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Leptospirosis in animals and human contacts in Egypt: broad range surveillance

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ABSTRACT

Introduction: Leptospirosis is a re-emerging zoonotic disease of humans and animals worldwide. The disease is caused by pathogenic species of the genus *Leptospira*. These organisms are maintained in nature via chronic renal infection of carrier animals, which excrete the organisms in their urine. Humans become infected through direct or indirect exposure to infected animals and their urine or through contact with contaminated water and soil. This study was conducted to investigate *Leptospira* infections as a re-emerging zoonosis that has been neglected in Egypt. **Methods:** Samples from 1,250 animals (270 rats, 168 dogs, 625 cows, 26 buffaloes, 99 sheep, 14 horses, 26 donkeys and 22 camels), 175 human contacts and 45 water sources were collected from different governorates in Egypt. The samples were collected from different body sites and prepared for culture, PCR and the microscopic agglutination test (MAT). **Results:** The isolation rates of *Leptospira serovars* were 6.9%, 11.3% and 1.1% for rats, dogs and cows, respectively, whereas the PCR results revealed respective detection rates of 24%, 11.3% and 1.1% for rats, dogs and cows. Neither the other examined animal species nor humans yielded positive results via these two techniques. Only six *Leptospira* serovars (Icterohaemorrhagiae, Pomona, Canicola, Grippotyphosa, Celledoni and Pyrogenes) could be isolated from rats, dogs and cows. Moreover, the seroprevalence of leptospiral antibodies among the examined humans determined using MAT was 49.7%. **Conclusions:** The obtained results revealed that rats, dogs and cows were the most important animal reservoirs for leptospirosis in Egypt, and the high seroprevalence among human contacts highlights the public health implications of this neglected zoonosis.

Keywords: Leptospirosis. Zoonosis. Egypt.

INTRODUCTION

Leptospirosis is a reemerging zoonotic disease of humans and animals worldwide. It is presumed to be the most widespread zoonosis in the world. The disease is caused by pathogenic species of spirochetes of the genus *Leptospira*. The organisms are maintained in nature by chronic renal infection of carrier animals, which excrete the organisms in their urine. Humans become infected through direct exposure to infected animals or their urine or through indirect contact with contaminated water or soil[1]. Animal species that serve as a maintenance host for one serovar can become an accidental host of another serovar. Moreover, every mammalian species is potentially an accidental host for leptospirosis, and no species has been identified as refractory to infection. Currently, there are more than 250 known serovars of *Leptospira* spp., which show a variable distribution by geographical region and host species[2].

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In developing countries, agriculture continues to be the main source of employment and income for a large number of rural residents. Living and working in close contact with animals or their waste may present greater opportunities for exposure and infection[3].

Few data are available on the incidence and prevalence of leptospirosis in the Middle East[1]. In Egypt, a pilot study conducted approximately 25 years ago revealed that 9% of sera collected from persons living in contact with carrier animals were seropositive for different *Leptospira* serogroups[4], and an earlier study[5] reported that 13% of the collected rodent sera were reactive for leptospirosis. More recent studies have been initiated to detect leptospiral antibodies among undiagnosed acute febrile illness (AFI) and hepatitis patients in Egypt[6] [7]. Approximately 16% of sera from both disease groups showed seroreactivity to *Leptospira* immunoglobulin M (IgM) by enzyme-linked immunosorbent assay (ELISA) and the microscopic agglutination test (MAT). These studies have revealed that the epidemiologic status of leptospirosis in Egypt needs to be defined and that difficulties in disease diagnosis need to be eliminated. Most patients with AFI are currently empirically diagnosed as typhoid or brucellosis cases and treated accordingly with chloramphenicol or tetracycline derivatives[6]. More recently, cross-species surveillance of *Leptospira* in animals has been

carried out in the vicinity of Mahalla, a portion of a governorate of Egypt, to determine the most common serovars affecting this region[3]. The findings of this study suggested that wild and domestic mammals are important sources of pathogenic leptospires in Mahalla City. Additionally, Hatem and Samir[8], recorded a recent outbreak of leptospirosis in sheep as the first epidemic in northern Egypt.

Therefore, the current study involved broad range surveillance of the zoonotic bacterial agent *Leptospira* in animals and human contacts in Egypt. The study aids in determining the most likely sources of leptospires infecting humans and the distribution of the various serovars in the region. This information is important to determine and optimize appropriate intervention strategies and methods for the diagnosis/management of leptospirosis cases in animals, especially in pet canines and livestock.

