Cross-Species Surveillance of *Leptospira* in Domestic and Peri-Domestic Animals in Mahalla City, Gharbeya Governorate, Egypt

Stephen A. Felt, Momtaz O. Wasfy,* Wael F. El-Tras, Ahmed Samir, Bassem Abdel Rahaman, Marie Boshra, Tina M. Parker, Mahmoud Essam Hatem, Ahmed Ahmed El-Bassiouny, Clinton K. Murray, and Guillermo Pimentel

Laboratory Unit, United States Naval Medical Research Unit-3, Cairo, Egypt; Department of Hygiene and Preventive Medicine, Faculty of Veterinary Medicine, Kafr El Sheik University, Egypt; Microbiology Department, Faculty of Veterinary Medicine, Cairo University, Egypt; Infectious Disease Service, Brooke Army Medical Center, Fort Sam Houston, Texas

Abstract. A survey of 179 animals (black rats, dogs, sheep, buffaloes, cattle, donkeys, weasels, and cats) for *Leptospira* infection was conducted in Mahalla City (Lower Egypt). Blood, urine, and kidney were collected and tested by culture, microscopic agglutination test (MAT), and/or polymerase chain reaction (PCR). Among rats, 26% were positive by PCR, including 7% that were also positive by culture for *L. interrogans* serovars Grippotyphosa, Pyrogenes, and Icterohaemorrhagiae. *L. borpetersenii* serovar Polonica was isolated for the first time in Egypt in three rats. MAT titers $\geq 1:800$ were observed in 11% of rats and 12% of dogs. *L. interrogans* serovar Grippotyphosa was detected in one cat. Sheep and donkeys were negative for leptospirosis by all methods. Buffaloes and cattle were seropositive in 20% and 44% of animals, respectively. Data indicate that several pathogenic serovars are circulating in the animals, which may pose exposure risks and account for high rates of acute febrile illness.

INTRODUCTION

Leptospirosis is a worldwide reemerging zoonotic disease caused by bacteria from the genus *Leptospira*. The natural reservoirs and carriers of *Leptospira* are domestic and wild animals, which may act as maintenance or accidental hosts. Maintenance hosts usually develop chronic infection of the renal tubules at an early age and remain asymptomatic while excreting leptospires for long periods of time.¹ The primary habitat for pathogenic serovars is the renal tubule, where they colonize, reproduce, and can be transmitted to humans or other mammalian hosts.^{2,3} Modes of transmission include direct contact of the mucous membranes (nose, mouth, and eyes) or compromised skin (cuts, abrasions, or waterlogged skin) with the urine or tissues of infected animals. Indirect transmission occurs because of exposure to contaminated water, soil, and mud. Congenital transplacental infections, including non-venereal environmentally acquired infection of pregnant females, have been shown, and leptospires were detected in the vaginal fluids and semen of many animal species.^{4,5} A species of animal that serves as maintenance host for one serovar can become an accidental host of another serovar, which can result in severe or fatal disease. Moreover, every mammalian species is potentially an accidental host for leptospirosis. Currently, there are more than 200 serovars of *Leptospira* spp. that are distributed worldwide.⁶

In animals, clinical signs associated with leptospirosis can range from insignificant to death. In acute disease, most mammalian species seem to develop similar general symptoms: lethargy, anorexia, fever, ruffled hair coat, suffusion, hemorrhages, icterus, agalactia, diarrhea, and abnormal posturing because of renal pain associated with nephritis. If pregnant, animals are infected *in utero*, and fetal death resulting in abortion may occur. The disease is seasonal, with peak incidence in summer or fall, where temperature and humidity are key factors in the survival of leptospires.^{2,3,5} In developing countries, agriculture continues to be the main source of employment, livelihood,

and income for a large number of rural residents. Living and working in close proximity to animals or their wastes may allow greater opportunities for exposure and infection.

