Role of *Mus musculus* in the transmission of several pathogens in poultry farms

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ARTICLE INFO	ABSTRACT
	This study aimed to analyze the role of *Mus musculus* as a host of *Leptospira* spp., lymphocytic choriomeningitis virus (LCMV) and *Toxoplasma gondii*, in poultry farms of Buenos Aires province, Argentina, and to assess the potential risk of transmission to humans and domestic or breeding animals. Samplings were performed between 2009 and 2011 (S1) and during 2016 (S2). In S1, we studied the prevalence of infection for *Leptospira* spp. and LCMV, whereas, in S2, we studied the prevalence of infection for *Leptospira* spp. and *T. gondii*. In S1, we found an overall *Leptospira* spp. prevalence in *M. musculus* of 18% (14/79) and no positive serum samples for LCMV (0/166). In S2, we detected no positive individuals for *Leptospira* spp. (0/56) and an overall *T. gondii* seroprevalence of 3.6% (2/56). The probability of *Leptospira* spp. infection in *M. musculus* was higher in reproductively active individuals and in samplings subsequent to months with high accumulated precipitation. Our results suggest that, in the poultry farms studied, the presence of *M. musculus* may be a risk factor in the transmission of *Leptospira* spp. and *T. gondii* to humans and domestic animals. The management of farms should include biosecurity measures for farm workers and more effective rodent control.

1. Introduction

Rodents can transmit a wide range of zoonoses caused by bacteria [(e.g., leptospirosis and salmonellosis (Seijo, 2001; Vanasco et al., 2003)], by viruses [(e.g., lymphocytic choriomeningitis, Argentine hemorrhagic fever (Busch et al., 1984; Saavedra et al., 2007)] and hantavirus pulmonary syndrome (Webster and Macdonald, 1995; Glass, 1997; Mills, 1999)], by helminthes [(e.g., trichinosis and fascioliasis (Ménard et al., 2000)], and those caused by protozoa [as toxoplasmosis and leishmaniasis (Kijlstra et al., 2008; Khademvatan et al., 2017)]. In addition, rodents are the zoonotic reservoirs of many infectious disease agents affecting humans (Mills and Childs, 1998; Taylor et al., 2001). Thus, synanthropic rodents as the house mouse (*Mus musculus*) and rats (*Rattus norvegicus* and *Rattus rattus*), which are frequently in contact with humans and domestic animals, represent a public health threat in both urban and rural habitats (Meerburg, 2006; Neiderud, 2015).

Among the diseases that can be transmitted by rodents, leptospirosis has a worldwide distribution and is considered to be re-emerging due to numerous outbreaks that have occurred worldwide during the last decades (Hartskeerl et al., 2011). It is caused by bacteria of the genus *Leptospira* (Adler and de la Peña Moctezuma, 2010), which can be genetically classified into 22 species, and more than 300 serovars. The most important rodent reservoirs of pathogenic *Leptospira* strains are *M. musculus*, *R. norvegicus* and *R. rattus*. The leptospires, are harbored in the rodent kidneys and excreted with the urine. Consequently, human beings and domestic and farm animals can be infected through contact with water or soil contaminated with urine from infected animals (Adler and de la Peña Moctezuma, 2010). The two *Leptospira* species responsible for most human infections are *Leptospira interrogans* and *Leptospira borgpetersenii*, which differ in their transmission routes (Bulach

et al., 2006). The infectious risk of *L. interrogans* is related to rainy periods and floodings (Guerra, 2009), whereas the transmission of *L. borgpetersenii* depends on the direct contact between hosts (Bulach et al., 2006).

In humans, leptospirosis causes a wide spectrum of clinical symptoms, ranging from mild fever to icteric Weil's disease and pulmonary hemorrhagic syndrome (Ko et al., 2009). The rate of mortality is related to delays in diagnosis and to the pathogenicity of some *Leptospira* strains (Bharti et al., 2003). While, in livestock production, leptospirosis may cause economic losses because infected animals are more prone to abortions and their newborns are weaker and grow more slowly than the offspring of non-infected animals (Lilenbaum and Martins, 2014).

