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Clazer, Marília; Vendrame Rodrigues, Graziela; Martins Ferreira, Bruna Paula; Marchi Zaniolo, Melissa; Bespalez Corrêa, Nilton Anderson; Salomão Fortes, Maíra; Navarro, Itamar Teodorico; Torres Chiderolli, Roberta; de Freitas, Julio Cesar; Dib Gonçalves, Daniela

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Toxoplasmosis, leptospirosis, and brucellosis seroepidemiology in veterinary medical students and their relation with unique health

Soroepidemiologia da toxoplasmose, leptospirose e brucelose em acadêmicos de medicina veterinária e sua relação com a saúde única

Marília Clazer¹; Graziela Vendrame Rodrigues¹; Bruna Paula Martins Ferreira¹; Melissa Marchi Zaniolo¹; Nilton Anderson Bsepalez Corrêa³; Maíra Salomão Fortes¹; Itamar Teodorico Navarro⁴; Roberta Torres Chiderolli⁵; Julio Cesar de Freitas⁴; Daniela Dib Gonçalves^{2*}

Abstract

Toxoplasmosis, leptospirosis, and brucellosis are global zoonoses, with humans as accidental participants in their transmission cycles. They can also be considered occupational diseases, because certain professionals are at greater risk of contact and infection by such zoonoses. These three diseases have different epidemiological characteristics because of the distinct environmental, social, cultural, and economic conditions where these pathogens circulate. Because of the importance of these diseases and their associations with specific occupations, we performed a seroepidemiological survey of *Toxoplasma*, *Leptospira*, and *Brucella* antibodies, with an analysis of the association between positive serum and certain occupational and environmental variables, in students of a veterinary medicine course (1st, 2nd, 3rd, 4th, and 5th year) in a university in the northwestern region in the state of Paraná, Brazil. From May to November 2014, blood samples were collected from 157 volunteers by professionals trained in nursing and biomedicine from the same university as the veterinary students. At the time of blood collection, the students did not present any clinical signs of the three diseases of interest. To detect anti-*Toxoplasma gondii*, anti-*Leptospira* spp., and anti-*Brucella* antibodies, indirect immunofluorescence test (IIF), microscopic agglutination test (MAT), fast agglutination test (a screening test), and enzyme-linked immunosorbent assay (ELISA) were performed. To determine variables associated with these infections, the students were interviewed to complete an epidemiological questionnaire with environmental, behavioral, and occupational information. The associations between these variables and infections were assessed by chi-square or Fischer's exact tests, with a 5% significance level (α). Of the 157 serum samples analyzed, 29.29% reacted to *Toxoplasma* antigens, with titers ranging from 16 to 4096 by IIF, 1.27% to *Leptospira* antigens, with titers ranging from 100 to 800 by MAT using Hardjo and Wolffi serovars, and 0.63% to *Brucella* antigens by ELISA; however, no variables were found to be associated with infection with any of these pathogens. The results of this study show that one-third of the students in the veterinary medicine course were exposed to *Toxoplasma gondii*, *Leptospira* spp., and *Brucella* spp. at some stage in their lives; however, it is not possible to determine whether these infections were acquired at the university, because no associations between occupational risk variables

¹ Discentes, Programa de Pós-Graduação em Ciência Animal com Ênfase em Produtos Bioativos, Universidade Paranaense, UNIPAR, Umuarama, PR, Brasil. E-mail: mariliacazer@bol.com.br; grazielavr@hotmail.com; bp_ferreira@hotmail.com; melissaz.vet@gmail.com; maira_salomao@yahoo.com.br

² Prof^a Dr^a, Programa de Pós-Graduação em Ciência Animal com Ênfase em Produtos Bioativos, UNIPAR, Umuarama, PR, Brasil. E-mail: danieladib@prof.unipar.br

³ Prof. Me., Curso de Graduação de Farmácia, UNIPAR, Umuarama, PR, Brasil. E-mail: nilton@prof.unipar.br

⁴ Profs. Drs., Programa de Pós-Graduação em Ciência Animal, Universidade Estadual de Londrina, UEL, Londrina, PR, Brasil. E-mail: itamar@uel.br; freitasj@uel.br

⁵ Discente, Programa de Pós-Graduação em Ciência Animal, UEL, Londrina, PR, Brasil. E-mail: robertaa_tc@hotmail.com

* Author for correspondence

and these infections were found. An understanding of the transmission of each etiological agent and methods to prevent infection is important to maintain low prevalence levels of these zoonotic diseases during the veterinary medicine course and extra-curricular internships, when there is increased exposure to these pathogens.