The disease has been reported from many countries throughout the world. Within the Middle Eastern region, leptospirosis caused severe illness among children in Eastern Turkey in 2003, emphasizing the importance of leptospirosis in rural areas where farming is the major source of income.⁷ In Egypt, recent surveillance studies conducted by the US Naval Medical Research Unit No. 3 (NAMRU-3) revealed that acute febrile illness (AFI) patients in the Mahalla city had an increased prevalence of leptospirosis compared with other regions of the country.^{8,9} The latest country serosurvey for leptospirosis in animals was conducted in Cairo more than 20 years ago.¹⁰

The aim of this study was to assess the proportion of wild and domestic animals with previous or acute leptospirosis in the Mahalla vicinity and to determine the most common *Leptospira* serovars. Knowledge of the predominant *Leptospira* serovars in animal carriers and reservoirs may be of epidemiological value in monitoring their circulation, determining potential exposure to animals, and implementing prevention and intervention measures.

MATERIALS AND METHODS

Study area. Mahalla is part of Gharbeya Governorate in Northern Egypt, approximately 100 km north of Cairo, with 500,000 inhabitants (2005 estimates) along a smaller canal off the Damietta branch of the Nile. Irrigation canals and basin irrigation practices continue to be used almost exclusively in this area. The economy of Mahalla is based on farming. This region produces the majority of the nation's rice and cotton. Many of the inhabitants who do not work directly in the fields often labor in one of the rice mills or any of the numerous textile plants within the immediate vicinity. No animal survey for leptospirosis has been conducted in this geographical area of Egypt.

The study was approved by the Institutional Animal Care and Use Committee of NAMRU-3, which was established in 1998 after accreditation by the International Association of Assessment and Accreditation of Laboratory Animal Care

*Address correspondence to Momtaz O. Wasfy, US Naval Medical Research Unit-3, 3A, Imtidad Ramses Street, Abbassia, Cairo, Egypt, 11517. E-mail: momtaz.wasfy@med.navy.mil

(AAALAC). A total of 100 black rats (*Rattus rattus*), 25 dogs (*Canis familiaris*), 25 sheep (*Ovis aries*), 12 buffaloes (*Bubalus bubalis*), 9 cattle (*Bos taurus*), 4 donkeys, 2 weasels (*Mustela nivalis*), and 2 cats (*Felis catus*) known to reside in close proximity to people in Mahalla were included in this study. This was ascertained through patient survey questionnaires from Institutional Review Board-approved surveillance studies of AFI.⁹

Specimen collections. All animal tissue collection and laboratory research for this study were conducted between September 2006 and October 2007.

Wild rodents and carnivores. Based on previous human surveillance studies in Mahalla, Tomahawk traps were placed in multiple field sites based on the proximity to houses or land property of patients known to have had a history of leptospirosis. For logistical reasons, an average of 25 traps were used during the study period to conduct one capture session every other week. The bait consisted of fresh tomatoes and cucumbers, and the capture success rate was in the range of 20–30%. Investigators wearing rubber or heavy-ply latex gloves collected the traps in the morning and placed them in double plastic bags with fenestrations. Animals were transported to the Veterinary Medicine College, Kafr-el-Sheikh University in a leak-proof plastic bag with isoflurane-soaked gauze inside. Blood was collected by aseptic intracardiac venipuncture for culture and serology test. Animals less than 200 g were cervically dislocated, and bilateral thoracotomies were performed on larger animals (> 200 g) to ensure death. Kidneys with intact capsule and urine were aseptically collected. Serum samples were kept at –20°C or in liquid nitrogen until processed.¹¹ Each captured animal had a unique identification number, with date and location of capture, species, age (juvenile or adult), lactational status, and any gross pathology noted on necropsy.

Cats and dogs. Tissue samples were collected from three populations of animals: feral dogs and cats that were euthanized by local animal control officials and veterinarians as population control or public safety measure, presumed feral animals found dead on the streets, and house pets that were euthanized or died under the care of local veterinarians. Sampled tissues included kidney biopsy (with renal capsule), urine, and blood.

Farm animals (sheep, cattle, water buffalo, and donkeys). Tissue samples were collected from animals that were being processed at local slaughterhouses or were being treated by local veterinarians. Samples from dead animals included renal biopsy (with intact capsules), urine, and blood. Sampling was limited to blood and in some cases, free catch urine from live animals.

Laboratory procedures. Animals were considered positive for leptospirosis infection if the organism was recovered from culture or when polymerase chain reaction (PCR)-specific assays for pathogenic and/or non-pathogenic *Leptospira* spp. were positive. Seropositivity was defined by titers $\geq$ 1:800 for one or more serovars using the microscopic agglutination test (MAT).

