

Original Article

Leptospirosis as a neglected burden at human-cattle interface in Mid-Delta of Egypt

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Abstract

Introduction: Leptospirosis is a neglected zoonosis in developing countries including Egypt where its burden is underestimated.

Methodology: A cross sectional study was carried out to estimate the seroprevalence and associated risk factors of *Leptospira interrogans* serovar Hardjo infection among cows and leptospirosis among human patients in Mid-Delta of Egypt.

Results: Out of 112 examined cows using ELISA, 3.6% were seropositive to *L. interrogans* serovar Hardjo infection. Seroconversion occurred in 5 animals (1 herd) of all examined animals in convalescent phase testing (5/112, 4.5%). Affected herd suffered acute outbreak with 43.3% within herd prevalence; signs of infection included abortions, bloody urine and sudden death of 2 cows. Highest risk for *L. interrogans* serovar Hardjo infection in cows was in animals drank from untreated surface water (6.7 times, $p = 0.06$). The seroprevalence of leptospirosis was 6.2% in all tested humans, 28.6% in nonspecific fever cases and 22.2% in non-viral hepatitis cases. The risk of leptospirosis among patients with nonspecific fever or non-viral hepatitis cases was 4 times higher than those with viral hepatitis ($p = 0.01$). Additionally, there was a significant association between leptospirosis and patients with livestock contact (Odds 8, $p = 0.01$).

Conclusions: This is the first report of *L. interrogans* serovar Hardjo outbreak in cows in Egypt. The study also highlighted the role of leptospirosis as neglected cause of nonspecific fever/non-viral hepatitis in humans in study region.

Key words: Leptospirosis; cattle; humans; risk factors; Egypt.

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Introduction

Leptospirosis is an emerging disease in developing countries with a little available information, which results in the disease being overlooked [1]. Leptospirosis was reported in many animal species including cattle, dogs, cats, rodents [2,3]. In cattle, the disease has a variety of symptoms; septicemia, haemoglobinuria, abortion, infertility, reduction in milk production and mastitis [4,5]. In humans, leptospirosis is associated with nonspecific fever, jaundice, pneumonia and haemoglobinuria [2,6]. The agricultural practices and frequent contact with livestock or their products are important risk factors for leptospirosis infection in humans as it increase the chances for humans to come into contact with the urine of infected animals or a urine-polluted environment [7-9]. Egypt is an agricultural developing country with a high

likelihood of contact between humans and ruminants specially cattle and sheep [10]. In Egypt, the relevant records on disease prevalence are scarce [11,12]. However, few recent reports suggested emerging high *Leptospira* infection in humans and animals in Egypt. For instance, [11] reported that around 49% of examined humans and 37.6% of the examined cows had antibodies to *Leptospira* infection. Cows are the main maintenance host of *Leptospira interrogans* serogroup Sejroe serovar Hardjo worldwide; a major cause of reproductive diseases and production losses in livestock [3,5]. *Leptospira interrogans* serovar Hardjo was the most common cause of cattle leptospirosis in several countries [3,5,13]. Records investigating prevalence of *L. interrogans* serovar Hardjo in Egypt are scarce. One study reported a very low rate (1%) in cattle in Egypt [12]; this study was conducted in one region, which

may not represent the epidemiological status of this serovar in Egypt. In addition, *L. interrogans* serovar Hardjo was associated with zoonotic transmission to dairy farmers [14], and was recovered at high rate from acute human infections in some regions [2], highlighting its public health risk as a zoonosis. Up to our knowledge, there is no study on the prevalence of *L. interrogans* serovar Hardjo among cows or on Leptospirosis prevalence among humans at Kafrelsheikh governorate. So, this study aimed at investigating the prevalence of *L. interrogans* serovar Hardjo infection among cows and the prevalence of leptospirosis among human population in Mid-Delta; Kafrelsheikh governorate, Egypt Potential risk factors of Leptospirosis in human-cattle population in study region was assessed as well.

