

SEROEPIDEMIOLOGY AND OCCUPATIONAL AND ENVIRONMENTAL VARIABLES FOR LEPTOSPIROSIS, BRUCELLOSIS AND TOXOPLASMOSIS IN SLAUGHTERHOUSE WORKERS IN THE PARANÁ STATE, BRAZIL

Daniela Dib GONÇALVES(1), Paulo Sérgio TELES(3), Célia Rosimar dos REIS(1), Fabiana Maria Ruiz LOPES(1), Roberta Lemos FREIRE(2), Italmar Teodorico NAVARRO(2), Lucimara Aparecida ALVES(4), Ernest Ekehardt MULLER(2) & Julio Cesar de FREITAS(2)

SUMMARY

Leptospirosis, brucellosis and toxoplasmosis are widely-distributed zoonosis, being the man an accidental participant of their epidemiological chains. The aim of this paper was to make a seroepidemiological report and identify occupational and environmental variables related to these illnesses in 150 workers in a slaughterhouse in the Northern region of Paraná. For the diagnosis of leptospirosis a microscopical seroagglutination test was applied; for brucellosis, the tamponated acidified antigen test and the 2-mercaptoetanol tests were used, and for toxoplasmosis the indirect immunofluorescence reaction test. For each employee an epidemiological survey was filled, which investigated occupational and environmental variables which could be associated with these infections. Positive results for leptospirosis were found in 4.00% of the samples, for brucellosis in 0.66% of samples and toxoplasmosis in 70.00%. From the three diseases researched, only the results for leptospirosis suggest occupational infection.

KEYWORDS: *Leptospira* spp.; *Brucella* spp.; *Toxoplasma gondii*; Occupational diseases; Slaughterhouse workers.

INTRODUCTION

Occupational diseases are those which emerge through apparent or non-apparent signs and can be related, in some way, to activities executed by men in their working environment³.

Leptospirosis, brucellosis and toxoplasmosis are zoonosis of great importance to public health. These diseases affect many groups of workers which keep direct or indirect contact with animals, mainly without adequately using protection measures^{12,16,21}.

Leptospirosis has cosmopolitan distribution and epidemiological studies have shown a clear predominance of this infection in low-remuneration professions¹⁶. In slaughterhouse workers transmission occurs through contact with urine, blood and organs from infected animals^{6,16}.

Brucellosis is a disease which can be accidentally transmitted to men. It is widely distributed, having high morbidity and low mortality. In slaughterhouse workers, contact with infection sources can be represented by carcasses and viscera of slaughtered animals and by formation of aerosols present in the slaughtering area¹³.

Toxoplasmosis is also a widely distributed zoonosis which affects animals and men, being one of the parasitary infections of greatest

interest to public health²¹. In slaughterhouse workers, the handling of carcasses and viscera from contaminated animals represent risk of infection by *Toxoplasma*²¹.

The aim of this paper was to prepare a seroepidemiological survey for leptospirosis, brucellosis and toxoplasmosis and identify occupational and environmental variables related to these illnesses in 150 workers of a slaughterhouse in the Northern region of Paraná.

MATERIALS AND METHODS

Sample collection: Blood samples were collected voluntarily from all 150 workers of a slaughterhouse with Federal Inspection Service in the Northern region of Paraná State, Brazil, which slaughtered only bovines until April, 2003 and only swine from May, 2003 onwards. All the workers wore gloves, rubber boots, waterproof aprons, helmets and masks as protection equipment. The blood collection was made by professional nurses from the local Health Center between July and September, 2003. The serological exams were performed in the Leptospirosis Laboratory, Microbiology and Infectious Diseases Laboratory and Zoonosis and Public Health Laboratory of the Department of Preventive Veterinary Medicine (DMVP), Universidade Estadual de Londrina (UEL).

Research tool: To obtain the epidemiological information, each

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(1) Post-Graduation Program, Animal Science, Department at Preventive Veterinarian Medicine (DMVP), Universidade Estadual de Londrina (UEL), Paraná State, Brazil.