Leptospira cultures. Cultures were performed using Ellinghausen McCullough Johnson Harris (EMJH) broth medium (3–4 tubes/sample) with 5-fluorouracil (200 µg/mL; Sigma Chemical Co., St. Louis, MO) to minimize contamination. Renal tissue was macerated before inoculation into culture media, and the remainder was stored at –20°C for PCR diagnostics. Only 2–3 drops of blood or urine (undiluted or diluted 1/10 and 1/100 in EMJH broth) were inoculated into

the medium. All cultures were kept at room temperature until transported to NAMRU-3, where incubation was continued at 28–30°C for up to 13 weeks. Cultures were examined weekly by dark-field microscopy to detect leptospiral growth.

PCR diagnostics. Briefly, DNA was extracted from animal tissues or *Leptospira*-positive culture using the QIAmp DNA Mini Kit (Qiagen, Valencia, CA) in accordance with the manufacturer's instructions. PCR was carried out in consecutive assays for amplifying the Lig1/Lig2 (detects all pathogenic leptospires) and G1/G2 (detects all *Leptospira* strains, including the non-pathogenic *L. biflexa*) genes as previously described.^{12,13} Although both assays were highly specific, the Lig1/2 PCR was shown to be sensitive to as few as six leptospires per 1 mL, and the G1/G2 PCR was sensitive to 50 leptospires.¹²

MAT. The test was performed as described by Kurtoglu and others and Parker and others.^{7,9} Briefly, test serum dilutions (1/50–1/12,800) were separately mixed with individual *Leptospira* cultures ($N = 23$ serovars; *L. biflexa* serovar Patoc and *L. biflexa* serovar Andamana were also included) and evaluated for the degree of 50% or more agglutination or lysis using dark-field microscopy. The serovar with the highest titer was considered to be the infecting serovar. Cultures of all *Leptospira* serovars required for the test were provided by Dr. Bajani from the Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention (Atlanta, GA).

Pulsed-field gel electrophoresis. Pure isolates were subcultured in EMJH broth and sent to the Brooke Army Medical Center, Fort Sam Houston, TX, for speciation and determination of serovars by comparison of pulsed-field gel electrophoresis (PFGE) profiles of *NotI*-digested genomic DNA of *Leptospira* isolates with known leptospiral serovars in the current Centers for Disease Control and Prevention database using the Dice band-based coefficients.¹⁴ *L. biflexa* serovar Patoc reference strain (US Department of Agriculture National Veterinary Services Laboratory [NVSL]) was used for control purposes.

Statistical analysis. Sample size was predicted based on the following assumptions: at least 10% of the animals were infected (based on similar studies conducted elsewhere),^{5,10,11} larger numbers of rodents would be necessary to detect more *Leptospira* serovars and minimize trap success bias when placed within or outside of particular microhabitats and near to houses or land property of patients known to have had a history of leptospirosis. Although the targeted numbers of some animal species (cats and donkeys) were suboptimal because of low clinical veterinary caseloads during this period, the obtained data may slightly tend to over- or underestimate the proportions of infected and carrier animal species, typical of most similar studies. The relative risks of animal exposure were determined at 95% confidence level using the method of Miettinen and Nurminen.¹⁵

RESULTS

Of the 100 rats captured and tested, 26% were positive for leptospires (either pathogenic or non-pathogenic serovars) (Table 1), including 7 that were detected in cultures (four *L. interrogans* belonging to serovars Grippotyphosa [$N = 1$], Pyrogenes [$N = 2$], and Canicola [$N = 1$] and three *L. borpetersenii* serovar Polonica) and 19 that were detected by PCR,

TABLE 1

Summary of culture, PCR, and MAT results for *Leptospira* spp. collected from September 2006 to October 2007 in domestic and peri-domestic animals from Mahalla City, Egypt