METHODS

The study was performed at the Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt, under the recommendations of the Institutional Animal Care and Use Committee Guidebook. **Table 1** shows the different animal species and the different tissues examined in this study.

Study area

The samples were collected from 14 different governorates: Alexandria (representing the coastal area); Gharbeya, Kafr El-Sheikh, Sharkya, Dakahleyya, Menufya, Behera and Qualyobya (representing the Delta); Giza, Fayoum, Beni-Swaif, 'Menia, and Assuit (representing upper Egypt); and El-Wadi El-Gedeed (representing the border area). The animals were randomly selected. Rodents and stray dogs were captured using traps. In the case of livestock, all specimens were collected either from farms, during presentation of the animals at veterinary clinics, or from animals owned by individuals. The examined camels were found in the Southern border areas.

TABLE 1 - Number of different sources and sites from which samples were collected.

Source of samples	Number	Body sites
Rats	270	blood, kidneys, urine
Dogs	168	blood, kidneys, urine
Sheep	99	blood, urine
Donkeys	26	blood
Cows	625	blood, urine
Buffaloes	26	blood, urine
Horses	14	blood
Camels	22	urine, blood
Contact humans	175	blood
Water sources	45	--
Total	1,470	

Specimen collection

Blood samples from rats and dogs were obtained via heart puncture, after which the animals were euthanized and subjected to necropsy to collect urine and kidney samples. For the other animals, urine samples were collected via catheterization and blood was then drawn from the jugular vein.

Serum samples

For the animals and human contacts involved in the study, 3 to 5ml of blood was collected in anticoagulant-free tubes and spun down at 5,000rpm for 20 min. All sera were transferred in cryovials and stored at -20°C until use for further serological testing (i.e., MAT).

Water sources

Water samples were collected from small water streams in regions where the examined animals were living in different governorates.

Laboratory procedures

Animals were considered positive for leptospirosis infection if the organism was recovered through culture, and/or polymerase chain reaction (PCR)-specific assays for pathogenic *Leptospira sp.* were positive, whereas a titer ≥ 200 against a pathogenic serovar was considered positive by the MAT.

Leptospira cultures

One drop of each blood and urine sample was inoculated into 3-4 tubes containing Ellinghausen, McCullough, Johnson and Harris (EMJH) broth medium containing 200µg/ml 5-fluorouracil (5-FU) to minimize contamination[9] [10].

The collected organs (kidneys) were macerated with sterile blades (or by passage through a 3ml syringe without a needle) and inoculated in the same manner. All cultures were incubated at 30°C for 6-8 weeks and were examined weekly using a dark-field microscope. Positive cultures were purified and subcultured in EMJH. Fletcher's semisolid medium was used for maintenance.

PCR diagnostics

Deoxyribonucleic acid (DNA) was extracted from the inoculated *Leptospira* culture media using the QIAmp DNA Mini Kit (Qiagen, Valencia, CA) in accordance with the manufacturer's instructions. PCR was carried out via amplification with specific primers for pathogenic leptospires (lig1/lig2) according to the protocol previously described by Palaniappan et al[11]. The PCR products were run on agarose gels containing ethidium bromide and visualized with a transilluminator at 468bp **(Figure 1)**.

Microscopic agglutination test

A standard MAT was performed on sera to determine the most reactive *Leptospira* serogroups. The test was carried out according to the methods described by WHO[12]. Briefly, live

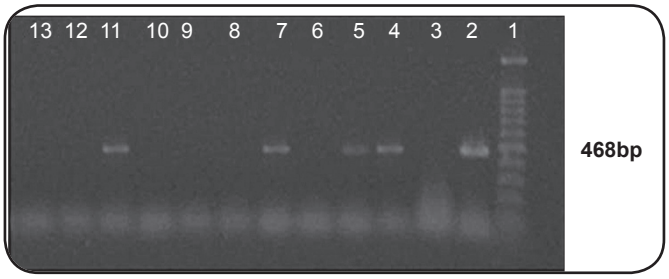

FIGURE 1 - PCR amplification of genomic DNA from samples. Lane 1: 100bp ladder; Lanes 2 and 3: positive (+) and negative (-) controls, respectively; Lanes 4, 5, 7, 11: positive samples; Lanes 3, 6, 8, 9, 10, 12, 13: negative samples. PCR products appeared at 468bp for the Lig1/Lig2 gene (a pathogenic gene). PCR: polymerase chain reaction; DNA: deoxyribonucleic acid.