Methodology

Ecology of the study area

Kafrelsheikh governorate lies in the north of Egypt. It is bordered by the Mediterranean Sea and Rosetta branch of the Nile at the north and west, respectively. It consists of 10 districts. The governorate is agriculture with high density of livestock population, and is famous for its rice production and wide spread of intensive fish farming (173,080 ton/year). In addition, plenty of stray dogs and rodents have access to water in agricultural lands and fish farms.

Sampling

Cow samples

Four districts were selected to represent the governorate (Kafrelsheikh and Qillin at the south; Biylaa and Elhamoul at the north). A cross sectional study was carried out to estimate the prevalence of *L. interrogans* serovar Hardjo infection among cows. The total cows' population in the 4 districts is 80,000 according the local animal census provided by the General Organization of Veterinary Services 2014. The sample size was estimated at expected prevalence of 50% and 9% accepted error as 119 cows using WinEpi Scope 2. One veterinarian in each of the selected districts was chosen to collect the samples. Data on the source of drinking water, history of signs of *Leptospira* spp. infection, pregnancy status, gender and age of each of the examined animals were recorded. Plain blood samples were collected aseptically by syringe aspiration from 119 cows and sent to lab where serum was separated by centrifugation (5000 rpm/5 min). Only 112 samples were used in serological testing because of hemolysis in some samples had occurred. Veterinarians were asked to collect a second sample from same

animal after 2-4 weeks from the first one to detect possible seroconversion (Acute infection). Acute case was defined by either seroconversion from negative to positive titer or a four-fold rise in titer in paired samples. Acute case was defined by either seroconversion from negative to positive titer or a four-fold rise in titer in paired samples. There is no official or commercial vaccine for leptospirosis among different ruminant species in the study area.

Human samples

A total of 129 serum samples were collected from humans as follows: 91 samples were collected from patients of the liver hospital in the governorate, while the other 38 serum samples were collected from 2 private hospital labs in the governorate. These serum samples were collected from patients attended in the hospital and the labs at the time of the authors' visit without previous differentiation of patients. Participant patients were categorized based on clinical manifestation and laboratory results to the following: viral hepatitis cases (positive for A, B, or C hepatitis viruses), non-viral hepatitis (negative for A, B, and C hepatitis viruses), and non-specific fever (negative for Brucellosis and Typhoid), and others cases (no hepatitis, and no fever). Information collected from examined patients included gender, residence (rural or urban), livestock contact (owning or working with livestock). Serum collection and clinical examination were conducted by medical staff of health facilities. Serum samples were preserved at - 20°C until being used for ELISA.

ELISA

ELISA for cows

L. interrogans serovar Hardjo Ab (cattle) ELISA kit (DRG International Inc., Springfield, USA) was used in this study. The test was carried out according to the steps provided with the kits. The positive/negative control sera were provided with the kits. The interpretation of results was as follow: Positive sample is the one scored optical density (OD) > mean OD of negative control plus 0.2. Negative sample is the one scored OD < mean OD negative control plus 0.1. Doubtful sample is the one scored OD between mean OD negative control and 0.2.

ELISA for humans

SERION ELISA classic *Leptospira* IgG/IgM (Virion-Serion, Warburg, Germany) was used to detect IgG as indicator of existing/previous exposure of participants to leptospirosis. The test was carried out

according to the kit manufacturer, and results were interpreted as follow: negative = IgG <10 IU/ml, borderline = IgG 10–15 IU/ml, and positive = IgG >15 IU/ml. Only Positive results were included in the analysis.