Animal	No. collected	No. positive by culture	Serovars isolated* (no. positive; specimen source)	PCR positive results		Serovars detected through MAT (no. positive)§
				All leptospirae†	Pathogenic leptospirae‡	
Rats	100	26	Grippotyphosa (1; kidney), Pyrogenes (2; kidney and blood), Canicola (1; blood), <i>L. borpetersenii</i> serovar Polonica (3; blood)	19	11	Grippotyphosa (4), Icterohaemorrhagiae (1), Australis (1), Hardjo (1), Hebdomadis (1), Patoc (3)
Water buffaloes	12	0	No growth	0	0	Hardjo (2)
Cats	2	1	<i>L. biflexa</i> serovar Patoc (1; kidney)	1	0	Grippotyphosa (1)
Cattle	9	0	No growth	0	0	Hardjo (1), Icterohaemorrhagiae (1)
Dogs	25	0	No growth	3	0	Australis (1), Canicola (1), Patoc (1)
Sheep	25	0	No growth	0	0	Negative
Weasel	2	0	No growth	0	0	Patoc (1)
Donkeys	4	0	No growth	0	0	Negative

* *L. interrogans* unless otherwise specified. Identification based on PFGE methodology.

† Includes non-pathogenic and pathogenic human leptospirae strains.

‡ Pathogenic to humans.

§ Titers $\geq 1:800$.

with 11 carrying pathogenic leptospirae. Four rat samples were positive in Lig1/Lig2 PCR only. Although 11% of the rats were seropositive in MAT at titers $\geq 1:800$, another 3% showed relatively lower titers and were also culture-positive. One rat was seropositive in MAT to four different serovars (Table 2). A total of 31 rats were juveniles, and two were seropositive. Of these two seropositive juvenile rats, only one was positive by culture and Lig1/Lig2 PCR.

Rats were also examined on necropsy for gross evidence of disease. Ectoparasites were observed in 96% of the rats necropsied, with fleas infesting the vast majority and lice and mites observed only occasionally. Of the rats with external parasites, 14.6% were positive for *Leptospira* by culture or PCR, and 9% were observed to have traumatic injuries to the integument system; of these, two (22%) were culture-positive for *Leptospira*. Six rats had renal and/or hepatic lesions; of these, four (66.7%) were positive by culture, whereas the rest were seropositive by MAT. When looking at the various host factors, rats that were adult, female, parasitized, and had

visceral lesions were at relatively increased risk of leptospire infection (Table 3), but this relationship was statistically non-significant ($P > 0.05$).

Of 25 dogs, only three animals were seropositive in MAT, with one being positive for serovars Canicola (titer $> 1:1,600$) and Pyrogenes (titer $> 1:800$), another being positive for Australis (titer > 800), and the third showing a high antibody titer against *L. biflexa* serovar Patoc (1:6,400). Three other canids were positive only by PCR (G1/G2 primers) for all types of leptospirae.

In bovids (water buffaloes and cattle; $N = 21$), all culture and PCR results were negative (Table 1). However, seroprevalence of *Leptospira* antibodies was 19% (4/21; all with titers $\geq 1:800$). *L. interrogans* serovar Hardjo was the most common serovar detected among these animals by MAT. *L. interrogans* serovar Icterohaemorrhagiae was detected in cows only (Table 2).

Although the number of captured weasels was limited ($N = 2$), only one animal showed a high antibody titer against

TABLE 2

Multiple MAT reactivity to different *Leptospira* serovars in wild and domestic animals in Mahalla, Egypt

Seropositive animals (%)	Number of animals MAT reactive regardless of titer
Rats = 14/100 (14%)	1 = Grippotyphosa (1:128,000) and Georgia (1:200) 1 = Grippotyphosa (1:3,200), Ballum (1:200), and Georgia (1:50) 2 = Grippotyphosa (1:1,600 and 1:800) 1 = Icterohaemorrhagiae (1:1,600) and Djasiman (1:100) 1 = Hardjo (1:12,800), Hebdomadis (1:3,200), Ictero haemorrhagiae (1:6,400), and Djasiman (1:400) 1 = Georgia (1:400) 4 = Patoc (1:3,200, 1:800, 1:200, and 1:50) 1 = Patoc (1:800) and Ballum (1:200) 1 = Australis (1:3,200) 1 = Djasiman (1:100)
Water buffaloes = 3/12 (25%)	1 = Hardjo (1:800) and Djasiman (1:50) 1 = Hardjo (1:6,400) and Pyrogenes (1:50) 1 = Hebdomadis (1:50)
Cows = 4/9 (44.4%)	2 = Icterohaemorrhagiae (1:400 and 1:3,200) 1 = Hardjo (1:3,200), Wolfii (1:100), and Djasima (1:100) 1 = Pomona (1:800) and Javanica (1:50)
Dogs = 3/25 (12%)	1 = Patoc (1:6,400) 1 = Australis (1:800), Wolfii (1:400), Bratislava (1:100), and Djasiman (1:400) 1 = Canicola (1:1,600) and Pyrogenes (1:800)
Weasels = 1/2 (50%)	1 = Patoc (1:800) and Icterohaemorrhagiae (1:400)
Cats = 1/2 (50%)	1 = Grippotyphosa (1:3,200), Hardjo (1:400), Patoc (1:200), and Canicola (1:100)