Leptospira cell suspensions representing 24 serovars (kindly obtained from US Naval Medical Research Unit - 3) were added to serially diluted serum specimens in 96-well microtiter plates and incubated at ambient temperature for 1.5 hours. Agglutination was then examined via dark-field microscopy at a magnification of 100X. A reactive MAT was determined by a titer ≥ 200. The reported titers were calculated as the reciprocal of the highest serum dilutions that agglutinated at least 50% of the cells for each tested serovar. Additionally, MAT is a tool through which the recovered isolates can be speciated using 20 different standard antisera.

RESULTS

Various prevalence rates of leptospirosis were determined using the applied culture techniques, PCR and MAT after examination of the collected samples and are shown in **Table 2**. In addition, the results of speciation analysis revealed that only six serovars (Icterohaemorrhagiae, Pomona, Canicola, Grippotyphosa, Celledoni and Pyrogenes) were the most predominant (**Table 3**). Moreover, of the 175 examined humans, 87 (49.7%) were serologically positive for *Leptospira serovars* antibodies, and all of the positive human sera showed reactivity with the isolated animal serovars (**Table 4**). However, the serological examination of animal sera using the MAT for the detection of specific antibodies against different serovars revealed that *Leptospira* Icterohaemorrhagiae and *Leptospira* Pomona were the predominant serovars in rats, cows, buffaloes and sheep, whereas *Leptospira* Icterohaemorrhagiae and *Leptospira* Canicola were more common among dogs. The serovars isolated from the two positive donkeys were identified as *Leptospira* Grippotyphosa and *Leptospira* Celledoni.

DISCUSSION

Leptospirosis is increasingly being recognized as an important cause of hemorrhagic fever in humans[13]. The classic presentation of severe leptospirosis (Weil's disease) is characterized by jaundice and acute renal failure. Leptospirosis is a worldwide, acute febrile zoonosis. Many animals are susceptible to the disease. The leptospires infecting humans are primarily maintained by warm-blooded vertebrates. In cities, Norway rats (*Rattus norvegicus*) and dogs have been regarded as major reservoirs. Humans can become infected when they come in contact with such animals or their contaminated excreta[14]. In Egypt, recent surveillance of AFI (acute febrile illness) patients revealed that among the areas tested, Mahalla city showed a higher frequency of leptospirosis compared with other regions of the country[7] [15]. Therefore, approximately four years later, another study was published providing data on the distribution of leptospirosis in animals in Mahalla[3]. Prior to this study, the most recent animal surveys in Egypt were conducted in Cairo over 20 years ago[4].

TABLE 2 - Total number of *Leptospira*-positive cultures compared with PCR- and MAT-positive samples from different sources.

Animal species	Number	Positive cultures		Positive by PCR		Positive sera by MAT	
		n	%	n	%	n	%
Rats	270	17	6.9	65	24.0	205	75.9
Dogs	168	19	11.3	19	11.3	98	58.3
Sheep	99	0	0.0	0	0.0	45	45.5
Donkeys	26	0	0.0	0	0.0	2	7.7
Cows	625	7	1.1	7	1.1	235	37.6
Buffaloes	26	0	0.0	0	0.0	4	15.4
Horses	14	0	0.0	0	0.0	0	0.0
Camels	22	0	0.0	0	0.0	0	0.0
Contact humans	175	0	0.0	0	0.0	87	49.7
Water sources	45	10	22.2	10	22.2	NA	

PCR: polymerase chain reaction; **MAT:** microscopic agglutination test; **NA:** not applicable.

TABLE 3 - Results of serotyping of the isolates recovered from different animal species.

Animal species	Positive isolates (n)	Serovar	Number
Rats	17	*L.* Icterohaemorrhagiae	5
		L. Pomona	4
		L. Grippotyphosa	3
		L. Celledoni	1
		L. Canicola	4
Dogs	19	*L.* Canicola	10
		L. Icterohaemorrhagiae	9
Cows	7	*L.* Icterohaemorrhagiae	2
		L. Pomona	5
Water sources	10	*L.* Icterohaemorrhagiae	2
		L. Grippotyphosa	1
		L. Celledoni	1
		L. Pyrogenes	1
		L. Pomona	5

n: number; ***L:*** *Leptospira.*

TABLE 4 - Number of cases positive by MAT correlated with the titers and serovars of the collected human contact sera.