(2) Professor at Preventive Veterinarian Medicine Department (DMVP), UEL, Paraná State, Brazil.

(3) Veterinarian; Federal Inspection Service in the Northern region of Paraná State, Brazil.

(4) Biochemist of the Leptospirosis Laboratory, DMVP, UEL, Paraná State, Brazil.

Correspondence to: Prof. Dr. Julio Cesar de Freitas, Universidade Estadual de Londrina (UEL), Departamento de Medicina Veterinária, Preventiva, Centro de Ciências Agrárias, CP 6001, 86051-990 Londrina, Paraná, Brasil, Phone/fax: 55.43.3371 4485. E-mail: freitasj@uel.br

worker was interviewed in order to fill in a questionnaire including information on occupational and environmental variables related to the studied diseases (Tables 1 and 2 and Annex 1).

Laboratory exams: Each serum sample was divided in three aliquots with equal volume, packed in sterile containers and kept at -20 °C, for later serological analysis.

In order to detect antibodies against *Leptospira* spp., all sera were submitted to a microscopical seroagglutination test (SAM), using 22 reference serovars¹⁹. The sera presenting 2+ or more in agglutination in the dilution 1:100 were considered positive, these being diluted progressively until the determination of the maximum positive dilution.

To detect antibodies against *Brucella* spp., all the samples were submitted to a trial test using the tamponated acidified antigen test (AAT) and as confirmatory test, the 2-mercaptoetanol (2-ME)¹. The AAT test was considered positive when a macroscopic agglutination occurred and on 2-ME when there was the formation of a precipitation with film at the bottom of the tube and a clear supernatant. The sample was only considered positive when it reacted on both serologic tests.

To detect antibodies against *T. gondii*, the indirect immunofluorescence test (IFI) was performed⁴. All titers presenting completely fluorescent tachyzoite from titer 16 were considered as being positive.

Statistical analysis: The results obtained after the study of variables were submitted to a statistical analysis by the Chi-Square Test (χ^2) corrected by Yates or Fisher Test, using the statistics program Epi6 version 6.04 (CDC, Atlanta, USA), adopting a 95% trust interval⁹.

RESULTS

From 150 serum samples from slaughterhouse workers analyzed, six (4.00%) were considered positive for leptospirosis, one (0.66%) for brucellosis and 105 (70.00%) for toxoplasmosis. Four samples (66.67%) presented antibodies only against one *Leptospira* serovar and two samples (33.33%) against two serovars simultaneously. Antibodies against serovars Hardjo, Wolffi and Castellonis were found, with titers between 100 and 400. The positive serum samples were from three employees from the waxing sector, one from the slaughtering room, one who operated machines and one from the animal and packages loading sector. The analysis of the variables indicated the living in an urban zone ($p = 0.033$) as a risk factor for leptospirosis (Table 1). For brucellosis, only one sample (0.66%) was considered positive, presenting agglutination on the AAT test and titer of 100 on 2-ME. This sample belongs to a worker in the administrative sector. In relation to toxoplasmosis, 105 samples (70.00%) were considered positive to IFI, with titers varying from 16 to 4096. These samples were from 63 workers from the slaughter room, six from the inspection room, five from the cauldron, 27 from general services and four from the office. There were no statistically significant differences among the variables studied and the presence of *T. gondii* (Table 2).

DISCUSSION

The percentage of workers in the slaughterhouse with positive

Table 1

Results of Fisher test in relation to the variables analyzed in order to determine the risk factors for leptospirosis in 150 serum samples of workers in a slaughterhouse with Federal Inspection Service of the Northern region of Paraná