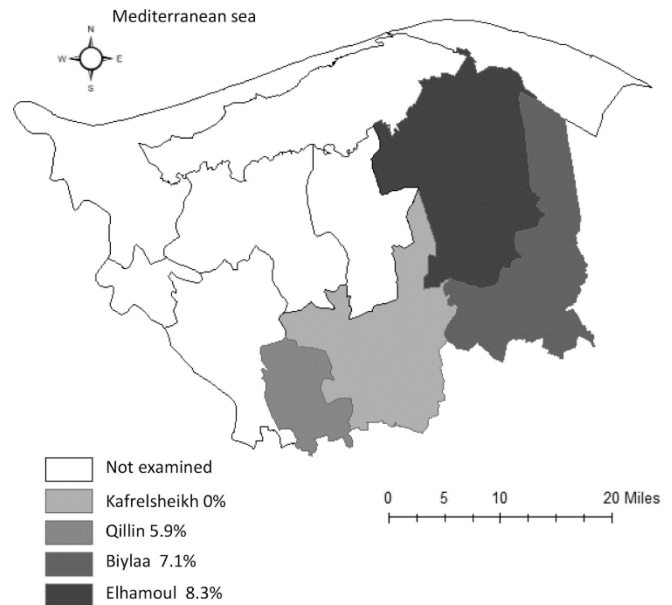
Epidemiological investigation

The seroprevalence of leptospirosis among either humans or cows were estimated by dividing the number of seropositive humans or cows by the number of tested humans or cows and multiply the result by 100 [15]. A univariate logistic regression was built to identify risk factors for leptospirosis among cows in the study area. Gender, age, type of breeding (either household animals or animals belonged to farms or herds), and location (southern vs. northern districts) were the examined variables; risk factors. Same model was used to estimate the degree of association between humans being seropositive to leptospirosis and risk factors including: gender, residence (rural vs. urban), livestock contact (caring or working), and clinical presentation (viral hepatitis, non-viral hepatitis and non-specific fever). This regression model was built on SAS 9.2. (SAS Institute, 2008) and significance level was set at $p < 0.05$.

Ethical approval

Ethical approval was obtained from the committee of Research, Publication and Ethics of the Faculty of Veterinary Medicine, Kafrelsheikh University. All procedures were explained to cows’ owners and owners’ informed verbal consents were obtained. Additionally, all human samples were collected by medical staff of hospital and private labs. Protocol, objectives and risks of the study were explained to all

Figure 1. Map of Kafrelsheikh governorate, Egypt showing *L. interrogans* serovar Hardjo seroprevalence per district among cows in study region.



participant humans and written consents were obtained prior to sampling.

Results

Out of 112 cow’ serum samples examined, 4 (3.6%) were seropositive to *L. interrogans* serovar Hardjo infection. Prevalence rates were higher in animals bred in herds/farms (7.1%) and lives in northern districts (7.5%) than those reared in households (2.4%) and lives in southern districts (1.4%) (Table 1, Figure 1). Acute infection was evidenced by seroconversion in the second test after 2-4 weeks (convalescent phase) in 5 animals (1 herd) from of all examined animals (5/112, 4.5%). In this herd, seroprevalence rate in examined

Table 1. Potential risk factors associated with leptospirosis status among cows in Kafrelsheikh governorate.

Variable	Number	Positive (%)	OR	SE	p <	95% CI
Housing						
Households	84	2 (2.4)	0.32	0.512	0.26	0.04 - 2.40
Farms/herds	28	2 (7.1)	-	-	-	-
Age*						
< 1 year	30	2 (6.7)	3.5	79.85	0.95	0.30 - 40.35
1-2 years	12	0 (0)	< 0.01	159.7	0.96	< 0.01 - > 99
> 2 years	50	1 (2)	-	-	-	-
Gender*						
Male	16	1 (6.3)	2.4	0.63	0.47	0.21 - 28.90
Female	76	2 (2.6)	-	-	-	-
Water source						
Surface water	16	2 (12.5)	6.71	1.04	0.06	0.87 - 51.58
Tap water	96	2 (2.1)	-	-	-	-
Location						
Northern districts	40	3 (7.5)	5.8	1.17	0.14	0.6 - 57.3
Southern districts	72	1 (1.4)	-	-	-	-

*There are some farmers who did not answer the question of the age or gender of their animals; Northern districts: Biylaa and Elhamoul; Southern districts: Kafrelsheikh and Qillin.

