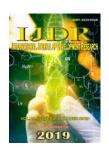


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SEROLOGICAL SURVEY OF *Leptospira spp.* AND Toxoplasma gondii IN COMPANION ANIMALS RESCUED FROM HOARDING BEHAVIOR CASES IN CURITIBA, PARANÁ, BRAZIL

¹Graziela Ribeiro da Cunha, ²Maysa Pellizzaro, ³Camila Marinelli Martins, ⁴Ana Carolina Yamakawa, ⁴Evelyn Cristine da Silva, ⁵Suzana Maria Rocha, ^{5,6}Vivien Midori Morikawa, ⁷Leila Sabrina Ullmann, ⁴Hélio Langoni and ^{*1}Alexander Welker Biondo

¹Departamento de Medicina Veterinária, Universidade Federal do Paraná, Curitiba – PR, Brasil

²Instituto de Saúde Coletiva, Universidade Federal da Bahia, Salvador – BA, Brasil

³Departamento de Enfermagem e Saúde Pública, Universidade Estadual de Ponta Grossa, Ponta Grossa – PR, Brasil

⁴Departamento de Higiene e Saúde Pública, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista,

Botucatu – SP, Brasil

⁵Departamento de Saúde Coletiva, Universidade Federal do Paraná, Curitiba – PR, Brasil ⁶Secretaria Municipal de Meio Ambiente, Prefeitura de Curitiba, Brasil ⁷Instituto de Biociências, Universidade Estadual Paulista, Botucatu – SP, Brasil

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*Corresponding author: Alexander Welker Biondo

ABSTRACT

This report has aimed to serosurvey *Leptospira spp*. and *Toxoplasma gondii* antibodies in two animal hoarding cases in Curitiba, Brazil. Blood samples from 15 dogs and 12 cats were tested, of which 3/15 (20.0%) dogs and 1/12 (8.3%) cat were seropositive to *Leptospira spp*. and 1/15 (6.6%) dog and 4/12 (33.3%) cats positive to *T. gondii*. The present report may be the first description of *T. gondii* and *Leptospira spp*. antibody status in dog and cat hoarding cases of Brazil. Despite low frequencies, these results have suggested that companion animals in hoarding conditions may act as sentinels for environmental contamination.

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INTRODUCTION

Hoarding disorder has been described as a mental illness characterized by a persistent difficulty to discard possessions, regardless their value (American Psychiatric Association, 2013). In animal hoarding cases, disease may result in animal accumulation and lack of adequate nutrition, sanitation, shelter and veterinary care (Patronek, Loar, and Nathanson, 2006). Hoarder household may negatively impact on neighborhood's public health, safety and welfare, mostly due to poor sanitary conditions, which may favor disease harboring and spreading, particularly zoonoses when involving animals (Frost, Steketee, and Williams, 2000).

In Brazil, animal hoarding cases have been reported in only two capital cities of southern states: Curitiba, with a total of 1,114 animals in 40 cases (Cunha *et al.*, 2017) and Porto Alegre, with 1,357 animals in 33 cases (Ferreira *et al.*, 2017). Such absence of studies may represent an underreport and/or underestimation of cases, which, consequently, may negatively affect proper establishment of public policies. In addition, companion animals in hoarding conditions may be more susceptible to transmissible diseases due to the unsanitary and overcrowded living conditions, with zoonotic gastrointestinal infections as ascarides and hookworms already described in cats seized from animal hoarders (Polak, Levy, Crawford, Leutenegger, and Moriello, 2014). Despite clinical conditions commonly observed in hoarding animals may include

respiratory infections, skin diseases, starvation, parasites and other negligence signs, no study to date has focused on zoonotic risk of hoarder cases. Accordingly, this report was aimed to assess anti-*Leptospira* spp. and anti-*Toxoplasma gondii* antibodies in two animal hoarding cases (one of dogs and one of cats) following animal rescue conducted by the Curitiba city, southern Brazil, due to unhealthy living conditions of hoarders and their pets.

CASE DETAILS

The two reported cases were chosen due to city emergency intervention, with animal rescue provided by the Animal Protection Service of Curitiba City Council, responsible for animal hoarding cases and other animal abuse complaints. Intervention and rescue have been considered the ultimate exception, only applied under refusal of mental support and animal assistance. Due to such extreme conditions, no animal medical record was available at the time. Animals of both cases were rescued, treated, spayed and neutered, and subsequently send for adoption. Whole blood samples were collected and submitted to serological analysis. Anti-Leptospira spp. antibodies were detected by microscopic agglutination test, with samples screened with a regional battery of 31 serovars and considered positive if titer was ≥ 100 (initial dilution of 1:100). Specific anti-T. gondii IgG antibodies were detected by indirect fluorescent antibody test, with samples considered positive if antibody titers ≥ 16 , determined to the last dilution at which $\geq 50\%$ of tachyzoites presented fluorescence. Data were analyzed in a descriptive way. Dog hoarding case was of a homeless man living along a Curitiba highway with 32 dogs, of which 15 adults and 17 puppies. Animals were kept with short leashes to their improvised muddy pens. Concomitant object accumulation worsened the local sanitary conditions. The hoarder was taken against his will to a city hospital for physical and mental treatment. A total of 15 blood samples were obtained from dogs, of which 9 (60.0%) females and 6 (40.0%) males. A total of 3/15 (20.0%) dogs were positive for anti-Leptospira spp. with titers ranging from 100 to 200. All Leptospira spp. positive samples of dogs were identified as serovar Gryppotyphosa (*Leptospira kirshneri*). A total of 1/15 (6.6%) dog was seropositive for anti-T. gondii antibodies with titer of 64. Cat hoarding case was of a woman living alone with 22 indoor cats, of which 10 kittens and 12 adults. Animals were found among object accumulation due to recent hoarder death, kept inside a crowded and unsanitary space and lacking proper food and water. In the rescue day, feces, urine, and dead animal carcasses were found into the household. A total of 12 blood samples were obtained from cats, of which 9 (75.0%) were from females and 3 (25.0%) from males. Seropositivity for anti-Leptospira spp. antibodies was found in 1/12 (8.3%) cat with titer of 100. Serovar Patoc (Leptospira biflexa) was detected in the single cat positive sample. Seropositivity for anti-T. gondii antibodies was found in 4/12 (33.3%) cats with titer ranging from 256 to 1024.