Serovar/titer	Icterohaemorrhagiae	Pomona	Grippotyphosa	Pyrogenes	Canicola	Total number
200	0	0	2	3	0	87
400	0	0	0	0	0	
800	3	3	0	0	4	
1,600	4	0	0	0	6	
3,200	6	10	0	0	0	
6,400	0	15	0	0	7	
12,800	4	15	0	0	5	
Total	17	43	2	3	22	

MAT: microscopic agglutination test.

In the current investigation, isolation was successful only from rats, dogs and cattle **(Table 2)**. The isolates recovered from rats were identified as Icterohaemorrhagiae (n=5), Pomona (n=4), Canicola (n=4), Grippotyphosa (n=3) and Celledoni (n=1), whereas those from dogs were Canicola (n=10) and Icterohaemorrhagiae (n=9), and the cow isolates were Pomona (n=5) and Icterohaemorrhagiae (n=2).

Notably, 9 rat isolates (Icterohaemorrhagiae and Pomona) were recovered from rats captured inside dairy farms. These serovars were also recovered from water sources supplying such farms (5 Pomona isolates and 2 Icterohaemorrhagiae isolates). In addition, the serological survey (MAT) demonstrated that the Icterohaemorrhagiae and Pomona serovars were the most prevalent among rats, followed by Celledoni, Canicola and Grippotyphosa. Icterohaemorrhagiae and Pomona were also the most prevalent serovars among cattle, which is in accord with the results obtained by Hatem et al.[16], while Icterohaemorrhagiae and Canicola were the most prevalent among dogs. However, the seroprevalence of *Leptospira* serovars observed in humans who had contact with captured animals showed that Pomona, Canicola and Icterohaemorrhagiae were the most common reactive serovars, followed by Pyrogenes and Grippotyphosa.

Regarding rats, a recent study[3] found that the detection rate of *Leptospira* antibodies was lower (23%) compared with an earlier study from Egypt (55.4%)[4] and many other reports from different parts of the world[17] [18] [19]. However, the same study[3] reported that dogs had never been evaluated as carriers of *Leptospira* in Egypt, and the observed seroprevalence in that study was 12%, which was similar to the seroprevalence reported in Italy[20] and Thailand[21] but much lower than that reported in Germany[22], Mexico[23] and the USA[24]. The same study found a high seroprevalence of leptospires in cows (44%). The authors added that this information supported the conclusions drawn by other researchers, who determined that livestock breeding confers

a major occupational risk[1] [25] [26]. Comparatively, in the present study, the seroprevalence of leptospirosis detected in rats, dogs and cows was 75.9%, 58.3% and 37.6%, respectively.

Our results showed that no isolates could be recovered from sheep, although the animals were serologically reactive (approximately 45%) for Icterohaemorrhagiae and Pomona, showing relatively high titers (800 and 6,400, respectively). This finding contrasts results previously obtained in Egypt[3], where sheep samples were found to be negative for leptospirosis by all of the applied diagnostic tools. However, earlier studies conducted in Egypt revealed that 4.2% of sheep were seropositive[25]. This discrepancy may be due to differences in the screened localities, the number of animals used, or the applied laboratory testing methods. Worldwide, the reported prevalence of leptospirosis in sheep has been found to be comparatively low in Canada[27], Portugal[26], Chile[28], Italy[29], and Brazil[30]. Only 7.7% of the donkey sera collected in this study were reactive for leptospirosis, particularly against Grippotyphosa and Celledoni. However, two earlier studies from Egypt showed that 17-29% of the tested donkeys were seropositive for *Leptospira*[4] [25]. Another recent study conducted in the Middle East determined that Iranian donkeys and horses showed a high rate of seroconversion (40% and 28%, respectively), particularly against *L. interrogans* serovars Icterohaemorrhagiae, Ballum and Pomona[31]. In contrast, the horse and camel specimens collected in the current study were negative for the presence of *Leptospira* cells, DNA and even antibodies indicating infection.

