

Original Article

Leptospiral infection: a serosurvey in urban and rural communities in Olavarría county, Argentina

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Abstract

Introduction: Leptospirosis is a neglected zoonotic disease, affecting mainly poor and vulnerable populations.

Methodology: A cross sectional-study was carried out in 557 subjects from Olavarría county (Argentina) to estimate the seroprevalence of leptospirosis and the factors associated with seropositivity. A survey was carried out to obtain clinical and epidemiological data. Serum was tested for anti-leptospiral antibodies by Microscopic Agglutination Test (MAT). Chi-square or Fisher Exact tests were used to assess association between the MAT results and the exploratory variables. For the variables statistically associated, odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated. Individuals from Olavarría city were georeferenced to describe the spatial distribution and to detect clusters of seropositivity for leptospiral antibodies.

Results: The overall prevalence of leptospirosis infection was 7.00%, higher in rural (19.66%) than in urban populations (3.64%) ($p < 0.001$). Sejroe was the most predominant serogroup in rural communities while Icterohaemorrhagiae was the most prevalent in urban populations. The factors associated with *Leptospira* infection were the presence of rodents inside households (OR = 3.9) in rural populations, while contact with cats (OR = 4.97) and male gender (OR = 7.75) represented higher risk of infection for the urban ones. Cases with positive serology have been found near Tapalqué stream or in the peripheral areas of Olavarría city.

Conclusions: The results from data obtained during the study period were similar to other reports and demonstrate the importance of continuous epidemiological surveillance system and specific community educational campaigns to prevent the leptospirosis infection in Olavarría county and other communities with similar characteristics especially in rural areas.

Key words: Leptospirosis; seroprevalence; risk factors.

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Introduction

Leptospirosis is a neglected zoonotic infectious disease presenting mostly in vulnerable populations. It is caused by spirochetes belonging to the genus *Leptospira*, which is currently divided into 64 species classified in 2 clades: “Saprophytes” and “Pathogens”, subdivided in two subclades each [1-4].

Humans most often acquire leptospirosis through direct contact with urine or tissues from infected animals, or from indirect contact with contaminated soil or water. Cuts and abrasions in skin or intact mucous membranes such as the conjunctival, oral, or genital surfaces are portals of bacterial entry. A broad range of

wild and domestic animals act as reservoirs, carriers or intermediate hosts of *Leptospira*. Particular associations have been established between certain serovars and infected species [5-8]. The epidemiological impact of each species as a reservoir varies according to the region, depending on the population density, rapid urbanization and occupational and recreational activities that local residents develop [6].

Pathogenic leptospires rapidly invade the bloodstream after penetrating skin or mucous membranes. The disease ranges from mild to lethal in the clinical spectrum and probably has a high

proportion (15%-40%) of subclinical and asymptomatic infections [9]. About 10% of the patients present with a severe form characterized by sudden onset of malaise, often with intense muscular pains, and high fever for several days, followed by jaundice, renal failure and hemorrhages. The case fatality rate ranges from 2.3% to 13%, being pulmonary hemorrhage the most fatal complication of leptospirosis [5-7,9-12].

Individuals with occupations at risk for direct or indirect contact with potential infected animals include veterinarians, abattoir workers, farmworkers, hunters, animal shelter workers, scientists, and technologists handling animals in laboratories or during fieldwork. Immersion in water or contact with soil/mud contaminated with animal body fluids (mainly rodent urine), during recreational activities are considered other risk factors [12].

Leptospirosis constitutes a serious public health issue. This disease may occur in urban environments of industrialized and developing countries, as well as in rural regions worldwide. Compared to urban ones, rural populations are more exposed to this disease due to various environmental factors and agriculture practices [12]. Natural disasters associated with flooding have also been related to an increase in the occurrence of leptospirosis in humans, as well as poor sanitation, high rat infestations, and the presence of stray dogs [1, 6,13-14]. Over the past few years, there has been an increase in reported cases in urban environments, particularly in peripheral neighbourhoods characterized by inadequate sanitation, poor housing and exposure to rodents [5-8]. This re-emergence is thought to be driven by anthropogenic actions, socio-economic factors and

climate changes that have occurred in the last decades. Prevention is largely dependent on sanitation measures that may be difficult to implement, especially in developing countries. [5-7,11,15-18].

Control of leptospirosis can be enhanced using space cluster analysis. This methodology allows analysis of the spatial distribution of zoonoses at different scales, establishing their relationship with sociodemographic and environmental conditions and identifying areas with different epidemiological risk [19].