TABLE 3
Host factors and leptospirosis in black rats

Criteria (host factors)	<i>Leptospira</i> - positive*	<i>Leptospira</i> - negative	Relative risk	95% confidence interval†
Adult	20/26	49/74	1.16	0.85–1.49
Juvenile	3/26	28/74	0.30	0.10–0.81
Male	10/26	39/74	0.73	0.41–1.17
Female	13/26	38/74	0.97	0.60–1.50
Trauma	2/26	7/74	0.81	0.20–3.12
Visceral lesions	4/26	4/74	2.84	0.82–9.64
Ectoparasites	22/23	61/74	1.03	0.79–1.22

* Positivity was determined by culture or PCR.

† Using the approximation of Miettinen and Nurminen,¹⁵ relative risk (RR) of 1 means that there is no difference in risk between the two groups, RR of < 1 means that the event is less likely to occur in the experimental group than in the control group, and RR of > 1 means that the event is more likely to occur in the experimental group than in the control group.

L. biflexa serovar Patoc (1:800). Likewise, one of two cats studied was culture-positive for an isolate showing PFGE profiles comparable with *L. biflexa* serovar Patoc, whereas the other was seropositive against *L. interrogans* serovar Grippotyphosa (1:3,200) and Hardjo (1:400). All samples collected from sheep and donkeys were negative for leptospirosis by all diagnostic methods used (Table 1).

DISCUSSION

A wealth of studies exists on the role of domestic and wild animals in the transmission of leptospirosis to humans. A recent surveillance for AFI patients in Egypt revealed that, of the areas tested, Mahalla city had an increased frequency of leptospirosis compared with other regions of the country.^{8,9} Although the extent of animal exposure or carriage in a certain area requires continuous monitoring, there is no data on the distribution of leptospirosis in animals in Mahalla, and the latest animal surveys in Egypt were conducted in Cairo over 20 years ago.¹⁰ Of the animal species tested in Mahalla, rats, dogs, cats, cattle, and water buffaloes tested positive by culture or PCR, and many showed high antibody titers against *Leptospira* serovars. Weasels, donkeys, and sheep tested negative in these methods, which, for weasels and donkeys, could merely be a result of inadequate sample size.

The results obtained in this study show that sheep were negative for leptospirosis by all laboratory methods used, suggesting that this species may not be an important reservoir of leptospires in the Mahalla region. Early studies in Egypt revealed that 4.2% of sheep were seropositive.¹⁰ This discrepancy may be caused by differences in localities screened, number of animals used, or laboratory testing methods. Worldwide, the reported prevalence of leptospirosis in sheep has been comparatively low in Canada,¹⁶ Portugal,¹⁷ Chile,¹⁸ Italy,¹⁹ and Brazil.²⁰ However, Ciceroni and others¹⁹ claimed that carrier sheep in Italy had atypical serovars (e.g., serovar Poi) that have not been looked for in this study and rarely elsewhere. Whether unusual serovars not being tested for are being harbored in sheep remains unknown.

Results from donkeys in this study were also negative for leptospirosis, although earlier studies from Egypt showed that 17–29% of the donkeys were seropositive for *Leptospira*.¹⁰ Another recent study conducted elsewhere in the Middle East concluded that Iranian donkeys and horses have shown a high rate of seroconversion (40% and 28%, respectively), particularly against *L. interrogans* serovars Icterohaemorrhagiae, Ballum, and Pomona.²¹ Our findings suggest that donkeys

are not significant reservoirs of infection in Mahalla, but low sample size number, geographical, temporal, or climatic differences, and unique intrinsic host defense factors may have contributed to this disparate finding.