A few previous studies have screened buffaloes for leptospirosis, including an earlier report from Egypt that demonstrated a seroprevalence of 26%[25]. This result is similar to that obtained in a previous study in Mahalla[3], but it is considerably higher than that reported from South Africa (1.7%)[32]. The present study found that approximately 15% of the collected buffalo sera were reactive to *Leptospira* antibodies.

The current investigation provides an indication of the current situation regarding the epidemiology of this zoonosis in Egypt. It is clear that leptospirosis has become a problem in large cities in Egypt and is not limited to small areas. Similar studies have confirmed this prevalence based on surveillance conducted in other regions of the world, such as Brazil[13]. Our results are helpful for determining which isolates can be found in particular animals in Egypt. Furthermore, our serosurvey of humans who were in contact with such animals revealed a high prevalence rate of 49.7%, and *Leptospira* Pomona, Canicola and Icterohaemorrhagiae were the most prevalent serovars identified in human infections. Interestingly, we were also able to recover *Leptospira* serovars from water streams; such contaminated water constitutes a potential hazard for humans.

This information is helpful for determining strategies for leptospirosis prevention and control in humans and animals by eradicating the disease in animals (source of infection) via the application of appropriate vaccination programs. A targeted vaccination program for dairy cows and dogs using a multi-serovar vaccine manufactured from the recovered isolates (bivalent or trivalent) would limit the disease burden among livestock and pets, thereby decreasing environmental contamination and accordingly reducing human exposure to this pathogen.

A significant effort is needed to increase community awareness that clean water sources should be employed for drinking, cooking and cleaning, rather than 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. Effective diagnostic methods should be established as needed, as the disease has generally been left undiagnosed and underestimated.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

1. Levett PN. Leptospirosis. Clin Microbiol Rev 2001; 14:296-326.
2. Plank R, Dean D. Overview of the epidemiology, microbiology, and pathogenesis of *Leptospira* spp. in humans. Microbes Infect 2000; 2:1265-1276.
3. Felt SA, Wasfy MO, El-Tras WF, Samir A, Rahaman AB, Boshra M, et al. Cross-species surveillance of *Leptospira* in domestic and peri-domestic animals in Mahalla City, Gharbeya Governorate, Egypt. Am J Trop Med Hyg 2011; 84:420-425.
4. Sebek Z, Sixl W, Valova M, Schaffler R. Leptospirosis in man, in wild and in domestic animals at waste disposal sites in Cairo. Geogr Med 1989; 3:141-150.
5. Tawfik S, Hamed O, El-Karamani R. Leptospirosis in Egyptian rodents. Zoonoses and Public Health 1977; 24:728-732.
6. Ismail TF, Wasfy MO, Abdul-Rahman B, Murray CK, Hospenthal DR, Abdel-Fadeel M, et al. Retrospective seroservey of leptospirosis among patients with acute febrile illness and hepatitis in Egypt. Am J Trop Med Hyg 2006; 75:1085-1089.
7. Parker TM, Murray CK, Richards AL, Samir A, Ismail T, Fadeel MA, et al. Concurrent infections in acute febrile illness patients in Egypt. Am J Trop Med Hyg 2007; 77:390-392.
8. Hatem ME, Samir A. The first recorded epidemic of leptospirosis in sheep in Egypt. Rev Sci Tech Off Int Epiz 2014; 33:889-892.
9. Ellinghausen HC, McCullough WG. Nutrition of *Leptospira pomona* and growth of 13 other serotypes. A serum free medium employing oleic albumin complex. Am J Vet Res 1965; 26:39-51.
10. Johnson RC, Harris VG. Differentiation of pathogenic and saprophytic leptospires. I. Growth at low temperature. J Bact 1967; 94:27-31.
11. Palaniappan RUM, Chang YF, Chang CF, Pan MJ, Yang CW, Harpending P, et al. Evaluation of lig-based conventional and real time PCR for the detection of pathogenic leptospires. Mol Cell Probes 2005; 19:111-117.