Mus musculus is also a reservoir of lymphocytic choriomeningitis virus (LCMV), the only member of the family *Arenaviridae* with demonstrated activity in every continent. Human infection occurs through exposure to secretions or excretions of LCMV infected animals. Although lymphocytic choriomeningitis is asymptomatic or mild, and rarely fatal, prenatal infection with LCMV is important because of its impact on the fetus and because first-trimester LCMV infection is associated with an increased risk of spontaneous abortion (Barton and Mets, 2001). In Argentina, most epidemiological studies on LCMV have been conducted in urban areas (Riera et al., 2005), while the risk and extent of LCMV infections in rural areas is unknown.

As mentioned above, rodents may also be involved in the transmission of toxoplasmosis, a widespread zoonosis caused by the obligate intracellular protozoan *Toxoplasma gondii* (Lindsay and Dubey, 2011). The definitive hosts of *T. gondii* are individuals of the Felidae family, including domestic cats, but other homeothermic animals such as rodents may act as amplifying host (Tenter et al., 2000). Transmission to humans occurs congenitally or by consumption of undercooked meat containing tissue cysts or food or water contaminated with oocysts from felid feces (Tenter et al., 2000). Although infection with *T. gondii* in immunocompetent humans is asymptomatic, it becomes important in pregnant women and immunosuppressed people (Sibley and Boothroyd, 1992). Rodents play an important role as intermediate hosts of *T. gondii*, and are involved in the domestic, peridomestic and wild infection cycles (Rendón-Franco et al., 2014).

Although many studies have focused on congenital toxoplasmosis in humans, little is known about the reservoirs of the parasite in nature (Tenter et al., 2000).

Risk factors for infection by the mentioned pathogens vary among countries, and depend on environmental and ecological variables, mostly related to farming activities, contact with animals (rodents and livestock) and/or poor sanitation (Elbers et al., 1999; Bhardwaj et al., 2008). In establishments that breed animals for human consumption, as poultry farms, rodents can be hosts and disseminators of several pathogens, including *Leptospira* spp., LCMV and *T. gondii* (Acha and Szyfres, 2001). In most poultry farms of Buenos Aires province, Argentina, *M. musculus* is abundant, but its role in the transmission of these pathogens has been scarcely studied. Thus, the aim of this study was to analyze the role of *M. musculus* in the transmission of *Leptospira* spp., LCMV and *T. gondii* in poultry farms of Buenos Aires province, with the following specific objectives: i) to estimate the prevalence of these pathogens in *M. musculus*, ii) to evaluate the relationship of prevalence according to rodent characteristics such as sex, age and breeding status and iii) to study the relationship between the prevalence of *Leptospira* spp. and environmental characteristics as precipitation, temperature and season.

2. Materials and methods

2.1. Study area and poultry farms studied

The work was carried out in poultry farms located in the departments of Exaltación de la Cruz (34°17′S, 59°14′W) and San Antonio de Areco (34°27′S, 59°27′W), in the province of Buenos Aires, Argentina. The study area is located within the Pampean region and is characterized by a temperate climate (mean annual temperature of 16 °C and mean annual rainfall of 1000 mm) and grassland-type vegetation, which has been replaced by implanted crops. Currently, the study area is mostly devoted to agricultural activities, in which soybean, wheat and corn represent the main crops and is an area of intensive breeding of cattle, pigs and poultry. This last activity began in 1980 and increased rapidly, reaching more than 130 poultry farms in the study area and its surroundings in 2003 (Miño, 2003).

The poultry farms where we conducted the rodent samplings are devoted to grow - out broiler chickens, and occupy about 2–4 ha, surrounded by wire fences, under which there is a plant community that grows spontaneously. They have a variable number of rectangular sheds (between 3 and 16), which are about 100 × 10 m and are separated from each other by dirt roads for vehicles, pedestrian trails, and/or vegetated areas, which can be pruned or unattended depending on the particular maintenance of each farm or on the time of the year.