Although several species of rodents and carnivores have been reported to inhabit the Mahalla region, black rats and weasels were the only wild animals captured in this study. The detection rate of *Leptospira* antibodies in rats was lower (11%) (Tables 1 and 2) compared with an earlier study from Egypt (55.4%)¹⁰ and many other reports from different parts of the world.^{22–24} The observed disparity may be because of the use of considerably lower cutoff points (< 1:200) or the collection of brown rat (*R. norvegicus*) in previous studies.^{10,23} The brown rat is considered by some as more versatile than the black rat in the transmission and carriage of leptospirosis.²⁵ Also, the high urbanization and construction activities that are currently ongoing in Mahalla may have limited the dispersion of rats and reduced their role as maintenance hosts for *Leptospira*.

Vertical transmission of the circulating serovars in this region seems to be very limited, with the possible exception of serovar Pyrogenes, evidenced by the low carriage rates of leptospirosis in juvenile animals compared with adults (Table 3).^{26,27} Almost all of the surveyed black rats were infested with ectoparasites, which may have directly or indirectly allowed for the percutaneous entry of leptospires. Rats with traumatic skin injuries had slightly higher positivity rates than animals without injuries: 22% versus 15%, respectively. Of the rats with gross abnormalities of either the kidney or liver, 66.7% were found to be infected with leptospires by culture or PCR.

L. borpetersenii serovar Polonica was isolated from three rats. This serovar was identified by PFGE, but serum was not screened specifically for this serovar, which has not been previously cultured from Egyptian human or animal tissues. Interestingly, it was isolated only in blood of infected rats in this study, despite attempts to isolate it from urine and renal tissue. Its presence may signify an emerging or previously undetected human or animal pathogen in the region. Follow-up studies should consider incorporating it into MAT serovar panels and PCR assays for screening.

Dogs have never been evaluated as carriers for *Leptospira* in Egypt, and the observed seroprevalence in this study was 12%. This value is similar to that described in Italy¹⁹ and Thailand²⁸; however, it is much less than that reported in Germany,²⁹ Mexico,³⁰ and the United States.³¹ No particular serovar was predominant in our study, although other reports have claimed a more frequent distribution of serovars Grippotyphosa, Icterohaemorrhagiae, Australis, and Canicola.^{28–32} Such discrepancies may be because of the relatively limited number of animals used or differences in climatic or geographical conditions.

A few previous studies have screened water buffaloes for leptospirosis, including an earlier report from Egypt that showed a seroprevalence of 26%.¹⁰ This finding is similar to that obtained in the present study, but it is considerably higher than that reported from the world-renowned wildlife Kruger National Park in South Africa (1.7%).³³ So far, serovar Hardjo has been the most commonly recognized serovar in buffaloes.³⁴ In cattle, the seroprevalence was higher (44%; serovar Icterohaemorrhagiae being most common), although the number of animals screened in this study was small. These data certainly support the conclusions drawn by other studies

that determined that livestock breeding and farming are major occupational risks.^{1,10,17} Although culture and PCR results were negative in both animal species, leptospires have been claimed to survive in these animals for many years at antibody levels below the marginal values used in serological screening.^{1,34} However, one study suggested that there was no consistent relationship between the presence of antibodies and the carrier state in cattle owing to the rapid decline in titers and varying persistence of infection.¹

Among weasels, only one animal showed high antibody titers against *L. interrogans* serovar Icterohaemorrhagiae (1:400), in agreement with studies that claimed that these animals act as incidental hosts for certain leptospires.^{35,36} However, some reports indicated that weasels were seronegative for leptospirosis and suggested further investigations to explore their role in disease transmission.³⁷ In cats, an isolate showing PFGE profiles comparable with *L. biflexa* serovar Patoc was collected from one animal whose serum was reactive to many serovars, including *L. interrogans* serovars Grippotyphosa (1:3,200), Hardjo (1:400), and *L. biflexa* serovar Patoc (1:200) (Table 2).³⁸ Detection of this isolate certainly warrants further investigation, because saprophytic leptospires do not normally colonize the kidney. Titers observed against *L. biflexa* serovars are customarily attributed to shared antigenicity.¹