Key words: Antibodies, *Brucella* spp. Occupational disease. *Leptospira* spp. *Toxoplasma gondii*. Zoonosis.

Resumo

A toxoplasmose, leptospirose e a brucelose são zoonoses de ampla distribuição mundial, tendo o homem como participante acidental da sua cadeia epidemiológica, entretanto, podem se apresentar como doenças ocupacionais, em diferentes categorias profissionais, despertando grande preocupação, por estarem constantemente expostos ao risco de contato e contágio com essas zoonoses. Estas três enfermidades possuem características epidemiológicas distintas, como reflexo das diferenças ambientais, sociais, culturais e econômicas encontradas em cada localidade. Considerando a importância destas três enfermidades e sua relação com o aspecto ocupacional o objetivo deste trabalho foi realizar um levantamento soropidemiológico para toxoplasmose, leptospirose e brucelose e identificar variáveis ocupacionais e ambientais relacionadas a estas três enfermidades em acadêmicos do curso de medicina veterinária de uma universidade da região noroeste do estado do Paraná, Brasil. No período de maio a novembro de 2014, foram coletadas amostras de sangue de forma voluntária de 157 acadêmicos do curso de Medicina Veterinária (1º, 2º, 3º, 4º e 5º ano). A coleta de sangue foi realizada por profissionais habilitados da área de enfermagem e da biomedicina da respectiva universidade. No momento da coleta de sangue, os acadêmicos não apresentavam manifestação de qualquer sinal clínico das enfermidades deste estudo. Para detectar anticorpos anti-*Toxoplasma gondii*, anti-*Leptospira* spp. e anti-*Brucella* foram realizadas as técnicas de imunofluorescência indireta (RIFI), aglutinação microscópica (SAM), aglutinação rápida (triagem) e ensaio imunoenzimático (ELISA) respectivamente e para a detecção de variáveis associadas às infecções, os acadêmicos foram entrevistados, respondendo a um questionário epidemiológico com informações ambientais, comportamentais e ocupacionais relacionadas às enfermidades, sendo a associação verificada pelos testes de qui-quadrado ou exato de Fischer, adotando-se um nível de significância (α) de 5%. Das 157 amostras de soro analisadas 29,29% foram reagentes para toxoplasmose com títulos variando de 16 a 4096 na IFI, 1,27% para leptospirose com títulos de 100 e 800 na SAM com os sorovares Hardjo e Wolffi e 0,63% para brucelose no ELISA, entretanto não houve variáveis associadas a nenhuma infecção. Os resultados deste trabalho demonstram que 1/3 dos acadêmicos do curso de medicina veterinária foram expostos ao *Toxoplasma gondii*, *Leptospira* spp. e *Brucella* spp. em algum momento de suas vidas, entretanto não podemos afirmar se esta infecção foi adquirida nas dependências da universidade já que não foi possível associar as variáveis de risco com as respectivas infecções. A conscientização desta população quanto às particularidades de cada agente etiológico e suas medidas de prevenção são fundamentais para manter as baixas taxas de prevalências das respectivas enfermidades zoonóticas durante o decorrer do curso de graduação e também quando da realização de estágios extra-curriculares, momento este de maior exposição aos diferentes agentes etiológicos.

Palavras-chave: Anticorpos. *Brucella* spp. Doença ocupacional. *Leptospira* spp. *Toxoplasma gondii*. Zoonose.

Introduction

Toxoplasmosis, leptospirosis, and brucellosis are zoonoses of great importance to public health, affecting humans who have direct or indirect contact with animals through various occupations (GONÇALVES et al., 2006).