In all the poultry farms studied, five-day-old chicks, medicines and food are provided by large integrated breeding companies. Chickens receive food and water *ad libitum* and are maintained at a comfortable temperature, ranging between 20 °C and 30 °C (Donald, 2009), for 45–50 days, after which they are removed for selling. During the next 15–20 days, sheds are reconditioned for the arrival of new chicks and some farms apply chemical control for rodents. In general, during cleaning procedures, farm workers do not wear any personal protective equipment (PPE) and are thus exposed to the inhalation of particles from poultry bedding.

2.2. Rodent community

Despite the periodic rodent chemical control, *M. musculus* and *Rattus* spp. are very abundant in farms, mainly around poultry sheds (Gómez Villafañe et al., 2007; León et al., 2018), but rare in cropfields and natural habitats. Native species, including the sigmodontine species *Akodon azarae*, *Calomys laucha*, *Calomys musculinus*, *Oligoryzomys flavescens* and *Oxymycterus rufus*, and the caviomorph *Cavia aperea* (Busch and Kravetz, 1992; Bilenca et al., 1995), are mostly found in the weedy borders that border the farms (Miño et al., 2007).

2.3. Rodent samplings

A first seasonal rodent sampling (S1) was conducted in 23 poultry farms in October 2009 (Spring), January (Summer), April (Autumn) and October (Spring) 2010, and January (Summer) and June 2011 (Autumn-early Winter), whereas a second sampling (S2) was conducted in 15 poultry farms in March (Summer), April–May (Autumn), September (late Winter) and October–December (Spring) 2016. Seven of the farms were sampled in both S1 and S2.

Rodents were captured by Sherman type traps (15 × 16 × 31 cm) placed along the external walls of sheds, and spaced approximately 10 m apart. Traps were baited with a mixture of peanut butter, rolled oats and animal fat, and were active for three consecutive nights and checked every morning.

Species, sex, breeding status, body mass, and body and tail length were recorded for each captured rodent. Native species were released at the capture site while *M. musculus* and *Rattus* spp captured were anesthetized via intramuscular injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Males were considered as reproductively active if they had scrotal testes, whereas females were considered reproductively active if they were lactating, pregnant or with an open vagina. *Mus musculus* individuals were classified in three age classes according to their head-body length: juvenile (<72 mm), subadult (≥72 and ≤77 mm), and adult (>77 mm) (Smith et al., 1993). Finally, the synanthropic rodents captured were euthanized to collect tissue samples by cervical dislocation after retro-orbital bleeding.

Trapping, handling and euthanasia were performed according to the

procedures and protocols of the Argentine National Law for Animal Care 14 346 and the Ethics Committee for Research on Laboratory, Farm and Wild Animals of the National Council for Scientific and Technological Research (CONICET, resolution 1047, section 2, annex II). This work was the result of projects approved by the CONICET (PIP 1410) and Universidad de Buenos Aires (UBACyT X47), which were evaluated by an ethics committee. In addition, the protocol was approved by the institutional committee for animal handling and use (CICUAL; protocol number 125–2019).

2.4. Pathogen detection

For *Leptospira* spp. detection, two types of analyses were conducted: one for S1 and another one for S2. In S1, we collected kidney and/or urine samples in sterile conditions and immediately placed them in Ellinghausen, McCullough, Johnson, Harris (EMJH) liquid medium. However, due to the amount of EMJH liquid medium available to us, we analyzed samples from only 12 out of the 23 poultry farms sampled. In addition, because of contamination of some of the samples, we analyzed samples of all sampling dates, except those of January and October 2010. Once in the laboratory, all cultures were incubated at 30 °C, adding 5-fluorouracil aseptically as cytostatic, and then examined weekly for growth, by dark field microscopy, for up to 8 weeks (Ellinghausen and McCullough, 1965; Johnson and Harris, 1967).

