasis in sheep and goats was 8.2% and 7.5% respectively. The highest titer in sheep and goat leishmanisis was 1/512 with percentage of 20.5%. In SHARQIA Governorate, the most of titer in sheep Lieshmaniasis was 1/32 with percentage of 42.9% while the most of titer in goats Lieshmaniasis was 1/512 with percentage of 100%. In Ismailia Governorate, the most of titer in sheep Lieshmaniasis was 1/32 with percentage of 60% while the most of titer in goats Lieshmaniasis was 1/32 and 1/512 with percentage of 33.3%. totally in the two governorates, Sheep and goat Leishmaniasis infection titers were 1/32, 1/64, 1/128, 1/256 and 1/512 with percentage of 46.2%, 10.3%, 15.4%, 7.7% and 20.5% respectively, table (1). Sheep Leishmaniasis infection in young age, old age, males and females was 4.2% (7/165), 13.1% (22/168), 6.9% (13/189) and 11.1% (16/144) respectively and in goats Leishmaniasis infection in young age, old age, males and females was 1.9% (1/54), 7.1% (9/126), 2.8% (1/36) and 6.3% (9/144) respectively.

Table 1: Titers of visceral Leishmaniasisinfection in Sheep and Goats

Sharqia Governorate													
				Titers									
				1/32		1/64		1/128		1/256		1/512	
Species	Examined	Positive	Positive (%)	N	%	N	%	N	%	N	%	N	%
Sheep	150	14	9.3	6	42.9	2	14.3	1	7.1	2	14.3	3	21.4
Goats	60	1	1.7	0	0	0	0	0	0	0	0	1	100
Total[1]	210	15	7.1	6	40	2	13.3	1	6.7	2	13.3	4	26.7
Ismailia Governorate													
Sheep	183	15	8.2	9	60	1	6.7	4	26.7	0	0	1	6.7
Goats	120	9	7.5	3	33.3	1	11.1	1	11.1	1	11.1	3	33.3
Total[2]	303	24	7.9	12	50	2	8.3	5	20.8	1	4.2	4	16.7
Total [1, 2]	513	39	7.6	18	46.2	4	10.3	6	15.4	3	7.7	8	20.5

Table 2: Seroprevalence of *Leishmaniasis*infection in Sheep and Goats in relation to age and sex

Sharqia Governorate								
	Sheep				Goats			
	Age*		sex		Age*		sex	
	A	B	♂	♀	A	B	♂	♀
Examined Numbers	30	120	30	120	15	45	15	45
Number of positive	1	13	1	13	0	1	0	1
Positive (%)	3.3	10.8	3.3	10.8	0	2.2	0	2.2
Ismailia Governorate								
Examined Numbers	135	48	159	24	39	81	21	99
Number of positive	6	9	12	3	1	8	1	8
Positive (%)	4.4	18.8	7.5	12.5	2.6	9.9	4.8	8.08
Total (%)	7/165 (4.2)	22/168 (13.1)	13/189 (6.9)	16/144 (11.1)	1/54 (1.9)	9/126 (7.1)	1/36 (2.8)	9/144 (6.3)

* age: (A) young age below one year (B) old age above one year

In Sharqia Governorate, the sheep Leishmaniasis infection in young age, old age, males and females was 3.3% (1/30), 10.8% (13/120), 3.3% (1/30) and 10.8% (13/120) respectively. While in the goat Leishmaniasis infection in young age, old age, males and females was 0% (0/15), 2.2% (1/45), 0% (0/15) and 2.2% (1/45) respectively. On other hand, In Ismailia Governorate, the sheep Leishmaniasis infection in young age, old age, males and females was 4.4% (6/135), 18.8% (9/48), 7.5% (12/159) and 12.5% (3/24) respectively. While in the goat Leishmaniasis infection in young age, old age, males and females was 2.6% (1/39), 9.9% (8/81), 4.8% (1/21) and 8.08% (8/99) respectively, Table (2). In Sharqia Governorate the prevalence rate of Leishmaniasis infection was significantly higher (P<0.05) in sheep than in goats.According to The t-test and one-way Analysis of Variance (ANOVA) analysis, the overall prevalence rate of Leishmaniasis infection was significantly higher (P<0.05) in old age and females than young age and males. Also, there was no statistically significant differences in sheep and goat Leishmaniasis seroprevalence in Ismailia Governorate (p-value> 0.05).In Sharqia Governorate, the most of goat infection was old aged females. While in sheep infection the prevalence rate was significantly higher (P<0.05) in female and old ages than in males and young ages. In Ismailia the prevalence rate of sheep and goat Leishmaniasis infection was significantly higher (P<0.05) in female and old ages than in males and young ages, Table (2).

DISCUSSION

One of the main goals of our study is to screen serologically sheep and goat domestic animals for their possible involvement in the epidemiology of Visceral Leishmaniasis in Sharqia and Ismailia Governorate. Cattle, donkeys, goats and sheep serve as sources of blood for L. donovani vectors especially cattle [15, 16]. Even if these domestic animals were not reservoir of L. donovani species, they play as risk factor to transmission of L. donovani by sand flies with indirect way to susceptible mammals [17, 19]. In the Egypt and other places of North

Africa, the epidemiological transmission of *L. donovani* is not well understood. Thus the epidemiological studies focusing on possible reservoir hosts are important for providing contributions to control measures and prophylaxis to be employed by public health authorities [20]. In the present study, we report for the first time the seroprevalence detection of visceral sheep and goat *Leishmaniasis* in Sharqia and Ismailia Governorate, Egypt. There are many African countries had domestic animals reservoirs of Leishmaniasis including sheep and goats [21-23]. In addition, The Egyptian mongoose Herpestes ichneumon is a possible reservoir host of visceral leishmaniasis in eastern Sudan [24]. The present study reports the presence of *L.donovani* antibodies in the samples taken from the sheep and goat serum for the first time in Egypt and there is Similar study in India indicates that goats are potential animal reservoirs of human Visceral Leishmaniasis [25]. Furthermore in the Indian subcontinent Nepal, Visceral Leishmaniasis was 6.1% in human , 5% in cows, 4 % in buffaloes and 16% in goats. The main risk factor animal reservoir of human Visceral Leishmaniasis infection in this study was goats although this data do not necessarily mean that goats constitute a reservoir host of *L. donovani*, these observations indicate the need for further investigation of goats possible role in VL transmission [26].

In conclusion, In the Sharqia and Ismailia Governorate and other places of Egypt, the animal transmission cycle of *L. donovani* is not well understood. Thus the epidemiological studies focusing on possible reservoir hosts are important for providing contributions to control measures and prophylaxis to be employed. Finally, this study suggests the possibility for a variety domestic animals to harbor the parasite and hence play a central role in the transmission. Therefore, more work is needed to isolate and to characterize Leishmania spp. causative agents.

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