animals increased from 7.7% (1/13) in the first ELISA to 46.2% (6/13) in the second test (5 animals seroconverted from negative to positive). Serum was collected from all animals in this herd; 26 out of 60 animals were seropositive (43.3%) indicating active outbreak. This herd showed clinical signs of hemoglobinuria in some of its animals and sudden death of 2 cows with a history of abortion. All cows reared in household and farms drink usually tap water (96/112, 85.7%) but cows bred in movable herds (16/112, 14.3%) drinks usually from surface water canals used for land irrigation. Risk odds of getting *L. interrogans* serovar Hardjo infection in cows were higher in animals bred in farms/herds vs. household animals (3 times), drank from surface water vs. tap water (6.7 times), and lived in northern vs. southern districts (5.8 times). However, none of these associations was significant ($p = 0.06 - 0.96$) (Table 1). Also, males and young cows less than 1 year age were at risk of infection 2.4 and 3.5 times more than females and old cows > 2 years, respectively. Yet, it was not significant difference. In humans, out of 129 serum samples examined, 8 (6.2%) samples were seropositive to leptospirosis infection. Leptospirosis antibodies were reported in 28.6% of cases with non-specific fever, and 6.3% of cases with hepatitis (4.7% of viral hepatitis cases, and 22.2% of non-viral hepatitis cases) (Table 2). The risk odds of leptospirosis in humans were higher, but not significant, in males vs. females (7.1 times) and rural vs. urban residents (1.98 times) as shown in Table 2. Also, infection with leptospirosis was significantly 8 times higher in patients with livestock contact ($p = 0.013$). Among clinical cases, odds of Leptospirosis in patients with non-specific fever or non-viral hepatitis were 4 times higher than in those with viral hepatitis ($p = 0.01$) (Table 2).

Discussion

Cattle are considered the maintenance host for *L. interrogans* serovar Hardjo worldwide [3,5,13], yet studies carried out in Egypt demonstrated that the most circulating isolates are *L. icterohaemorrhagiae* and *L. pomona* [11,12]. In the current study, the prevalence of infection with *L. interrogans* serovar Hardjo among cows was studied for the first time in Kafrelsheikh governorate. Results of this study declared that *L. interrogans* serovar Hardjo is endemic at low levels among cows in the study area; 3.6%. This result was almost similar to another report (3.1%) in Colombia [13]. Lower prevalence (1%) of this strain was reported in cattle from other region in Egypt [12]. The reason for this low prevalence may be attributed to the use of tap water (treated) for drinking of majority of tested animals reared in household or farms (85.7%), while only few tested animals from movable herds have access to contaminated surface water sources; a recorded risk factor for leptospirosis [7]. Additionally, our recorded higher odds (7 times) for leptospirosis in animals drank from surface water vs. those drank from tap water may support this hypothesis. Higher prevalence rate (7.1%) and risk odds (3 times) were recorded in animals reared in farms/herds than those in household. Cows reared in herds cross long distances for pastures, which expose them to many infection sources as other carrier wild animals, and contaminated water sources. This is supported by the finding of [3] who recorded high association between leptospirosis and increase in grazing acres. Also [16] reported that animals reared in high numbers (as farm setting) were more exposed than animals reared in small numbers (< 5), which agreed with our finding as utmost 1-5 animals are reared per household in Egypt. Leptospirosis prevalence in cows reared in northern districts was

Table 2. Potential risk factors associated with leptospirosis status among humans in Kafrelsheikh governorate.

Variable	Number	Positive (%)	OR	SE	p <	95% CI
Gender						
Male	67	7 (10.4)	7.12	0.54	0.07	0.85 – 59.61
Female	62	1 (1.6)	-	-	-	-
Residence*						
Rural	44	5 (11.4)	1.98	0.87	0.43	0.36 - 10.95
Urban	33	2 (6.1)	-	-	-	-
Livestock contact*						
Yes	14	4 (28.6)	8	0.84	0.013**	1.55 - 41.23
No	63	3 (4.8)	-	-	-	-
Hepatitis -Fever cases						
NS-Fever	7	2 (28.6)	8.2	0.98	0.03**	1.19 - 56.08
NV-Hepatitis	9	2 (22.2)	5.86	0.95	0.06	0.91 - 37.79
NS-Fever/NV-Hepatitis	16	4 (25)	6.8	0.77	0.01**	1.51 - 31.01
Viral Hepatitis	86	4 (4.7)	-	-	-	-