Although our positive results for G1/G2 PCR (10.6%; sensitivity ≤ 50 cells/mL) and Lig1/Lig2 PCR (7.26%; sensitivity ≤ 6 cells/mL)¹² from different animal species show a greater sensitivity than culture (4.5%), MAT seropositivity data (titer $\geq 1:200$) was generally higher (12.8%), suggesting more pronounced exposure than incidental or accidental rates. Antibodies have been shown to develop after the disappearance of *Leptospira* from the blood.^{1,24} However, the obtained PCR and culture results were relatively lower than those reported in many other studies,^{23,24} partly because of differences in disease prevalence or the number of animals examined and stage of infection. Primary serovars that reacted in MAT were also detected by culture. However, one rat showed serovar Pyrogenes by culture and serovar Hardjo by MAT, suggesting previous exposure.

Overall, findings suggest that wild and domestic mammals are important sources of pathogenic leptospires in the Mahalla region. The observed multiple reactivity against *Leptospira* serovars in MAT may reflect the magnitude of cross-reactivity among bacteria, particularly in the early stage of the disease,^{1,6} or show the history of previous exposures. The obtained seroprevalence data are an alert against a potentially important veterinary problem in Mahalla and could provide an effective measure for the extent of carriage and disease transmission to humans. This requires the development of weighted plans to reduce the carriage rates among animal species that constitute a major risk for disease spread in the Nile Delta region—cattle, buffaloes, dogs, and rats. A targeted vaccine program for cattle and water buffaloes using a multiserovar vaccine (serovars Icterohaemorrhagiae, Hardjo, and Pomona) would limit disease burden among livestock and decrease environmental contamination. Although serovars Grippotyphosa and Canicola, which are known to cause significant disease in cattle,³⁹ have not been detected in these animals in this study, they were identified in other species (black rats and dogs) and should be considered in potential vaccines for bovids. Although the vast majority of dogs in this region are feral, a similar targeted vaccine strategy could be applied to owned dogs.

A significant effort is needed to increase community awareness to use clean water sources for drinking, cooking, and cleaning as opposed to using untreated water directly from canals or streams. Farm laborers should consider the feasibility and practicality of wearing personal protective equipment when performing high-risk tasks. Rats and feral and nuisance dogs (and cats) should be humanely eliminated by the local animal control officials, particularly in areas where there is likely contact with other domestic mammalian species (e.g., farms). In addition, effective diagnostic methods should be established as needed, because the disease has been generally undiagnosed and underestimated.

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Authors' addresses: Stephen A. Felt, Comparative Medicine Department, Stanford University, Stanford, CA, E-mail: felt@stanford.edu. Momtaz O. Wasfy, Lab Unit, US Naval Medical Research Unit-3, Cairo, Egypt, E-mail: momtaz.wasfy.eg@med.navy.mil. Bassem Abdel Rahaman, Lab Unit, US Naval Medical Research Unit-3, Cairo, Egypt, E-mail: bassem.abdel-rahman.eg@med.navy.mil. Marie Boshra, Lab Unit, US Naval Medical Research Unit-3, Cairo, Egypt, E-mail: marie.boshra@gmail.com. Tina M. Parker, Tuberculosis, Leprosy and Other Mycobacterial Diseases Respiratory Diseases Branch DMID/NIAID/NIH/DHHS 6610 Rockledge Drive, Bethesda, MD, E-mail: parkerti@niaid.nih.gov. Guillermo Pimentel, Lab Unit, US Naval Medical Research Unit-3, Cairo, Egypt, E-mail: wael_el-tras@yahoo.com. Ahmed Ahmed El-Bassiouny, Department of Hygiene and Preventive Medicine, Faculty of Veterinary Medicine, Kafr El Sheik University, Egypt, E-mail: bassiounya@yahoo.com. Ahmed Samir, Department of Microbiology, Faculty of Veterinary Medicine College, Cairo University, Egypt, E-mail: ahmedsamir121@hotmail.com. Mahmoud Essam Hatem, Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt, E-mail: essamhatem@yahoo.com. Clinton K. Murray, Infectious Disease Service, Brooke Army Medical Center, Fort Sam Houston, TX, E-mail: clinton.murray@us.army.mil.

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