In Argentina, notification of leptospirosis is mandatory. Santa Fe, Buenos Aires and Entre Ríos are the provinces where most cases occur. Due to sub-registration and sub-diagnosis (related to delays in diagnosis due to lack of infrastructure and inadequate clinical suspicion), the disease is often ignored and the true incidence and prevalence are unknown [7,13-14,20].

The following study was conducted in Olavarría county for several reasons: i) the hydrography of the region; ii) the history of floods due to the overflow of the Tapalqué stream (which goes through the entire county); iii) the main economic activities carried out, which represent important risks for the occurrence of leptospirosis; and iv) the lack of studies on the epidemiology of this zoonosis in the region.

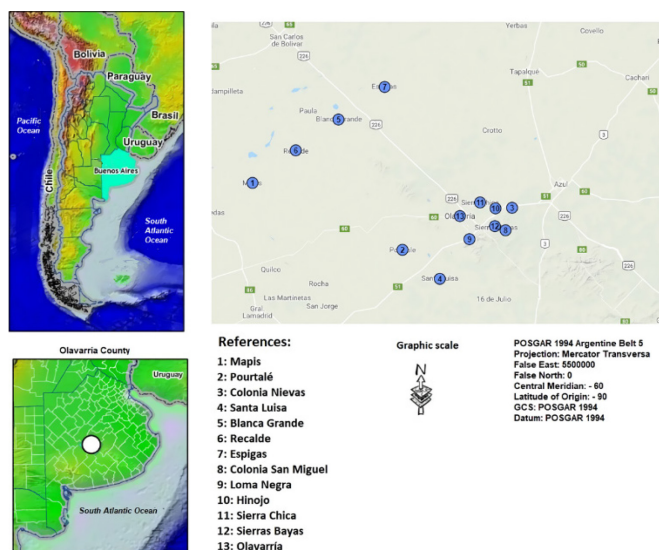
The aims of the study were a) to determine the seroprevalence of *Leptospira* sp. infection in rural and urban populations from Olavarría county; b) to identify the presumptive infecting *Leptospira* serogroups according to rural and urban area; c) to establish the factors associated with the seropositivity according to the area of residence; and d) to analyze the spatial distribution of the presence of anti-*Leptospira* antibodies of seropositives human cases in Olavarría city.

Methodology

Study area and population

Olavarría is located in the central region of Argentina, in Buenos Aires province (Figure 1). This county has a total population of 111,708 inhabitants (89,721 belonging to Olavarría city) [21]. The main hills that are part of the landscape are in the range of 250 - 300 meters high. It is defined by a flat plain, with difficult or impeded drainage. The subzone presents a sub-humid to humid water regime, with an average annual precipitation that ranges between 800-900 mm. The temperature regime is moderate, being January the warmest month and July the coldest. The average annual temperature is 13.8 °C.

Figure 1. Geographical distribution of localities in Olavarría county, Buenos Aires province, Argentina.



The hydrography of the region includes the Laguna Blanca Chica, the Laguna Blanca Grande and the Tapaqué stream, among other water courses.

The main economic activities of Olavarría are: industry (50% of the national production of cement), commerce, agriculture, mining and livestock. Olavarría is one of the counties in Buenos Aires province with the largest stock of cattle (658,230 head of cattle as of March 31, 2019) [22]. The main healthcare facility in Olavarría city is the Hector M. Cura Municipal Hospital. Both in Olavarría city and in the other localities of the county, there are an additional 27 Primary Health Care Centers (PHCC) strategically distributed to provide health coverage to all people. In Argentina, public health centers provide free care to anyone who needs it, particularly those in the lower income quintiles who lack social security coverage or who cannot pay for medical services (36% of the population) [23].

Design of the study

A cross sectional-study was performed from May 2013 to March 2014, including residents from Olavarría who spontaneously attended public health facilities (Hector M. Cura Municipal Hospital and PHCC). Immunosuppressed patients and those previously treated with antibiotics were excluded. Since the real seroprevalence was unknown, a seroprevalence of 50% was assumed to calculate the minimum sample size required, and an absolute error of 5% and a confidence level of 95% were used. The population size from which the sample was selected was 111,708 and the minimum sample size calculated was 383.

After obtaining written informed consent, serum samples were collected. Five mL of blood was drawn from each subject by antecubital venipuncture into tubes without anticoagulant. Sera were separated and stored at -20 °C and then transported to the Leptospirosis Laboratory of the Department of Rural Zoonoses, Ministry of Health in Azul, Buenos Aires province. To obtain information on clinical and epidemiological data, a trained interviewer conducted a survey.

