

Seroprevalence of *Toxoplasma gondii* and associated risk factors in operators of a slaughter plant in Bío-Bío (Chile)

Ignacio Eduardo Troncoso Toro¹ / Karen Constanza Arrué Brenet² / Natalia Angélica Soto Alvear³ / Adela Antonieta Valenzuela Contreras⁴ / Álvaro Fabrizzio Luzio Quiroga⁵ / Christof Fischer Wiethuchter⁶ / Fabiola Andrea Rivas Betancour⁷

Abstract

Toxoplasmosis is a worldwide zoonosis caused by *Toxoplasma gondii*, a protozoan whose definitive hosts are cats, among them domestic cat, which can transmit the infection to humans. In Chile, there are no published studies on seroprevalence in people with occupational risk. Thus, this study aimed to determine the seroprevalence of *Toxoplasma gondii* and associated risk factors in operators of a slaughter plant in Bío Bío (Chile). Serum samples from 39 operators were collected and studied by chemiluminescence analysis in order to detect IgG and IgM antibodies, with a sensitivity and specificity of 93 and 96%, respectively. An epidemiological survey was conducted and odds ratio was calculated for the analysis of the variables of hygiene, food, and exposure. Evidence showed that 24 individuals had IgG antibodies for an apparent seroprevalence of 61.5%, while this was 0% for IgM. In addition, the highest seropositivity was observed in operators who did not use masks (64%) and did not disinfect the working material (100%), as well as in those who consumed undercooked meat (62.5%). Regarding exposure time, 72.7% was obtained for the group of more than 10 years, and 62.2% of seropositivity was found in those exposed between four and seven days a week. There were no significant differences for any of the analyzed variables ($p > 0.05$). The study concludes that there is a high seroprevalence of *Toxoplasma gondii* in workers with occupational risk at the Bío-Bío slaughter plant.

Keywords: immunoanalysis, operators, occupational risk, seroprevalence, toxoplasmosis.

- 1 Veterinarian, Universidad Santo Tomás, School of Veterinary Medicine, Concepción, Chile.
✉ ignaciotroncoso@santotomas.cl
- 2 Veterinarian, Animal Lover Veterinary Clinic, San Carlos, Chile.
✉ kabvet@gmail.com
- 3 Veterinarian, Universidad Santo Tomás, School of Veterinary Medicine, Concepción, Chile.
✉ nataliasotoalvear@gmail.com
- 4 Veterinarian, Universidad Santo Tomás, School of Veterinary Medicine, Concepción, Chile.
✉ adela.medvet@gmail.com
- 5 Veterinarian, MSc., Universidad Santo Tomás, School of Veterinary Medicine, Concepción, Chile.
✉ aluzio@santotomas.cl
- 6 M, Ph.D., Universidad Santo Tomás, School of Veterinary Medicine, Concepción, Chile.
✉ cfischer@santotomas.cl
- 7 Veterinarian, Clínica Veterinaria Iquique, Chile.
✉ mvvet.fabiola.rb@gmail.com

Seroprevalencia de *Toxoplasma gondii* y factores de riesgo asociados en operarios de una planta de beneficio animal del Bío-Bío, Chile

Resumen

La toxoplasmosis es una zoonosis de distribución mundial causada por *Toxoplasma gondii*, protozoo que tiene como hospederos definitivos a los felinos, entre estos el gato doméstico, el cual puede transmitir la infección al ser humano. En Chile no existen estudios publicados de seroprevalencia en personas con riesgo ocupacional. Por eso el presente estudio tuvo como objetivo determinar la seroprevalencia de *Toxoplasma gondii* y factores de riesgo asociados en operarios de una planta de beneficio animal del Bío-Bío, Chile. Se muestrearon 39 sueros de operarios y se estudiaron mediante análisis quimioluminiscente para la