Toxoplasmosis, one of the most common parasitic infections in the world, is caused by the protozoan *Toxoplasma gondii*, with domestic and/or wild felines serving as infection reservoirs (FRENKEL et al., 1995). This zoonosis can result in severe disease in humans, particularly in its

congenial form. It is of increasing importance in pregnant women and immune-suppressed patients who are infected for the first time (CAPOBIANGO et al., 2014; LANGONI et al., 2009).

Humans can be infected by ingestion of oocysts in contaminated water and food, by ingestion of bradyzoites in raw or undercooked meat, or by transplacental transmission of tachyzoites, transfusion of leukocytes, transplantation of organs, ingestion of non-pasteurized goat milk, or accidents in the laboratory (DUBEY et al., 2002; DUBEY, 2010). *T. gondii* infection is highly prevalent in humans in Brazil, with 50% of children and 80% of women in fertile age present antibodies against this protozoan (DUBEY et al., 2012).

Another very important zoonosis globally is leptospirosis, a contagious disease with endemic and seasonal transmission. It is transmitted directly or indirectly by as many as 250 bacterial species in the genus *Leptospira*, which can infect humans and various wild and/or domestic animal species (CONFALONIERI; DUTRA, 2014; FAINE et al., 1999). Humans are considered accidental and terminal hosts in the transmission chain of this disease; they can be infected by direct exposure to blood, urine, or tissue from infected animals or indirectly through water, mud, pasture soil, trough and stable surfaces, or food contaminated with the microorganism (FAINE et al., 1999).

Among the factors that favor persistence of *Leptospira*, its antigenic variability, relative degree of survival in environmental level with the absence of parasitism, and wide variety of susceptible vertebrates are particularly important. Rodents are the main reservoirs, sheltering *Leptospira* in kidneys and excreting the organisms alive in urine to the environment, contaminating water, soil, food, other animals, and even humans (FAINE et al., 1999).

Another frequent global zoonosis is brucellosis, caused by bacteria belonging to the genus *Brucella*, which are Gram-negative facultative intracellular microorganisms that do not form spores. A total of nine species (and their hosts) have been described,

namely *Brucella melitensis* (goats), *Brucella abortus* (cattle), *Brucella suis* (pigs, reindeer), *Brucella ovis* (sheep), *Brucella canis* (dogs), *Brucella neotomae lepida* (*Neotoma lepida*, desert rodents), *Brucella ceti* (dolphins and whales), *Brucella pinnipedialis* (seals), and *Brucella microti arvalis* (*Microtus arvalis*, wild mice) (DIAS, 2012; GUIMARÃES, 2011).

Human infections may occur through direct exposure to an infected animal, including its tissues, blood, urine, vaginal or other excretions, or carcass, or through indirect exposure, including the ingestion of raw or undercooked meat, non-pasteurized milk, or dairy products originating from an infected animal (POESTER et al., 2009). Brucellosis is a global public health problem: it is underdiagnosed and underreported, with an estimated 25 cases for every diagnosed case (CARDOSO; COSTA, 2012). The prevalence of *Brucella* infections in humans is closely associated with the prevalence of the pathogen in animals, and, for this reason, in (BRASIL, 2001), the National Program for the Control and Eradication of Animal Brucellosis and Tuberculosis (Programa Nacional de Controle e Erradicação da Brucelose e da Tuberculose Animal, PNCEBT) was created by the Ministry of Agriculture, Livestock, and Food Supply (MAPA), with the purpose of reducing the prevalence of these infections in animals, and, consequently, in human beings (BRAZIL, 2001).

The three diseases of interest in this study are occupational; that is, humans can acquire the infection from their working environment through direct or indirect exposure to infected animals or contaminated animal products and by-products. Cases of these disease have been recognized in workers in slaughterhouses, dairy factory, and laboratories, in insemination and rural workers, in veterinarians, in other professionals working in the clandestine slaughtering of animals, vaccination of herds, and other occupations (GONÇALVES et al., 2006, 2013; GRESSLER et al., 2012; PELISSARI et al., 2011; RAMOS et al., 2008; SANTOS et al., 2007).