Detection of antibodies by Microscopic Agglutination Test (MAT)

To determine the presence of anti-*Leptospira* antibodies, MAT was performed following standard procedures on all serum samples, using a panel of live antigen suspensions of locally circulating reference strains of *L. interrogans* serovars Canicola, Copenhageni, Pomona, Pyrogenes and Hardjo; *L.*

borgpeterseni serovars Tarassovi, Wolffii and Castellonis and *L. kirschneri* serovar Grippotyphosa, developed at 28-30 °C in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium and with no more than 15 days of growth [3]. Serial serum dilutions were performed with Phosphate-Buffered Saline (PBS, pH 7.2) starting from a 1:50 dilution. The plates were incubated at 37 °C for 90 minutes. After incubation, the serum-antigen mixtures were checked for agglutination by dark field microscopy. Tests were interpreted as positive when agglutination at dilutions \geq 1:50 of at least 50% of the leptospires for any serovar was observed. The highest serum dilution that had greater than 50% agglutination or less than or equal to 50% free leptospires (as compared to negative control) was considered the endpoint titer of the quantitative MAT. In positive sera, the presumptive infecting serovar was the one with the highest agglutination titer. The cross-reaction of different *Leptospira* were taken as the cases in which a serum reacted with two or more serovars at the same titer [6,24].

Data entry and statistical analysis

Clinical and epidemiological information and laboratory results were entered into Epi-Info database version 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Descriptive statistics were expressed in terms of proportions. The exposure variables were analyzed by contingency tables. The Chi-square, or the Fisher exact test when appropriate, was used to evaluate associations ($\alpha = 5\%$). The outcome variable was positivity to MAT and the independent variables were the clinical and epidemiological data obtained in the survey. Odds ratios (OR) and 95% confidence interval (CI) were calculated separately for each variable that was significant in the Chi-square or the Fisher exact test. All the analyses were performed using the Epi-Info database version 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA).

Given the characteristics of the areas studied, georeferencing the participants' addresses was only possible in the city of Olavarría, where most samples were collected and where the highest number of inhabitants of the entire county live. The longitude and latitude of the addresses of citizens from Olavarría city were determined using Google Maps (2016 version). Outcome data (MAT results) were plotted on maps to describe the spatial distribution of seropositive and seronegative cases using QGIS 2.18.7 software. Potential spatial clustering was investigated with space scan statistics using SaTScan software, v.9.3. The

Bernoulli model was applied [25]. Each subject positive to MAT was classified as a case, and each MAT negative subject as a control. The level of significance for all analyses was set as < 0.05 .

Ethical approval

Ethics approval was obtained from the Ethics Committee of the National Institute of Epidemiology “Dr. Juan H. Jara,” Mar del Plata, Argentina. Prior to enrolment, the researchers read an information sheet describing the study to the subjects, answered any questions and asked for written consent to participate. The study participants received no compensation for their participation and were free to withdraw from the study at any time. Anonymity was ensured through the use of subject-generated identification codes.

Results

Sociodemographic characteristics

A total of 557 patients (230 males) with a mean age of 41 years were included in the study. Most of them (79%) resided in urban localities with more than 2,000 inhabitants (Olavarría, Sierras Bayas, Sierra Chica, Loma Negra, Hinojo), while 21% came from rural areas (with less than 2,000 inhabitants) Blanca Grande, Colonia Nuevas, Colonia San Miguel, Espigas, Mapis,

Table 1. Communities included in the study, numbers of citizens and classification. Olavarría county, 2013-2014.

Community	Total of inhabitants and population type
Mapis	Dispersed rural
Pourtalé	Dispersed rural
Colonia Nuevas	10 (Aggregated rural)
Santa Luisa	40 (Aggregated rural)
Blanca Grande	65 (Aggregated rural)
Recalde	385 (Aggregated rural)
Espigas	492 (Aggregated rural)
Colonia San Miguel	902 (Aggregated rural)
Loma Negra	3,451 (Urban)
Hinojo	3,841 (Urban)
Sierra Chica	4,812 (Urban)
Sierras Bayas	6,856 (Urban)
Olavarría	89,721 (Urban)
Total	110,575

Pourtalé, Recalde and Santa Luisa). The communities included in the study, with the total number of inhabitants and the type of population (dispersed rural population/aggregated rural population/ urban population) are shown in Table 1.