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detección de anticuerpos inmunoglobulina G (IgG) e inmunoglobulina M (IgM), con una sensibilidad y especificidad del 93 y 96 %, respectivamente. Se aplicó una encuesta epidemiológica y se calculó el *odds ratio* para el análisis de las variables higiénicas, alimenticias y de exposición. Se evidenciaron 24 personas con anticuerpos IgG para una seroprevalencia aparente de 61,5 %, mientras que para IgM esta fue del 0 %. Se determinó además la seropositividad más alta en los operarios que no usaron mascarillas (64 %) y no desinfectaron el material de trabajo (100 %), así como en aquellos que consumieron carne poco cocida (62,5 %). Respecto al tiempo de exposición, se obtuvo un 72,7 % para el grupo mayor de 10 años y 62,2 % de seropositivos expuestos entre cuatro y siete días a la semana. No existieron diferencias significativas para ninguna de las variables analizadas ($p > 0,05$). Se concluye que existe una seroprevalencia elevada de *Toxoplasma gondii* en operarios con riesgo ocupacional en la planta de beneficio animal del Bío-Bío.

Palabras clave: inmunoanálisis, operarios, riesgo ocupacional, seroprevalencia, toxoplasmosis.

Soro prevalência de *Toxoplasma gondii* e fatores de associados em operários de uma planta de beneficiamento animal de Bío Bío, no Chile

Resumo

A toxoplasmose é uma zoonose de distribuição mundial causada por *Toxoplasma gondii*, protozoário que tem como hospedeiros definitivos os felinos, entre estes o gato doméstico, que pode transmitir a infecção ao ser humano. No Chile não existem estudos publicados de soro prevalência em pessoas com risco ocupacional. Por isso o objetivo deste estudo foi determinar a soro prevalência de *Toxoplasma gondii* e fatores de risco associados em operários de uma planta de beneficiamento animal de Bío Bío, Chile. Foram coletadas amostras de 39 soros de operários estudados por meio de análise quimioluminescente para a detecção de anticorpos IgG e IgM, com uma sensibilidade e especificidade do 93 e 96 %, respectivamente. Se aplicou uma enquete epidemiológica e se calculou o *odds ratio* para a análise das variáveis higiénicas, alimentícias e de exposição. Se evidenciaram 24 pessoas com anticorpos IgG para uma soro prevalência aparente do 61,5 %, em quanto que para IgM esta foi do 0 %. Pôde-se determinar além do mais, a soro positividade mais alta nos operários que não usaram máscaras (64 %) e não desinfectaram o material de trabalho (100 %), assim como naqueles que consumiram carne pouco cozida (62,5 %). No que se refere ao tempo de exposição, se obteve um 72,7 % para o grupo maior de 10 anos e 62,2 % de soro positivos expostos entre quatro e sete dias por semana. Não existiram diferenças significativas para nenhuma das variáveis analisadas ($p > 0,05$). Conclui-se que existe uma soro prevalência elevada de *Toxoplasma gondii* em operários com risco ocupacional na planta de beneficiamento animal de Bío Bío.

Palavras chave: imuno-análise, operários, risco ocupacional, soro prevalência, toxoplasmose.

INTRODUCTION

Diseases or infections that are transmitted from animals to humans under natural conditions are called *zoonosis* (1). An example of this is toxoplasmosis, a disease of worldwide distribution caused by *Toxoplasma gondii* (*T. gondii*), an obligate intracellular protozoan parasite which owes its name to *Ctenodactylus gundi*, a rodent in North Africa, where it was first isolated in 1908 (2).

The definitive hosts of this disease are cats, and among them the domestic cat (*Felis catus*), which has an important influence on the transmission of the disease to humans, being a preferred animal of company. These cats shed oocysts in their feces, which then, under favorable environmental conditions, sporulate and become infective for a wide variety of mammals and birds that can participate as intermediate hosts in the biological cycle (3). The most common ways of transmission are consumption of meat contaminated with tissue cysts; improper handling of the same; intake of food contaminated with feline feces, and vertical-transplacental transmission. Other most common mechanisms are transfusion, transplants of tissues and organs, via water, mechanical transmission by arthropods and insects, and via sex (4).