Serological investigations of anti-*T. gondii*, anti-*Leptospira* spp., and anti-*B. abortus* antibodies have already indicated risk factors in veterinary students in different regions of Brazil (LACERDA et al., 2000; LIMA, 2008; LANGONI et al., 2009). Owing to the importance of these three diseases and their association with occupation, we performed a seroepidemiological survey for *Toxoplasma*, *Leptospira*, and *Brucella* antibodies, as well as analyzed occupational and environmental variables related to infection with these organisms, in students in a veterinary medicine course in a university in the northwestern region in the state of Paraná, Brazil.

Material e Methods

Study location and sample collection

From May to November 2014, blood samples were collected from 157 student volunteers in a veterinary medicine course (1st, 2nd, 3rd, 4th, and 5th year) from a university located in the northwestern region in the state of Paraná. Blood sampling was performed in an appropriate location at the university by a trained professional. At the time of blood collection, the students did not present any clinical signs of the diseases studied.

A total of 157 serum samples was collected, with 38 (24.23%) belonging to students in the first year, 33 (21.01%) to students in the second year, 35 (22.29%) in the third year, 27 (17.19%) in the fourth year, and 24 (15.28%) in the fifth year; 74 (47.14%) were from females and 83 (52.86%) were from males.

Serological diagnoses

Each serum sample was divided into three aliquots of equal volume, stored in sterile flasks, and held at -20°C for later serological examination.

To detect anti-*Leptospira* spp. antibodies, the serum samples were submitted to microscopic agglutination testing (MAT) (RYU, 1970) at the Laboratory of Leptospirosis in the Department of

Preventive Veterinary Medicine (DMVP) at the State University of Londrina (UEL), with 22 reference serovars, namely Australis, Bratislava, Autumnalis, Butembo, Fortbragg, Castellonis, Bataviae, Canicola, Whitcombi, Cinoptery, Grippothyphosa, Hebdomadis, Copenhageni, Icterohaemorrhagiae, Panama, Pomona, Pyrogenes, Hardjo, Wolffi, Shermani, Tarassovi, and Sentot. The samples were stored at 28°C for 5 to 10 days in modified EMJH (Difco, USA) medium (ALVES et al., 1996). Serum samples showing at least 50% agglutination at 1:100 dilution were considered reactors and sera were two-fold serially diluted to determine the maximum positive dilution. The probable infecting serovar was considered the one associated with the highest agglutinating titer; those showing co-agglutination at the highest dilution were considered as only reagent to *Leptospira* spp. (BATISTA et al., 2005).

To detect anti-*Brucella abortus* antibodies, the serum samples were sent for a fast agglutination screening test at the Laboratory of Preventive Veterinary Medicine and Public Health at Universidade Paranaense (UNIPAR). They were tested using commercial kits prepared with *B. abortus* antigen (proteins and lipopolysaccharides) produced by Ebram Produtos Laboratoriais Ltd., São Paulo (SP). Samples showing agglutination were considered as reactors. To confirm these results, reactive samples were tested for IgM and IgG antibodies by enzyme-linked immunosorbent assay (ELISA) in a private laboratory, with results greater than 11.0 for both IgM and IgG considered positive. A serum sample was only considered positive when it was reaction in both serological tests.

To detect antibodies against *T. gondii*, an indirect immunofluorescence test (IIF) was performed according to methods described by Camargo (1974). The slides containing antigens (tachyzoites) of the RH strain were prepared in the Zoonosis and Public Health laboratory at DMVP-UEL. The sera were four-fold diluted, starting at a titer of 1:16, with sera from humans known to be positive and negative for *Toxoplasma* used as controls. IIF slides were read under an epi-

fluorescence microscope (Leica, DMLS) at 400× magnification. Samples showing fluorescence at a titer of 1:16 were considered positive.

Questionnaire

To determine variables associated with infection by each of the three pathogens, the study participants were interviewed to complete a questionnaire on environmental, behavioral, and occupational variables related to the diseases addressed in this study.