12. World Health Organization. (WHO). Human leptospirosis: guidance for diagnosis, surveillance and control. Geneva: WHO; 2003. (Cited 2015 April). Available at: http://www.who.int/csr/don/en/WHO_CDS_CSR_EPH_2002.23.pdf
13. Gouveia EL, Metcalfe J, Carvalho AF, Aires TS, Villasboas-Bisneto JC, Queiroz A, et al. Leptospirosis-associated severe pulmonary hemorrhagic syndrome, Salvador, Brazil. Emerg Infect Dis 2008; 14:505-508.
14. Childs JE, Schwartz SB, Ksiazek TG, Graham RR, LeDuc JW, Glass GE, et al. Risk Factors Associated with Antibodies to Leptospires in Inner-city Residents of Baltimore: A Protective Role for Cats. Am J Public Health 1992; 82:597-599.
15. Kurtoglu MG, Tuncer O, Bozkurt H, Caksen H, Berktas M, Ceylan E, et al. Report of three children with leptospirosis in rural area of the east of Turkey. Tohoku J Exp Med 2003; 201:55-60.
16. Hatem ME, Ata NS, Abdou AM, Ibrahim ES, Bakry MA, Samir A, et al. Surveillance of bovine leptospirosis: isolation and serodiagnosis. Global Veterinaria 2014; 13:127-132.
17. Michel V, Branger C, Andre-Fontaine G. Epidemiology of leptospirosis. Rev Cubana Med Trop 2002; 54:7-10.
18. Doungchawee G, Phulsuksombat D, Naigowit P, Khoaprasert Y, Sangjun N, Kongtim S, et al. Survey of leptospirosis of small mammals in Thailand. Southeast Asian J Trop Med Public Health 2005; 36:1516-1522.
19. Priya CG, Hoogendijk KT, Berg M, Rathinam SR, Ahmed A, Muthukkaruppan VR, et al. Field rats form a major infection source of leptospirosis in and around Madurai, India. J Postgrad Med 2007; 53:236-240.
20. Ciceroni L, Bartoloni A, Pinto A, Guglielmetti P, Valdez VC, Gamboa Barahona H, et al. Serological survey of leptospiral infections in sheep, goats and dogs in Cordillera province, Bolivta. New Microbiol 1997; 20:77-81.
21. Meeyam T, Tablerk P, Petchanok B, Pichpol D, Padungtod P. Seroprevalence and risk factors associated with leptospirosis in dogs. Southeast Asian J Trop Med Public Health 2006; 37:148-153.
22. Geisen V, Stengel C, Brem S, Muller W, Greene C, Hartmann K, et al. Canine leptospirosis infections - clinical signs and outcome with different suspected *Leptospira* serogroups (42 cases). J Small Anim Pract 2007; 48:324-328.
23. Jimenez-Coello M, Vado-Solis I, Cardenas-Marrufo MF, Rodriguez-Buenfil JC, Ortega-Pacheco A: Serological survey of canine leptospirosis in the tropics of Yucatan Mexico using two different tests. Acta Trop 2008; 106:22-26.
24. Stokes JE, Kaneene JB, Schall WD, Kruger JM, Miller R, Kaiser L, et al. Prevalence of serum antibodies against six *Leptospira* serovars in healthy dogs. J Am Vet Med Assoc 2007; 230: 1657-1664.
25. Maronpot RR, Barsoum IS. Leptospiral microscopic agglutinating antibodies in sera of man and domestic animals in Egypt. Am J Trop Med Hyg 1972; 21:467-472.
26. Rocha T. A review of leptospirosis in farm animals in Portugal. Rev Sci Tech 1998; 17:699-712.
27. Kingscote B. Leptospirosis in Sheep in Western Canada. Can Vet J 1985; 26:164-168.
28. Zamora J, Riedemann S, Tadich N. A serological survey of leptospirosis in sheep in Chile. Rev Latinoam Microbiol 1999; 41:73-76.
29. Ciceroni L, Lombardo D, Pinto A, Ciarrocchi S, Simeoni J. Prevalence of antibodies to *Leptospira* serovars in sheep and goats in Alto Adige-South Tyrol. J Vet Med B Infect Dis Vet Public Health 2000; 47:217-223.
30. Silva EF, Brod CS, Cerqueira GM, Bourscheidt D, Seyffert N, Queiroz A, et al. Isolation of *Leptospira noguchii* from sheep. Vet Microbiol 2007; 121:144-149.
31. Hajikolaei MRH, Haidari MM, Abdollapour G. Comparison of leptospiral infection in the horse and donkey. Bull Vet Inst Pulawy 2005; 49:175-178.
32. Myburgh JG, Bengis RG, Bester CJ, Chaparro F. Serological reactions to *Leptospira* species in buffalo (*Syncerus caffer*) from the Kruger National Park. Onderstepoort J Vet Res 1990; 57: 281-282.