In S2, to determine the presence of *Leptospira* spp., kidneys of *M. musculus* were taken in sterile conditions and placed on dry ice (−80 °C). Once in the laboratory, aliquots of renal tissue were incubated in EMJH and Fletcher semi-solid medium at 30 °C and examined regularly every 15 days by dark-field microscopy for six months.

Samples from S1were analyzed at the Departamento de Bacteriología, Instituto Nacional de Enfermedades Infecciosas, ANLIS Dr. Carlos G. Malbrán, Buenos Aires, Argentina, while samples from S2were processed at the Laboratorio de Leptospirosis, Instituto de Patobiología, INTA, Buenos Aires, Argentina.

For detection of antibodies against LCMV, we conducted ELISA tests described by Riera et al. (1997) to serum samples of *M. musculus* from all farms studied in S1. Samples were analyzed at the Instituto Nacional de Enfermedades Virales Humanas (INEVH-ANLIS), Buenos Aires, Argentina.

The study of the presence of *T. gondii* in *Mus musculus* from poultry farms arose after the samplings of S1 period. Busch and Burroni (2015) found that *M. musculus* individuals from poultry farms showed a tendency to use sites with odour of cat urine and feces. This result could be due to the manipulation of rodent behavior by *T. gondii*, as has been reported by some authors in other parts of the world (Webster, 2007). Because of that, the presence of *T. gondii* was assessed in *M. musculus* captured during S2. A blood sample (approximately 0.25 ml) was taken from each mouse through retro-orbital bleeding and their brain removed after euthanasia. Blood samples were kept refrigerated until they were centrifuged and used for serological diagnosis through indirect immunofluorescence, which detects anti-*T. gondii* IgG antibodies. Regarding brain samples, one hemisphere was preserved in dry ice (−80 °C) for molecular diagnosis whereas the other was preserved in 10% formaldehyde in phosphate buffer solution (PBS) for histopathological studies in search of tissue cysts. With respect to serology, cell culture-derived tachyzoites of RH strains (Dubey, 2010) were used as antigens and processed as previously described (More et al., 2008), using anti-mouse IgG (IgG, whole molecule)–FITC-conjugate (Sigma–Aldrich, St. Louis, USA) for all rodent species samples. Sera were tested at 3 different dilutions: 1/50; 1/200 and 1/800 (Huang et al., 2004). DNA was extracted from CNS samples using a commercial kit (Wizard® Genomic DNA Purification Kit, Promega) according to the manufacturer's protocol at the LAINPA. The specific PCRs using Tox5-Tox8 primers were used to identify *T. gondii* DNA as previously described (More et al., 2012). Each amplification routine was conducted with the positive control (DNA from *T. gondii* RH strain), and negative control (control process sample

DNA) and a no template control (NTC). The PCR products were visualized in 1.5% agarose gels (Biodynamics), and stained with SYBR Safe (Invitrogen) using 100 bp standard (Cien Marker, Biodynamics) (More et al., 2012). These analyses were performed in the Laboratorio de Inmunoparasitología, Facultad de Ciencias Veterinarias Universidad Nacional de La Plata (LAINPA –FCV – UNLP), Buenos Aires, Argentina.

2.5. Data analysis

The prevalence of infection was calculated as the percentage of hosts found to be infected with a particular parasite.

The data of accumulated rainfall (total millimeters of rainfall in the month) and average monthly temperatures used for the analyses were obtained from the website of the agro-meteorological information station of the National Institute of Agricultural Technology (INTA) San Pedro, Buenos Aires province (available http://inta.gob.ar/document os/informacion-agrometeorologica-eea-san-pedro/view).