Exposure factors and clinical characteristics

The main exposure factors differentiated according to the type of population of individuals included in the study are presented in Table 2. The presence of rodents

Table 2. Exposure factors according to type of population. Olavarría county, 2013-2014.

Exposure	Urban population FREQUENCY	Rural population FREQUENCY	<i>p</i> value; PR (CI 95%)
	n/N (%)	n/N (%)	
Electricity supply network	407/413 (98.55)	104/114 (91.23)	< 0.001 ; 1.08 (1.01-1.14)
Public water supply system	341/393 (86.77)	66/72 (91.67)	0.240
Cement walls	371/438 (84.70)	100/114 (87.72)	0.420
Tile or cement floor	313/437 (71.62)	97/117 (82.90)	0.0135; 0.86 (0.78-0.95)
Public gas network	310/413 (75.06)	76/114 (66.67)	0.073
Tin roof	248/437 (56.75)	88/115 (76.52)	< 0.001 ; 0.74 (0.65-0.84)
Septic tank	113/256 (44.14)	47/59 (79.66)	< 0.001 ; 0.55 (0.45-0.66)
Peridomestic rodents	255/437 (58.35)	73/113 (64.60)	0.227
Living close to flooded areas	143/370 (38.65)	49/107 (45.79)	0.184
Presence of rodents inside households	174/437 (39.82)	45/115 (39.13)	0.893
Presence of rodents in the workplace	86/321 (26.79)	30/104 (28.85)	0.683
Wastelands near the residences	259/436 (59.40)	53/110 (48.18)	0.034; 1.23 (1.00-1.52)
Streams and other water courses near the residences	176/435 (40.46)	51/113 (45.13)	0.369
Animal corrals near residences	139/437 (31.80)	36/110 (32.72)	0.853
Landfill near the residences	125/436 (28.70)	13/110 (11.81)	< 0.001 ; 2.42 (1.42-4.12)
Livestock productions near the residences	54/426 (12.70)	23/108 (21.29)	0.022; 0.59 (0.38-0.92)
Contact with canines	338/434 (77.88)	93/113 (82.30)	0.306
Contact with felines	36/346 (10.40)	35/99 (35.35)	< 0.001 ; 0.29 (0.19-0.44)
Contact with horses	53/431 (12.30)	23/113 (20.35)	0.028; 0.6 (0.38-0.94)
Contact with cattle	42/431 (9.75)	22/112 (19.64)	0.004; 0.49 (0.3-0.79)
Contact with sheep	30/431 (6.96)	16/111 (14.41)	0.012; 0.48 (0.27-0.85)
Contact with swine	34/431 (7.9)	13/113 (11.50)	0.223
Contact with fowls	18/346 (5.20)	3/99 (3.03)	0.590*

*: Fisher exact test.

(burrows or rodent excrements) and risk of flood were reported by the respondents. The presence of wastelands or landfills near the households prevailed in urban areas, while livestock farms near the residences predominated in rural areas. Exposure risk factors related with housing characteristics, services and infrastructure were more common in rural areas. Regarding contact with domestic animals, contact with canines was frequent both in urban and rural areas but contact with horses, cattle, sheep, and felines predominated in the rural areas. In terms of exposure to water sources, recreational activities such as swimming (13.1%) and rowing/canoeing (2%) in fresh water, mainly in rivers or streams, were the most frequent. Occupational risk-exposures were: rural working (21.3% in rural areas and 12.7% in urban areas), gardening (8.6%), construction work (4.5%), plumbing (3.4%) and garbage collection (1.1%).

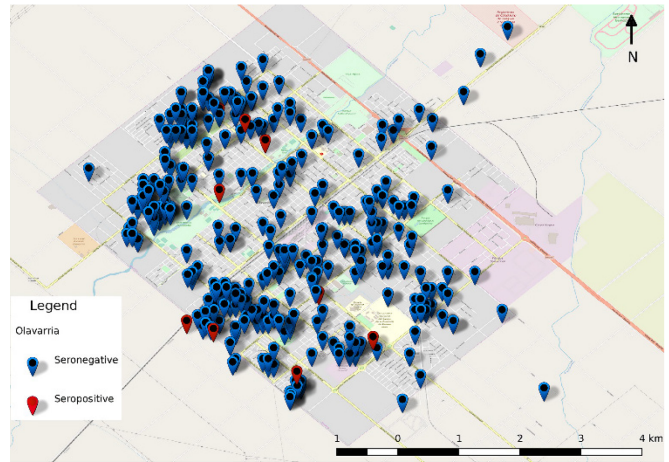
The main signs and symptoms referred by individuals were: headache (37.80%), asthenia (31.5%), myalgia (27.89%), cough (25.87%) and cold (24.77%).