DISCUSSION

Frequencies of anti-*Leptospira* spp. antibodies in dogs have been reported ranging from 9.3% (35/378) (Morikawa *et al.*, 2015) to 38.9% (35/90) (Martins *et al.*, 2013) in Curitiba and metropolitan area and ranging from 12.2% (41/335) (Fonzar, and Langoni, 2012) to 21.6% (51/236) (Caldart *et al.*, 2015)

considering other studies performed at the Paraná State, corroborating with the herein results. Previous studies were performed with dogs from different sources and a variety of environmental infection levels, such as domiciled, stray and shelter dogs. However, considering the hoarding unsanitary conditions which predispose rodent infestation (Bratiotis, 2013), a higher frequency of anti-Leptospira spp. antibodies was expected in hoarded dogs, and further studies should be conducted to fully establish the dog role on leptospirosis in hoarding cases. The low titers found in dogs may indicate recent contact with the agent, or residual titer from a previous infection (Morikawa et al., 2015), with no pet showing clinical signs of disease at the time of sampling. Furthermore, animals may keep low titers when in constant contact with the agent, thus each case should be individually evaluated considering all related variables. Serovar Gryppotyphosa found in dog samples has been previously reported in dogs at a lower prevalence (Martins et al., 2013; Morikawa et al., 2015).Regarding seropositivity to T. gondii antibodies, previous studies have reported a frequency of 30.7% (8/26) in neighborhood dogs in Curitiba (Constantino et al., 2016) and 20.6% (56/271) in domiciled dogs from Londrina (Caldart et al., 2015), which is higher than a frequency reported in hoarded dogs. This probably may be related to outdoor and free-roaming habits of neighborhood dogs, which may increase their exposure to infection (Constantino et al., 2016), while hoarded dogs have been described usually in long-term confinement at restricted and overcrowded spaces (Patronek et al., 2006).

In overall, these findings suggest that hoarded dogs have been in contact with Leptospira spp. and T. gondii at some point in their life, showing a possible environmental contamination, which may represent a potential source of infection to hoarders. Despite anti-Leptospira spp. antibodies were found in only one cat in this report, frequency was higher than previous reported in Curitiba metropolitan area, with a frequency of 4.6% (3/65) in domiciled cats (Cordeiro, Vieira, and Oliveira, 2017). Detection of serovar Patoc in the only positive cat sample in this report was unexpected, once a previous study found serovar Pomona in domiciled cats of Curitiba (Cordeiro et al., 2017). Although serovar Patoc has been reported as a saprophytic strain of Leptospira biflexa, bacteria may cross-react with pathogenic serovars (Pellizzaro et al., 2017). Both findings have shown that cats from Curitiba and metropolitan area have been susceptible to *Leptospira* spp. infection and post an alert for further investigation.

Seropositivity for anti-T. gondii antibodies was found in 4/12 (33.3%) cats with titer ranging from 256 to 1024. Regarding T. gondii antibodies found in cats, results reported herein have shown a higher frequency of seropositive cats (33.3%) than previously reported in Curitiba and Londrina, which found anti-*T. gondii* antibodies in 16.3% (46/282) (Cruz *et al.*, 2011) and 20.9% (87/415) (Caldart et al., 2015) of owned cats, respectively. These results may be related to differences in environmental oocyst contamination, since hoarder households may be under cluttered and unsanitary conditions. Despite the frequency differences, data reported herein have suggested cat contact with environmental or food-borne T. gondii. Regardless, cats have been definitive hosts of T. gondii and may post infection risk for hoarders, particularly due to feces contact under littered conditions. Although a total of 65 animal hoarding cases has been identified in Curitiba (Cunha et al., 2017), no specific management protocol has been successfully

established to date. The city taskforce has been working to identify and reduce risks in each case, preferentially keeping pets at the hoarder household. Both cases herein were considered as emergency exceptions, with animals rescued due to extreme risk of living conditions. Severe cases with tragic endings have been previously reported in Curitiba, including a hoarder household fire causing 43 dog deaths and a hoarder found dead at his household and partially eaten by own dogs. A multidisciplinary and specific public policy for animal hoarding cases should be established, particularly in developing countries, to reduce such negative impacts and prevent diseases and other disasters. The present report may be the first description of T. gondii and Leptospira spp. antibody status in dog and cat hoarding cases of Brazil. Despite low frequencies, the results reported herein have indicated that companion animals in hoarding conditions may act as sentinels for environmental contamination and potential source of these zoonotic infection for hoarders.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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