Statistical analysis

To determine the association between serological results the different variables, Fischer's exact test and chi-square tests were performed in Bioestat 5.0 (AYRES et al., 2007), with the significance level set at 5%.

Results

Of 260 students in the Veterinary Medicine program, 157 (60.38%) participated in the study voluntarily. Of these, 49 (31.21%) were considered

reactors to at least one of the three zoonotic diseases in this study (Table 1).

Of the 49 positive samples subjected to serological testing, 17 (34.69%) samples were obtained from students in their first year, eight (16.33%) from students in their second year, nine (18.37%) from students in their third year, six (12.24%) from students in their fourth year, and nine (18.36%) from students in their fifth year. Of the seroreactive samples, 26 (53.06%) were from female and 23 (46.93%) from male students (Table 2).

For *Toxoplasma*, 46 (29.29%, 46/157) samples were considered positive by IIF, presenting antibody titers ranging from 16 to 4096 (Table 3). No variable studied was considered statistically significantly associated with *Toxoplasma* infection (Tables 4 and 5).

For leptospirosis, two (1.27%, 2/157) samples were considered positive by MAT, and antibodies against the following serovars were detected: Hardjo (50%, 1/2), with a titer of 100, and Wolffi (50%, 1/2), with titer of 800. Both sample were from male subjects. No variable studied was found to be statistically significantly associated with *Leptospira* infection (Tables 4 and 5).

Table 1. Results of indirect immunofluorescence (IIF) assay for *Toxoplasma gondii*, microscopic agglutination test (MAT) for *Leptospira*, and immunoenzymatic assay (ELISA) for *Brucella* antibodies by gender in 46 serum samples from students in a veterinary medicine course in a university located in the northwestern region in the state of Paraná, Brazil, 2014.

Gender	Antibody Frequency		
	Indirect immunofluorescence assay (IIF)	Microscopic seroagglutination test (MAT)	Immunoenzymatic assay (ELISA)
Female	26 (56.53%)	--	--
Male	20 (43.47%)	02 (1.27%)	01 (0.63%)
Total	46 (100%)	02 (100%)	01 (100%)

Table 2. Results of indirect immunofluorescence (IIF) for *Toxoplasma gondii*, microscopic seroagglutination test (MAT) for *Leptospira*, and immunoenzymatic assay (ELISA) for *Brucella* antibodies by university year (1st, 2nd, 3rd, 4th, and 5th year) in 46 serum samples from students in a veterinary medicine course in a university located in the northwestern region in the state of Paraná, Brazil, 2014.

University Year Frequency			
	Indirect immunofluorescence assay (IIF)	Microscopic seroagglutination test (MAT)	Immunoenzymatic assay (ELISA)
1 st year	16 (32.65%)	--	01 (2.04%)
2 nd year	08 (16.33%)	--	--
3 rd year	07 (14.29%)	02 (4.08%)	--
4 th year	06 (12.24%)	--	--
5 th year	09 (18.36%)	--	--
Total	46 (93.88%)	02 (4.08%)	01 (2.04%)

Table 3. Frequency of specific antibody titers detected by indirect immunofluorescence assay (IIF) for *Toxoplasma gondii* in 46 serum samples from students in a veterinary medicine course in a university located in the northwestern region in the state of Paraná, Brazil, 2014.

Antibody Titers	Frequency		Total (%)
	Female	Male	
16	01	02	03 (06.52%)
64	07	03	10 (21.74%)
256	15	12	27 (58.69%)
1024	03	02	05 (10.87%)
4096	--	01	01 (02.18%)
Total	26 (56.53%)	20 (43.47)	46 (100%)

Table 4. Result of the chi-square (χ^2) testing of the association between specific variables and the presence of *Toxoplasma gondii*, *Leptospira* or *Brucella* antibodies in 157 serum samples from students in a veterinary medicine course in a university located in the northwestern region in the state of Paraná, Brazil, 2014.