*There are some patients who did not answer the question of residence or livestock contact; NS: non-specific; NV: non-viral; **: Significant at $p \leq 0.05$.

higher than those reared in southern ones (7.5% vs. 1.4%). Northern districts are close to Burullus Lake and many rice fields and fish farms are concentrated in these districts of the governorate. Leptospirosis was highly associated with exposure to rice fields and contaminated surface water [7,9,17], which could explain our findings. Seroconversion was recorded in 1 herd that uses surface water canals for drinking and moved around the governorate all the year for pastures. This herd suffered from active *L. interrogans* serovar Hardjo outbreak with 43.3% of its animals tested positive in convalescent ELISA test. The signs of infection include bloody urine, abortions and sudden death of 2 animals.. Same *L. interrogans* serovar Hardjo outbreak pattern was previously recorded in two outbreaks (41.8 - 48.4% prevalence rate) in Brazil [5]. This is the first recorded *L. interrogans* serovar Hardjo outbreak among cattle in Egypt. These findings highlight the role of surface water sources for distributing the *Leptospira* infection in cattle population in study area and the importance of preventing different animal species from access to these high risk sources. The prevalence of *Leptospira* spp. antibodies among humans of study area was estimated at 6.2%, which was comparable with other reports (1.4-14%) in Americas [2,18]. Much higher prevalences (46.3 - 49.7%) were recorded in Egypt [11] and in Malaysia [19], however these reports tested only high risk groups who either owned or worked with live livestock or their products. Hepatitis is among the top 10 leading causes of death in Egypt [20]. Majority of hepatitis cases in Egypt are due to Hepatitis viruses A, B and C, however negative-ABC Hepatitis cases also exist with mostly unknown etiologies and consequent difficulties in control. Leptospirosis was reported in 22.2% of hepatitis cases of unknown cause. This finding was higher than previous reports (2 - 16.1 %) in Egypt [21] and other African countries [6]. Fever is a common sign of leptospirosis in humans that occurs in almost all acute cases [2,22]. This study showed that leptospirosis was found in 28.6% of febrile cases of unknown cause. This was comparable with other reports (16 - 29.9%) from Egypt and elsewhere [6,21,23]. Leptospirosis detection is not a routine test for febrile patients in Egypt and most developing countries, thus underreporting and misdiagnosis is highly expected in these countries and may contribute to the neglected burden of Leptospirosis worldwide. Notably, Leptospirosis was almost 7 times ($p = 0.01$) more likely associated with fever or hepatitis cases of unknown cause than with cases of viral hepatitis, which highlighted the important role of Leptospirosis as a possible cause of these unidentified

cases in Egypt. Our results showed that males showed higher rate (10.4%) and odds (7 times) for Leptospirosis than females, which agreed with other reports worldwide [2,22]. In Egypt, males are more associated in professions as agriculture farming, sewer, abattoirs or animal caring than females; high risk occupations [7,9]. There was no significant difference in leptospirosis risk between rural and urban patients which agreed with [22]. Despite expected high *Leptospira* exposure for rural residents linked to more agricultural activities or animal contact, there was no significant difference in leptospirosis risk between rural and urban patients ($p = 0.4$). This agreed with [22], and could be attributed to frequent exposure of urban residents to stray animal carriers (dogs, rodents) or to contaminated stagnant water (gutter water) especially in urban slums as previously reported [7,17]. Frequent contact with livestock was significantly associated with leptospirosis (OR 8, $p = 0.01$), which agreed with other reports [8,9]. This highlights the importance of direct zoonotic transmission of leptospirosis and emphasis the role of disease awareness in population at risk to avoid infection.

Conclusions

This study reported the first outbreak of *L. interrogans* serovar Hardjo in 1 cattle herd with fatalities and production loss, yet this serovar seemed to spread at low level in Egypt. The study also highlighted the importance of leptospirosis as a neglected or underestimated cause of febrile illness or hepatitis in Egypt. More attention should be taken by national authorities to take practical actions towards estimating the disease burden and improving public awareness campaigns of the disease in Egypt.

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