Variables	Positive samples / n (%)	p*
Sex		
Feminine	0/37 (0.00)	0.336
Masculine	6/113 (5.30)	
Living		
Urban zone	2/8 (25.00)	0.033
Peri-urban / rural zone	4/142 (2.81)	
Presence of pets at home		
Yes	4/110 (3.63)	0.657
No	2/40 (5.00)	
House with sewage system		
Public service	4/140 (2.85)	0.052
Septic tank	2/10 (20.00)	
Abandoned plot near the house		
Yes	3/72 (4.16)	1.000
No	3/78 (3.84)	
Presence of rodents at home		
Yes	3/88 (3.40)	0.691
No	3/62 (4.83)	
Direct contact with blood/organs in slaughterhouse		
Yes	4/111 (3.60)	0.650
No	2/39 (5.12)	
Suffered work-related accident		
Yes	3/86 (3.48)	0.700
No	3/64 (4.68)	
Habitude of eating during work		
Yes	5/145 (3.44)	0.186
No	1/5 (20.00)	
Habitude of washing hands before/ after eating during work		
Yes	6/140 (4.28)	1.000
No	0/10 (0.00)	
Contact with urine on inspection table		
Yes	2/40 (5.00)	0.657
No	4/110 (3.63)	
Previous work in farms		
Yes	6/107 (5.60)	0.182
No	0/43 (0.00)	
Helped animal births		
Yes	3/26 (11.53)	0.064
No	3/124 (2.41)	
Muscular pain		
Yes	3/96 (3.12)	0.667
No	3/54 (5.55)	

Fisher's exact test - probability

serological results for leptospirosis was of 4.00%. These results were higher than those found in Minas Gerais State, Brazil¹⁷, São Paulo State, Brazil⁷ and in Turkey² which described, respectively, 2.70%; 2.95% and

Table 2

Results from Chi-square test (χ^2) in relation to variables analyzed to determine risk factors for toxoplasmosis in 150 serum samples in workers of a slaughterhouse with Federal Inspection Service in the Northern region of Paraná

Variables	Positive samples / n (%)	χ^2	p
Sex			
Feminine	25/37 (67.56)	0.03	0.868
Masculine	80/113 (70.79)		
Living			
Urban zone	6/8 (75.00)	-	1.000*
Peri-urban / rural zone	99/142 (69.01)		
House with sewage system			
Public service	98/140 (70.00)	-	1.000*
Septic tank	7/10 (70.00)		
Presence of rodents at home			
Yes	60/88 (68.18)	0.16	0.690
No	45/62 (72.58)		
Presence of pets at home			
Yes	77/110 (70.00)	0.04	0.840
No	28/40 (70.00)		
Direct contact with blood/organs in slaughterhouse			
Yes	76/111 (68.46)	0.24	0.625
No	29/39 (74.35)		
Suffered work-related accident			
Yes	66/86 (76.74)	3.65	0.056
No	39/64 (60.93)		
Habitude of washing fruit/vegetables before consumption			
Yes	102/145 (70.34)	-	0.636*
No	3/5 (60.00)		
Habitude of handling soil			
Yes	75/109 (68.80)	0.10	0.749
No	30/41 (73.17)		
Habitude of ingesting raw /rare meat			
Yes	49/65 (75.38)	1.16	0.280
No	56/85 (65.88)		
Habitude of ingesting raw milk			
Yes	93/132 (70.45)	0.00	0.956
No	12/18 (66.66)		
Previous work in a farm			
Yes	74/107 (69.15)	0.02	0.874
No	31/43 (72.09)		
Muscular pain			
Yes	64/96 (66.66)	1.00	0.316
No	41/54 (76.34)		

p = probability; * = Fisher's exact test

1.96% positivity among the employees from the slaughterhouses studied. The different results obtained could have been influenced by the difference in the prevalence of animal leptospirosis in the respective regions and countries studied and could also reflect the periods studied, which leads to higher or lower probabilities of infection in workers who handle carcasses, organs and viscera from animals infected with different