It is important to note that only 17.4% (93/533) of the subjects had received prior information about the disease (via the internet, in an educational institution, from social media, at work, from health professionals, or through friends / family).

Seroprevalence, serovar distribution and titers

The overall prevalence of anti-*Leptospira* spp. antibodies was 7.00% (95% CI = 4.79- 9.21); it was higher in rural area (prevalence = 19.66%; 95% CI = 12.03-27.28) than in urban areas (prevalence = 3.64%; 95% CI = 1.77-5.50). The risk of having anti-*Leptospira* antibodies was 6.48 times higher in rural than urban residents (95% CI = 3.30-12.75, *p* < 0.001). Colonia San Miguel was the community with the highest seropositivity rate (56.76%). The frequency of *Leptospira* spp. serovars varied according to the area of residence (rural/urban) (Table 3). The cross reaction to

Figure 2. Spatial distribution of seropositive and seronegative individuals in Olavarría city, 2013-2014.



≥ 1 serovar was measured in 41% of serum samples. Titers ranged from 1:50 to 1: 200.

Risk factors

After analyzing the association between positive serology and the exposure variables under study, the risk factor most related to leptospirosis infection in rural population was the presence of rodents inside households (odds ratio, OR = 3.9; 95% CI = 1.21-12.5) while the main risk factors for urban population were the contact with felines (OR = 4.97; 95% CI = 1.58-15.62) and male gender (OR = 7.75; 95% CI = 1.68-35.71). None of the signs and symptoms reported by the patients was found to be statistically associated with the presence of anti-*Leptospira* sp. antibodies.

Spatial distribution and analysis

The spatial distribution of the people analyzed in the city of Olavarría is shown In Figure 2. This map shows that cases with positive serology have been found near Tapalqué stream or in the peripheral areas of the city. Although, no statistically significant cluster of greater risk for the presence of anti-*Leptospira* antibodies was detected.

Table 3. Frequency of *Leptospira* spp. serovars according to area of residence (rural/urban). Olavarría county, 2013-2014.

Serogroup	Serovar	Rural	Urban	p value	Total n/N*(%)
		n/N*(%)	n/N*(%)		
Tarassovi	Tarassovi	7/22 (31.81)	10/17(58.82)	0.092	17/39 (43.58)
Sejroe	Wolffi	14/22 (63.63)	2/17 (11.76)	0.001	16/39 (41.02)
Sejroe	Hardjo	10/22 (45.45)	3/17 (17.64)	0.067	13/39 (33.33)
Ballum	Castellonis	1/22 (4.54)	5/17 (29.41)	1.000*	6/39 (15.38)
Icterohaemorrhagiae	Copenhageni	0/22 (0.00)	4/17 (23.53)	0.029*	4/39 (10.25)
Hebdomadis	Hebdomadis	4/22 (18.18)	0/17 (0.00)	0.118*	4/39 (10.25)
Canicola	Canicola	1/22 (4.54)	1/17 (5.88)	1.000*	2/39 (5.12)
Pomona	Pomona	0/22 (0.00)	1/17 (5.88)	0.436*	1/39 (2.56)

*: Fisher exact test.

Discussion

Distribution of leptospirosis depends on geographic and socio-economic features [12]. Therefore, differences between urban and rural exposure should be expected. Our study provide evidence that overall prevalence of anti-*Leptospira* spp. antibodies was 7.00%, higher in the rural areas (19.66%). Similar findings were reported by Vanasco *et al.* [10], Schelotto *et al.* [26], Mwachui *et al.* [18] and Vimal Raj *et al.* [27] who identified rural activities or living in rural areas as risk factors for leptospirosis. In concordance, according to Schneider *et al.* [28], the risk for contracting leptospirosis was eight times higher in rural populations within the state compared to the urban populations due to various environmental factors and agriculture practices.

In Buenos Aires province, there are three risk areas for acquisition of leptospirosis being the peri-urban area the one that represents a greater risk of infection (prevalence close to 40%), followed by the rural area (prevalence close to 10%) and finally the urban area (prevalence less than 5%) [29]. The high infection rate detected in Colonia San Miguel (56.8%) could be explained by the demographic conditions, since this rural population seems to be a peri-urban area, the population tends to urbanize, but it does it in a disorderly way, and even the rural habits of the people remain intact.