Due to differences in the methodology used in the detection of *Leptospira* spp. in S1 and S2, the two samplings were considered separately. In S1, the relationship between the probability of *Leptospira* spp. infection of rodents and environmental and rodent characteristics was studied by Generalized Linear Mixed Models (GLMMs) with binomial error structure and a logit-link function (Bolker et al., 2009; Zuur et al., 2009; Crawley, 2012). The presence or absence of the given pathogen (1 or 0, respectively) was the response variable, whereas the season of the year in which the sample was taken (warm (spring-summer) or cold (autumn-winter), the accumulated monthly precipitation (mm) of the month prior to each rodent sampling, the accumulated precipitation (mm) of the six months prior to each sampling, and the rodent characteristics (sex, age class, and breeding status) were the explanatory fixed factors. Poultry farms were considered as a random factor (Zuur et al., 2009).

GLMMs were conducted using the lme4 (Bates et al., 2015) and MuMI n packages (Barton and Barton 2015) from the R software version 3.2.2 (R Core Team, 2013). The MuMIn package was used to run all possible model combinations based on a global model. Previously, we checked continuous variables for collinearity by using the variance inflation factor (VIF) for mixed models (Schweinberger, 2014). According to Zuur et al. (2010), a VIF value > 3 indicates collinearity. Variables that showed such values were removed from the analysis. Models were selected according to the Akaike's Information Criterion corrected (AICc) for small sample size (Burnham and Anderson, 2002). The model with the lowest AIC value was considered the most parsimonious, i.e. the model which explained the majority of the variance with the fewest parameters. We chose the models with smaller AICc values than the null model and those with a ΔAICc <2 in relation to the model with the lowest AICc value (Richards, 2005). The models chosen were averaged to obtain a final model using the "Averaged Model" function (Symonds and Moussalli, 2011; R Core Team, 2013). To estimate the efficiency of this model, we used the concordance index "Kappa (κ)" (Cohen, 1960), with which a valuation scale can be made according to the values of κ: "model with no agreement": $\kappa < 0$; "negligible model": $0.00 < \kappa < 0.20$; "discrete model": $0.21 < \kappa < 0.40$; "moderate model": $0.41 < \kappa < 0.60$; "substantial model": $0.61 < \kappa < 0.80$ and "almost perfect model": $0.81 < \kappa < 1.00$ (Landis and Koch, 1977). We then calculated the values of sensitivity and specificity for the averaged model (Lalkhen and McCluskey, 2008).

3. Results

A total of 177 and 85 rodents were captured during S1 and S2, with sampling efforts of 4446 and 4928 trap-nights, respectively. In S1,we captured 166 *M. musculus*, 4 *R. norvegicus*, 2 *R. rattus*, 3 *A. azarae*, 1 *C. musculinus* and 1 *O. flavescens*, whereas in S2 we captured56 *M. musculus*, 12 *Calomys* spp., 9 *R. rattus,* 5 *C. aperea*, 2 *R. norvegicus* and 1 *O. rufus*. The presence of *M. musculus* was recorded in 87% (20/23) of

the farms studied in S1and in 86.6% (13/15) of those studied in S2. In both samplings, the sex ratio of *M. musculus* was 53% females and 47% males and, in both sexes, all age classes and breeding status were represented.

Regarding pathogen detection, in S1 all serum samples analyzed (N = 166) were negative for LCMV and the prevalence of *Leptospira* spp. in *M. musculus* was 18% (14/79; CI$_{95\%}$: 9.3–26.1). In addition, two kidney samples analyzed for *R. rattus* were positive for *Leptospira* spp. There was at least one positive *M. musculus* individual in 50% (6/12) of the farms examined for *Leptospira* spp. in S1. The *M. musculus* positive for *Leptospira* spp. belonged to the three age classes (Table 1) and the prevalence was higher for reproductively active than for reproductively inactive individuals (Table 1). The proportion of positive individuals in the warm season was higher than that in the cold season (Table 1). In S2 no individuals were positive for *Leptospira* spp. (0 out of 56 samples).