Variable	Percent Positive (%)	P-value	OR (95% CI)
<i>Toxoplasma gondii</i>			
Gender			
Male	20/46 (43.48%)		
Female	26/46 (56.52%)		
Ingestion of raw meat			
Yes	33/46 (71.74%)	0.201**	1.623 (0.770 – 3.420)
No	13/46 (28.26%)		
Ingestion of raw non-sanitized vegetables			
Yes	45/46 (97.83%)	0.111*	5.814 (0.734 – 46.056)
No	1/46 (2.17%)		

continue

continuation

Contact with cats during academic activities	Yes	38/46 (82.61%)	0.520**	1.337 (0.550 – 3.249)
	No	8/46 (17.39%)		
Habit of sanitizing the hands after contact with animals	Yes	44/46 (95.65%)	0.586	0.430 (0.593.146)
	No	2/46 (4.35%)		
<hr/>				
AIs aware of the transmission and prevention of <i>T. gondii</i>	Yes	40/46 (86.96%)	0.952**	1.032 (0.371 – 2.869)
	No	6/46 (13.04%)		
<i>Leptospira</i> spp.				
Gender	Male	2/2 (100%)		
	Female	--		
Presence of rodents at home	Yes	1/2 (50%)	1.000*	0.742 (0.66 – 8.360)
	No	1/2 (50%)		
Habit of keeping the trash closed	Yes	1/2 (50%)	0.567*	0.632 (0.56 – 7.175)
	No	1/2 (50%)		
Cleans the kennel	Yes	1/2 (50%)	0.579*	2.667 (0.237 – 30.036)
	No	1/2 (50%)		
Contact with domestic animals during academic activities	Yes	2/2 (100%)	1.000*	--
	No	--		
Is aware of the transmission and methods to prevent infection with <i>Leptospira</i>				
	Yes	2 (100%)	1.000*	1.116 (1.058 – 1.178)
	No	--		
<i>Brucella</i> spp.				
Gender	Male	1/1 (100%)		
	Female	--		
Frequent ingestion of raw milk/fresh cheese	Yes	1/1 (100%)	0.623*	--
	No	--		
Has vaccinated animals against brucellosis	Yes	-	1.000*	--
	No	1/1 (100%)		
Injured while administering this vaccine	Yes	-	1.000	--
	No	1/1 (100%)		
Assisted in dystocia/fetotomia	Yes	--	1.000*	--
	No	1/1 (100%)		
Is aware of the mode of transmission and methods to prevent infection with <i>Brucella</i>				
	Yes	1/1 (100%)	1.000*	--
	No	--		

P = probability; *Fischer's exact test; **chi-square corrected by Yates (comparison between 1 and 2 and 1 and 3) OR = odds Ratio; CI = confidence interval.

Table 5. Result of chi-square (P^2) testing of the association between common variables and the presence of *Toxoplasma gondii*, *Leptospira* or *Brucella* antibodies in 157 serum samples from students in a veterinary medicine course in a university located in the northwestern region in the state of Paraná, Brazil, 2014.

Disease Variable	Percent Positive (%)	P-value	OR (95% CI)
Use of personal protective equipment for clinical practice with domestic animals			
Yes	49/49 (100%)	0.353**	--
No	--		
Use of personal protective equipment for handling of biological materials (urine, feces, blood, semen)			
Yes	49/49 (100%)	0.353**	--
No	--		
Injured when collecting biological material			
Yes	10/49 (20.41%)	0.850**	0.925
No	39/49 (79.59%)		(0.414–2.069)
Performed extracurricular internship			
Yes	21/49 (42.86%)	0.387	0.743
No	28/49 (57.14%)		(0.378–1.459)

P = probability; *Fischer's exact test; **chi-square corrected by Yates (comparison between 1 and 2 and 1 and 3) OR = odds ratio; CI = confidence interval.

For brucellosis, two (1.27%, 2/157) samples were considered positive by the screening test. However, only one sample (0.63%, 1/157) was confirmed positive, presenting IgG and IgM levels of 20.1 and 18.5, respectively. This sample was from a male subject. No variable studied was considered statistically significantly associated with *Toxoplasma* infection (Tables 4 and 5). However, students with positive sera reported frequent ingestion of raw meat and fresh cheese.