serovars of *Leptospira*. Antibodies against serovars Hardjo, Wolffi and Castellonis with titers between 100 and 400 were found. Similar results in titers and antibodies against serovars were obtained in São Paulo State, Brazil⁶ and Colombia¹⁸. The serological results of this paper suggest these workers had already had contact with some *Leptospira* serovars and probably been infected for some time. The low serological titers found on the workers, associated with the antibodies detected against the serovars traditionally related to cattle, suggest chronic infections occurring when this slaughterhouse worked only with bovines. In relation to the different sectors of the slaughterhouse, from six positive employees in MAT, three (50.00%) worked in the waxing sector. These results are similar to those found in workers in the same sector in Minas Gerais State, Brazil and S. Paulo State, Brazil, also with 50.00% positivity²⁰. These results confirm this sector as having a high infection risk due to the exposure of organs and viscera of possibly infected animals. Despite leptospirosis being an occupational disease in slaughterhouse workers, the occupational variables analyzed were not significant. However, we should not discharge the possibility of this infection having occurred by other environmental and behavioral situations, once that, even though the variable living in urban zone (p = 0.033) was being considered as a risk factor, the infection could have occurred in the working environment, when the slaughterhouse still killed only bovines.

For brucellosis, one sample was considered positive (0.66%) on both serological tests. This employee worked in administration and did not have direct contact with the animals. However, he had the habit of ingesting non-pasteurized milk, which could indicate the probable source of infection. This low prevalence of human brucellosis among the slaughterhouse workers researched in this paper is probably related with the low prevalence (3.02%) of bovine brucellosis in the State of Paraná, Brazil¹⁰.

The 105 (70.00%) employees who were found positive for toxoplasmosis indicate that the infection by this microorganism is still in high levels when compared to others in the region studied. In Jaguapitã, Paraná State, Brazil¹⁴ the prevalence of human toxoplasmosis is of 66.00% seroreagents. In Londrina, Paraná State, Brazil¹¹ a positivity of 59.50% was detected in employees of a pork sausage factory and in Pato Branco, Paraná State, Brazil⁸ a positivity of 67.20% among workers of slaughterhouses. The titers of antibodies found by IFI in this paper varied from 16 to 4096, with 11 (10.48%) employees presenting titers of 4096, suggesting recent infection by *T. gondii*. The sector of the slaughterhouse which had most workers affected was the inspection room, with 75.00% positivity, followed by the slaughtering room, with 70.00%. However, there were no statistically significant differences among the sectors, suggesting the infection also occurred in an external environment, not only in the working environment. Despite toxoplasmosis being considered an occupational disease, the occupational variables analyzed in this study were of no significance. Among the three diseases studied in this work, it is possible that the infection of the workers by *Leptospira* spp. had occupationally occurred, despite the variable living in urban zone (p = 0.033) having been considered as a risk factor, while the infections by *Brucella* spp. and *T. gondii* occurred probably outside working environment.

ETHICS COMMITTEE: The present paper was approved by the Ethics in Research Committee of the Hospital Universitário Regional do Norte do Paraná, Decision CEP 142/03.

ANNEX 1

EPIDEMIOLOGY SURVEY: LEPTOSPIROSIS, BRUCELLOSIS AND TOXOPLASMOSIS IN SLAUGHTERHOUSE WORKERS

- **RESIDENCE:** rural area () urban area ()

- **EDUCATION:**
 - Elementary School complete () incomplete ()
 - High School complete () incomplete ()
 - University complete () incomplete ()

- **ANIMALS:**
 - Do you have pets at home? yes () no () canine () feline ()
 - Do the pets have contact with other animals? yes () no ()
 - Has the pet been sick? yes () no ()
 - If yes, what were the symptoms?.....
 - Does the pet have access to the house? yes () no ()
 - In case you have cats, where do they defecate? home () yard () litter box ()
 - Which of the following does the pet have contact with? vegetable gardens () flower gardens () garbage () vacant lots () flooded areas ()

- **WATER SUPPLY:**
 - Does the house have treated water? yes () no ()
 - Is the water tank covered? yes () no ()

- **SEWAGE:**
 - Destination? public sewage system () cesspit () river/streams () in the open ()

- **GARBAGE:**
 - Destination? public collection system () vacant lots ()
 - Garbage storage? plastic bags () open bins ()
 - Does the pet have access to the house and/or street garbage? yes () no ()

- **VACANT LOTS:**
 - Are there vacant lots next to the house? yes () no ()
 - Is there garbage in the lot? yes () no ()