According to the GLMMs, two models were selected to explain the probability of *Leptospira* spp. infection in *M. musculus* in S1 (Table 2). The final averaged model included the breeding status (BC; BC Estimator: -0.96 ± 0.63; CI$_{95\%}$: $-0.21 - 0.29$) and the accumulated monthly precipitation of the month prior to each rodent sampling (AMP1; AMP1 Estimator: 0.41 ± 0.28; CI$_{95\%}$: $-0.14 - 0.97$). Active individuals were more prone to be infected and prevalence increased with higher precipitation but with a one month delay. According to the Landis and Koch classification (1977), the model had a moderate agreement, and its sensitivity was 0.43 ± 0.14 (Table 2), indicating that the model detected the presence of *Leptospira* spp. when it was actually present in 43% of cases. According to the specificity value (0.88 ± 0.04; Table 1), the model correctly classified 88% of cases with absence of *Leptospir*a spp.

Antibodies anti-*T.gondii* were found in two of the *M. musculus* captured during S2, by the IFAT technique at a titer of 1/200; with a total seroprevalence of 3.6% (2/56; 95% CI = -0.01–0.08). However, the molecular diagnosis by PCR was negative in all case.

Both of these positive rodents were captured in the same poultry farm in spring 2016 and were sexually inactive males (one subadult and one adult).

4. Discussion

This study describes the potential role of *M. musculus* in the transmission of three pathogens in rural areas of Argentina. Studies involving more than one pathogen in breeding production systems are rare (e.g. Le Moine et al., 1987; Meerburg et al., 2006; Lovera et al., 2017), and most focus mainly in the prevalence of pathogens (Giraldo de Leon et al., 2002; De Faria et al., 2008; Scialfa et al., 2010).

Mus musculus was found infected with *Leptospira* spp. in 50% of the poultry farms studied and during the three years of the S1sampling. The prevalences of *Leptospira* spp. infection reported for *M. musculus* show a high variability, even at local scales (Rahelinirina et al., 2010; Gamage et al., 2011; Muñoz-Zanzi et al., 2014; Torres Castro et al., 2014). In pig and poultry farms of Sweden it was 9% (6/68; Backhans et al., 2013),

while in other rural areas prevalences varied between 9.1% and 42% (Vanasco et al., 2003; Rahelinirina et al., 2010; Gamage et al., 2011; Muñoz-Zanzi et al., 2014).

Although in S2 we detected no *Leptospira* spp.-positive individuals, the presence of *Leptospira* spp. should not be ruled out, because, during 2016, cases of leptospirosis in humans were recorded in the area, indicating the persistence of the pathogen in the study area (Chiani et al., 2016). The absence of *M. musculus* positive for *Leptospira* spp. in S2 could have been due to the smaller sample size analyzed with respect toS1 (N = 56 and N = 79 respectively) or due to particular characteristics of the studied farms studied in S2 that restrict the persistence of the pathogen in the environment and, consequently, rodent infection. Although the analyses were conducted by different research groups in the two periods, there were minor differences in the methodology used to detect *Leptospira* spp. and we consider that this did not affect the probability of *Leptospira* spp detection.

According to Hartskeerl et al. (2011), leptospires are difficult to eradicate. In the present study, we found that two of the seven poultry farms sampled in both sampling campaigns were positive for *Leptospira* spp. in S1 but negative in S2. This could have been due to the lower number of captures in these two farms in the last sampling (16 and 12 *M. musculus* individuals in each farm, in S1 and 3 individuals in each farm in S2).

In agreement with that observed in many studies, here we found a higher prevalence in reproductively active than in inactive individuals, probably related to a higher probability of conspecific aggressive encounters due to territoriality, social hierarchy behaviors and copulatory encounters, which can lead to fighting and subsequent injuries (Brown, 1953; Mucignat-Carettau, 2004; Sommaro et al., 2010). In addition, in many mammal species, males have larger home ranges than females, a fact that may lead them to a higher exposure to pathogens (Cosson, 2014). This was not the case for the *M. musculus* infection with *Leptospira* spp. found in the present study, which was similar in both sexes, as was also observed by Lovera et al. (2017) in pig and dairy farms of the same area. *M. musculus* shows a social structure with relatively close demes with scarce contacts between members of different demes, and, in poultry farms, movements of both sexes are frequently limited within poultry sheds. This social behavior may also explain the absence of differences between age groups, and suggests that infection may occur at early stages of life, including vertical transmission, as suggested by Fentahun and Alemayehu (2012).