Exposure factors such as livestock productions near the residences, contact with horses, cattle and sheep were observed more frequently in rural areas. Also, there is evidence that rural residents experience health disadvantages compared to urban residents, associated with a higher prevalence of health risk factors and socioeconomic differences [30]. In this work, similarly reported by other authors, socioeconomic disadvantage, such as lack of access to infrastructure and basic services, are two of the greatest underlying influences on health status in rural areas [31].

Likewise, the factors associated with seropositivity varied in each area, showing that for rural residents, the presence of rodents inside households was a major risk factor, perhaps associated with inadequate sanitation and poor housing [5,7-8]. Coincident with the results founded by Leal-Castellanos *et al.*, for urban populations, the main risk factors were the close contact with felines and male gender [13].

Although *Leptospira* infection occurs in domestic cats' populations, studies about the role of cats in the epidemiology of this zoonosis has not received much attention [32]. Recently, Dorsch *et al.* proposed that cats can become infected with *Leptospira*, as well as be

chronic carriers. Urinary shedding of infectious *Leptospira* spp. has recently been proven [33]. Therefore, this species can play a role in the transmission of the zoonosis. That could explain that the close contact with this species could be a risk factor for human infection.

The increased proportion of anti-*Leptospira* antibodies in males is coincident with the findings of Vanasco *et al.* [10] and Scialfa *et al.* in Argentina [29] for confirmed cases of leptospirosis. Occupational exposures may increase the risk of contact with leptospire in men. Another hypothesis could be that women are more cautious when they are in contact with animals or at leisure, thus reducing the possibility of infection [34,35].

The most reactive serogroup was Tarassovi, this serogroup has usually been associated with the presence of pigs, but other reports have described the circulation of the Tarassovi serogroup in *Rattus norvegicus* [36]. In spite of the fact that pig farming is developed in rural areas, this species is usually raised in this locality in peri-urban areas and sometimes in the backyards of the houses. Animal corrals were present near residences in 31.80% of urban residences and 32.72% of rural ones. Moreover, 11.5% of rural inhabitants and 7.9% of urban residents reported having contact with swine, explaining the high prevalence of this serogroup in urban communities.

It was also observed that serogroups reacted differently according to area (rural or urban). In rural areas, the Sejroe serogroup (serovar Hardjo and Wolffi) were the most reactive. In Argentina, the serovar Wolffi (like the serovar Hardjo belonging to the Sejroe serogroup) is associated with the presence of livestock farms, which were more common in rural communities [29]. In urban areas, the most reactive serogroups were Tarassovi, Ballum and Icterohaemorrhagiae. These serogroups have been mainly associated with rodents (*Mus musculus* and *Rattus norvegicus*). Studies carried out in the region have shown that rodents captured in peri-urban areas presented 1.9 times more risk of acquiring the disease than those caught in rural areas [5,31,29].

The number of cross-reactions detected in about half of the respondents (41%) agree with other studies. It reflects the possibility of co-infection with multiple serogroups in endemic areas, due to exposure to multiple serogroups or the repeated infections to different serogroups. It could be also due to the presence of several common antigens between different leptospira [37].

In Olavarría city, cases with positive serology have been found in the peripheral areas, which are characterized by inadequate sanitation, poor housing and exposure to rodents. In addition, other cases were situated near Tapalqué stream, which causes the region to be usually flooded, increasing the risk of infection [5,7-8].

Only 17.4 of the subjects had received prior information about the disease (via the internet, in an educational institution, from social media, at work, from health professionals, or through friends / family). It shows the limited scope of the programs of prevention, not reaching the information to the inhabitants of more dispersed areas.

Conclusions

In conclusion, given the reemerging nature of leptospirosis and its explosive occurrence worldwide, especially after floods and other natural disasters related to climate change, we consider our outcomes to be affected by decision-making and efficient planning for disease prevention.

Based on our results, we believe that there is a need for an epidemiological surveillance system, particularly in the rural areas, to allow proper diagnosis. Also, specific prevention campaigns based on the risk factors detected in this study (rodents for rural populations and cat contact/male gender for urban ones) should be implemented.

In addition, due to the lack of knowledge about the disease in the majority of the population, we think that specific community educational campaigns are recommended to prevent leptospirosis infection.

This study provides useful information on leptospirosis distribution and risk factors that could also be considered in other communities with similar characteristics especially the rural ones.

Acknowledgements

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