Discussion

The general prevalence of *T. gondii*, *Leptospira*, or *Brucella* antibodies in the students was 31.21%, suggesting that these students were directly or indirectly exposed to *T. gondii*, *Leptospira* spp., or *Brucella* spp. at some stage in their lives.

The prevalence of *T. gondii* antibodies was 29.29%. This was similar to results by Araújo et al. (2000) (30.34%) and Lima (2008) (25.5%), who surveyed veterinary medicine students in Campo Grande (Mato Grosso do Sul) and western

Paraná (PR), respectively. This can be explained by the vastness of Brazil, with social-cultural and behavioral differences in each region and climate, which directly affect the survival and infectiousness of the etiological agent, and, consequently, directly impact the epidemiology of toxoplasmosis in each study location (BITTENCOURT et al., 2012; CAETANO, 2013; COUTO et al., 2003)

The antibody titers detected by IIF in six (13.04%) samples indicated that these infections were acute, because a dilution of greater than 1:1024 is generally considered evidence of a recent *T. gondii* infection. Goldsmith (1997) reported that IgM can be detected by IIF 1 or 2 weeks after infection, reaches a peak at 6 to 8 weeks, then declines.

T. gondii infection was not associated with any environmental, behavioral, or occupational variables in the survey. This result was similar to those found by Lima (2008) in western Paraná (PR) and Vicente et al. (2014) in Rio de Janeiro (RJ). Even though risk variables were not identified in this work, 82.60% of the reactors reported having contact with cats, 71.73% reported frequently

ingesting raw or undercooked meat, and 97.82% reported ingesting raw unsanitized vegetables. With these frequencies, it is clear that an awareness of the biological cycle of *T. gondii*, including temperature, humidity, and oxygenation, correct use of a cat litter box, sanitation of vegetables, and avoiding raw or undercooked meats are extremely important for the prevention of human infection.

Although leptospirosis is also considered an occupational disease (GONÇALVES et al., 2013), the prevalence of this infection in humans is largely unknown. Many cases of human leptospirosis are not reported, with the infection being frequently confused with colds and influenza (FAINE et al., 1999). Therefore, it is important to study professionals in certain occupations to understand its epidemiology.

The prevalence of *Leptospira* antibodies was of 1.27%. A higher prevalence (30.40%) was detected by Lima (2008) in western Paraná (PR), and a lower prevalence (0%) was reported by Langoni et al. (2009) in Botucatu (SP). These serological results might have been affected by the prevalence of *Leptospira* infections in animals in the respective locations and study periods. This low prevalence is similar to that reported in dogs and sheep in the same region as this study (DREER et al., 2013; OLIVEIRA, 2014). This can be explained by the fact that the northwestern region of the state of Paraná is located in a pedologic area, characterized by soil of medium to sandy texture (70%) sand composition, in a region known as Caiuá Sandstone), leading to greater water permeability that may have limited survival of the microorganism, because humidity is need for it to survive, grow, and multiply (EMBRAPA, 1999; FAINE et al., 1999; OLIVEIRA, 2014). However, by MAT, one sample showed an antibody titer of 1:800, which, according to the Ministry of Health, is evidence of an acute infection (BRASIL, 1995).

No environmental, behavioral, and occupational variables were found to be associated with *Leptospira*

infection. However, antibody against the Hardjo and Wolffi serovars in samples reactiveng to MAT suggests the possible involvement of the university students in an occupational relationship, since these two serovars are the ones most commonly detected in cattle, that are considered the main infection reservoirs (FAINE et al., 1999). Chronically infected and asymptomatic cattle can eliminate the bacteria through the urine for long periods of time, thus contributing to the persistence of infection focuses (GUIMARÃES et al., 1982). Therefore, the bovine species significantly contribute to the maintenance of infection in humans and in other animal species by direct exposure to urine, post-abortion uterine discharges, fetuses, placenta, uterine infections and/or infected semen (ELLIS, 1994).

Brucellosis is a disease of global distribution, endemic in several regions in Brazil; however, it presents very different prevalences due to the territorial dimension and the characteristics specific of each region (LAGE et al., 2008; RIBEIRO et al., 2008).