In our work, infection by *Leptospira* spp. in *M. musculus* was also explained by rainfall, as previously observed by many authors (Arango et al., 2001; Mwachui et al., 2015), who found that transmission was related to humid conditions because they favor the survival of *Leptospira* spp. in the environment (Hartskeerl et al., 2011). This is consistent with leptospirosis outbreaks reported worldwide related to aquatic transmission or in areas with high humidity and temperature (Muñoz-Zanzi et al., 2014; ANLIS, 2016; Castelar, 2016).

Although in this study we could not assess the *Leptospira* serovar present in the *M. musculus* individuals captured in the poultry farms sampled, we consider it can be that *L. interrogans* serovar Icterohaemorrhagiae, because this was the serovar found by Lovera et al. (2017) in individuals of *M. musculus* and *R. rattus* captured in pig and dairy farms of the same area. These authors also found *R. rattus* infected with *L. borgpetersenii*. Further research to confirm the serovars present in poultry farms of the study area are relevant because the prevalence (18%) and high abundance of *M. musculus* found contribute to enhancing the probability of infection in human beings as well as in breeding and domestic animals. Risk factors are related to the abundance and prevalence of infection of the reservoir as well as to environmental conditions, especially precipitation. In rainy years, with relative low abundance of rodents but with a prevalence of 18%, human illness can become epidemic (Babudieri, 1958).

Regarding *T. gondii*, seropositive rodents were found in one of the 15 poultry farms studied, finding a seroprevalence of 3.6% (2/56 rodents).

Table 1
Percentage of infection with *Leptospira* spp. of *M. musculus* individuals according to Age class, Breeding status and Sampling season.

	No. Positive/total (%)	CI 95%
Age class		
Juvenile	2/12 (22)	1.6–32
Sub-adult	4/30 (13)	4–22
Adult	8/37 (17)	12–32
Breeding Status		
Reproductively active	9/35 (26)	18–34
Reproductively inactive	5/44 (11)	8–14
Sampling season		
Warm	8/33 (24)	17–31
Cold	6/46 (13)	8–18

Table 2

Candidate models and averaged model for the prevalence of *Leptospira* spp. in *M. musculus*. BS: breeding status. AMP1: accumulated monthly precipitation of the month prior to each rodent sampling. SE: standard error. CI: confidence interval. AICc: Akaike's Information Criterion corrected for small samples.

| | Candidate Models | | | | IRV | Averaged Model | | |
| | 1 | | 2 | | | | | |
	Estimator	SE	Estimator	SE		Estimator	CI (95%)	SE
Intercept	−1.13	0.46	−1.63	0.37		−1.36		0.49
BS(Inactive)	−0.96	0.63			0.54	−0.96	(-0.21; 0.29)	0.63
AMP1			0.41	0.28	0.46	0.41	(-0.14; 0.97)	0.28
Likelihood (Log)	−35.446		−35.623					
AICc	76.9		77.2					
ΔAICc	0		0.35					
Weight	0.54		0.46					
Kappa						0.31		0.13
Sensitivity						0.43		0.14
Specificity						0.88		0.04

Previous reports of *T. gondii* prevalence in synanthropic rodents are very variable (Murphy et al., 2008; Gotteland et al., 2014). In addition, Mecier et al. (2013) determined that the same rodent species can show different prevalence according to localities or countries, indicating the complex epidemiology of *T. gondii*.