- **FLOODED AREAS:**
 - Is there a stream next to the house? yes () no ()

- **RODENTS:**
 - Are rodents found in the house? yes () no ()
 - Are rodent prevention mechanisms being used? yes () no ()

· SLAUGHTERHOUSE WORK:

- Sector?.....
- How long have you worked in this sector? 1 year () 3 years () 5 years () 10 years () more than 10 years ()
- Do you have any contact with animal organs, muscles, blood? yes () no ()
- Which sector did you work for before?.....
- How long did you work in this sector? 1 year () 3 years () 5 years () 10 years () more than 10 years ()
- Did you receive any training before you started working? yes () no ()
- Do you wear? gloves () rubber boots () waterproof aprons () masks () protection glasses ()
- Have you had any work-related accident? yes () no ()
- What happened?
- Where did you work before you started working for the slaughterhouse?.....
- How long did you work there?.....
- Do you smoke? yes () no ()
- Do you smoke during your work breaks? yes () no ()
- Do you wash your hands before and after you smoke? yes () no ()
- Do you eat during working hours? yes () no ()
- Do you eat during your work breaks? yes () no ()
- Do you wash your hands before and after you eat? yes () no ()
- Do you have any contact with urine in the inspection table? yes () no ()

· BEHAVIORAL HABITUDES:

- Do you fish or bathe in rivers? lakes () dams () dikes ()
- Do you carry out activities related to soil or sand manipulation? yes () no ()
- Do you keep animal food at home? yes () no ()
- Is the animal food kept in the open? yes () no ()
- Do you usually wash fruits and vegetables before you eat them? yes () no ()
- Eating habitudes: raw/rare meat () home made cheese () non-pasteurized milk ()
- Have you had any blood transfusion? yes () no ()
- Have you ever worked on farms or small rural properties? yes () no ()
- If yes, for how long?.....
- Do you own a place in the country or a small rural property? yes () no ()
- If yes, do you raise animals in the property? yes () no ()
- If yes, which animals do you raise? bovine () caprine () swine () poultry ()
- Have you ever participated in vaccinations ? yes () no ()
- Have you ever had any infectious disease (hepatitis, leptospirosis, toxoplasmosis, brucellosis)? yes () no ()
- Did you have any cold symptoms? a week ago () 15 days ago () a month ago () 3 months ago ()
6 months ago () more than 6 months ago ()
- If yes, which of these symptoms did you have? fever () muscle pain () headache () ? weight loss () bleeding ()
dry cough () diarrhea () sweating () shivering () insomnia () calf pain () arthralgia ()
- Do you have any children with some kind of deficiency? yes () no () physical () mental ()
- Are you familiar with the diseases that can be transmitted by animals? yes () no ()
Which ones?.....

RESUMO

Soroepidemiologia e variáveis ocupacionais e ambientais relacionadas à leptospirose, brucelose e toxoplasmose em trabalhadores de frigorífico do Estado do Paraná, Brasil

A leptospirose, brucelose e a toxoplasmose são zoonoses de ampla distribuição, sendo o homem participante acidental das suas cadeias epidemiológicas. O objetivo deste trabalho foi realizar levantamento soroepidemiológico e identificar variáveis ocupacionais e ambientais relacionadas a estas enfermidades em 150 trabalhadores de um frigorífico da região Norte do Paraná. Para o diagnóstico de leptospirose foi realizada a prova de soroaglutinação microscópica; para a brucelose, a prova do antígeno acidificado tamponado e do 2-mercaptoetanol e para toxoplasmose, a reação de imunofluorescência indireta. Para cada funcionário foi preenchido um questionário epidemiológico que investigou variáveis ocupacionais e ambientais que poderiam estar associadas a estas infecções. Resultados positivos para leptospirose foram encontrados em 4,00% das amostras, para brucelose em 0,66% das amostras e para toxoplasmose 70,00%. Das três enfermidades pesquisadas, somente os resultados obtidos para leptospirose sugerem infecção de modo ocupacional.

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