The prevalence of brucellosis in veterinary medicine students is little known, since there are few studies performed on university students. On the other hand, in the occupational character, Mendonça (1997) in the city of Garanhuns (Pernambuco) found 2.99% of individuals positive to *B. abortus* in workers from butcheries, cattle breeders and animal feeders. Higher results were found by Lacerda et al. (2000) in the northeastern region, where 10.17% seropositivity were observed in slaughterers in two slaughterhouses in the city of São Luis, Maranhão.

In this study, the prevalence of anti-*Brucella* spp antibodies in the university students was low, presenting a 0.63% rate, that is, only one sample was considered positive to this disease. This result is similar to the one found by Branco (2015), which detected a 0.86% prevalence in cows, but lower than the one in Dias et al. (2009) where the prevalence of bovine brucellosis was 1.70%, both in the northwestern region of the state of Paraná (PR).

The low prevalence detected in this study can be a reflection of the improvement of animal sanity in the bovine herd of the northwestern region in relation to the sanitary handling, as well as the awareness and adoption of practices demanded since 2001 by the National Plan for the Control and Eradication of Animal Brucellosis and Tuberculosis established by the Ministry of Agriculture, Livestock and Food Supply (MAPA) in Brazil.

Regarding antibody titers detected in ELISA, it is understood that the infection of the student is acute, since it presented IgM indexes above the cut-off point.

In this paper, it was not possible to associate the infection of university students with environmental, behavioral and occupational variables, although the student reported that he has the habit of ingesting raw or undercooked meat and fresh cheese in the epidemiological questionnaire. According to Paulin and Ferreira Neto (2008), eating habits is an indirect form of acquiring infection, such as: raw or undercooked animal products, raw milk or non-pasteurized dairy products (cheese, butter, yogurt, ice cream) originated from possibly infected animals.

The non-association of risk variables with the diseases in this study may have occurred due to the incomplete participation of the 260 students, or even by the low serological prevalences detected for the different diseases. A different result could have been found if all the students regularly enrolled in the course had taken part in this study, where only 1/3 of the students voluntarily took part. This study is an opportunity to systematize and discuss conceptual, educational and sanitary aspects of professionals who are exposed to different etiological agents, aiming at improving their attributions regarding the health of the professional x animal health, leading to the most modern concept of Unique Health currently being discussed by professionals in different areas in the world.

It is important to emphasize that from the 49 (31.21%) samples considered positive for the three

diseases studied, 26 (53.06%) were female students, which does not corroborate with Teixeira et al. (1998), who reported that the male gender is the most commonly infected, probably due to the highest exposure and professional/occupational activities they have, such as veterinarians, miners, farmers, slaughterhouse workers, public cleaning, animal handlers and fishermen, among other professions, since they are exposed to possibly infected animals or their byproducts (blood, urine, feces, placenta, tissues, mucus). This higher positivity related to the female gender may be due to change in the profile of freshmen in the Veterinary Medicine course.

Another important issue in this paper is that 100% of the students who took part in the survey reported using the PPEs (gloves, goggles, boots and overalls) during clinical service or the handling of biological material (blood, urine, feces, placenta, tissues, mucus), which could also have contributed to ensure the greater integrity of the students, animals and environment itself, since it found a low prevalence for toxoplasmosis, leptospirosis and brucellosis.

The results of this study indicate that there were 49 (31.21%) students in the veterinary medicine course previously exposed to *T. gondii*, *Leptospira* spp. or *Brucella* spp. at some moment in their lives. However, it was not possible to determine whether these infections were acquired during their time at the university, because we found no association between positive serum and risk variables. An awareness of these etiological agents and methods to prevent infection is important to maintain these low prevalence levels throughout the veterinary medicine course and during extra-curricular internships, when there is greater risk of exposure to various pathogens.

Additional studies on veterinary medicine students and other professionals should be conducted to better understand One Health (human and animal) in the northwestern region in the state of Paraná.

Ethics Committee

This project was submitted to the Ethics Committee on Research Involving Human Beings (CEPEH) at UNIPAR and Plataforma Brasil and was approved under protocol 443.790 on 01/Nov/2013.

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