The detection of anti-*T. gondii* IgG in the sera analyzed indicates that the infectious agent was present in any of its three infecting forms. However, this does not indicate whether the infection was recent or chronic, since a reinfection can occur in the circulatory system due to the release of bradyzoites from cysts originated in a previous infection and located in heart or striated muscle, as observed in chronic infections (Esteban-Redondo and Innes, 1998; Tenter et al., 2000; Hernández-Cortazar et al., 2015). *T. gondii* infection in small rodents does not always occur at the level of the nervous structures, as a consequence of the host's immune response, which is reflected in the ability to control the distribution of oocysts and to decrease their pathogenicity (Dubey and Frenkel, 1998). Isolation of *T. gondii* from brain samples depends on whether the brain portion analyzed contains cysts (Dubey et al., 1998). In rodents with a chronic infection, the parasite can also lodge in the heart and striated muscle (Hernández-Cortazar et al., 2015), tissues not analyzed in this work.

The presence of *T. gondii*-positive individuals in farms where domestic cats are also present constitutes a risk for farmers, enhanced by the presence of carcasses of chickens and rodents in the surroundings of breeding sheds. Dubey et al. (2003) found that chickens can become infected both by sporulated (infectious) oocysts present in the soil, spread by cats through their feces, and by cysts or tachyzoites, through the occasional pecking of remains of dead animals, such as rodents or other chickens.

Regarding LCMV, the absence of infection by this virus in poultry farms contrasts with the 12.9% prevalence (76/588 rodents) found by Riera et al. (2005) in urban areas of central Argentina. The non-detection of LCMV-positive rodents in the present study may have been due to the low prevalence of the virus or because it is really absent in the area. Nevertheless, more individuals of *M. musculus* should be analyzed to determine the role of this rodent as reservoirs of LCMV.

Synanthropic rodents that are attracted by foodstuffs and mild conditions of farms may infect the floor bedding with feces and urine, constituting a serious risk for farm workers who enter sheds without protection and are thus exposed to the inhalation of pathogenic particles. Usually, in poultry farms, there are other wild (rodents and opossums), domestic (dogs and cats) and breeding animals (cows, pigs, sheep and horses) that may become infected by direct or indirect contact with rodents and may function as an epidemiological bridge for transmission of pathogens to humans from infected rodents (Gay et al., 2014).

In summary, the findings of this study suggest that, in the poultry farms of the study area, the presence of *M. musculus* may be a risk factor for the transmission of *Leptospira* spp. and *T. gondii* to human beings and domestic animals. Consequently, farm work may include biosecurity measures, such as the use of disposable gloves, masks and goggles, along with a more effective rodent control. For this, we suggest eliminating potential sources of food and shelter, keeping plant cover low, and restricting rodent access to buildings structures.

The results of this study provide novel information about the role of *M. musculus* in the transmission of pathogens in intensive poultry breeding establishments. The microorganisms that cause zoonotic diseases, including those that are able to transit from wildlife to human beings, have public health implications. These studies are necessary to support the application of control and prevention measures for the rodent species involved in the transmission of zoonotic diseases, as well as to provide baseline knowledge of the health situation in the area.

Author contributions

Manabella Salcedo, Iris: Methodology, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization.

Fraschina, Jimena: Validation, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization.

Busch, María: Conceptualization, Methodology, Validation, Formal analysis, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

Guidobono, Juan Santiago: Formal analysis, Data Curation.

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Ethics approval

Trapping, handling and euthanasia were performed according to the procedures and protocols of the Argentine National Law for Animal Care 14 346 and the Ethics Committee for Research on Laboratory, Farm and Wild Animals of the National Council for Scientific and Technological Research (CONICET, resolution 1047, section 2, annex II). This work was the result of projects approved by the CONICET (PIP 1410) and Universidad de Buenos Aires (UBACyT X47), which were evaluated by an ethics committee. In addition, the protocol was approved by the Institutional Committee for Animal Handling and Use of the Facultad de Ciencias Exactas y Naturales-Universidad de Buenos Aires (CICUAL;

protocol number 125–2019).

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of competing interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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