In the global context, prevalence in humans is highly variable; it depends on the hygienic conditions, climate, and customs of each region or country. It is estimated that at least half of the world's population has been exposed to this infection, but without developing the disease. Thus, prevalence reaches 28.6% at the global level, and in Chile, it is 36.2% (5). The most important risk groups are pregnant women who previously have not been exposed to infection with *T. gondii* and individuals with a compromised immune system. Similarly, there is an elevated percentage of children (48%) with high susceptibility to infection between 6 and 14 years old (6).

Studies carried out in Colombia show that there is a high seroprevalence (71.8%) of anti-*T. gondii* immunoglobulin G (IgG) antibodies in slaughter plant workers (7). In

Chile, more specifically in the region of Bío-Bío, there are no published studies that demonstrate the presence of antibodies in this group of people. Therefore, the objective was to determine the seroprevalence of *Toxoplasma gondii* and associated risk factors in operators of a slaughter plant in Bío-Bío, Chile.

MATERIALS AND METHODS

Study site

The study was conducted in the city of Chillán, which is located 110 km north of Concepción, eighth region of Bío-Bío, Chile, specifically in a slaughter plant located on the outskirts of the city.

Sample size

A sampling of 39 individuals was carried out (two veterinarians and 37 operators of the slaughter and deboning areas), who voluntarily agreed to participate in the investigation. This procedure was authorized by signing a letter of consent and confidentiality of the results.

Sampling procedure

A blood sample of 4 ml without anticoagulant was collected from each individuals, obtained by venipuncture of the cephalic vein. Serum was obtained by centrifugation at 2000 rpm for 10 min; it was identified and stored at -20 °C until serological analysis.

Diagnostic method

Chemiluminescence immunoassay was used, which is a quantitative serological method for in vitro diagnosis with Immulite and Immulite 1000 analyzers for the measurement of anti-*T. gondii* IgG and IgM antibodies. The test has a sensitivity of 93% and a specificity of 96% (8). This result is greater or equal to 8 IU/ml, indicative of infection.

Distribution of variables

Parallel to blood sampling, a survey was applied to the workers under study, by which relevant data were obtained for the epidemiological investigation of risks, such as time of exposure to raw meat in days (3 to 7 days) and years (1, 3, 5, 10, and more than 10 years); hygienic habits such as the use of masks or disinfection of the working material, as well as alimentary habits such as consumption of undercooked meat and hand washing both inside and outside of the work establishment.

Statistical Analysis

Being a descriptive study, absolute and relative frequencies were calculated. In order to determine whether there were significant differences between the variables of alimentary habits, hygiene, and time of exposure, as well as their possible association, Fisher's exact test was applied,

which is very useful for the analysis of small samples. In addition, odds ratio (OR) was calculated as the quotient between the number of times the event occurs and the number of times it does not occur (magnitude of association between seropositivity and the presence of risk factors) (9), only for the alimentary and hygienic variables. Statistical significance was considered as $p < 0.05$ in the analysis of the results. All analyses were conducted using the InfoStat statistical program (version 2010).

RESULTS

Out of a total of 39 samples analyzed by chemiluminescence technique, 24 were found positive for *T. gondii* (IgG), which represents a seroprevalence of 61.5%, while all samples were negative for IgM, with a prevalence of 0% (Table 1).

Table 1. Absolute and relative frequencies of anti-*Toxoplasma gondii* antibodies in operators with risk exposure variable in a slaughter plant in Bío-Bío, Chile

Variables	Total (n=39)	Positive (n=24)	%	p-value
Hygienic				
<i>Mask</i>				
Used	14	8	57.1	0.73
Not used	25	16	64.0	
<i>Disinfection of material</i>				
Apply	34	19	55.9	0.13
Does not apply	5	5	100.0	
Alimentary				
<i>Consumption of undercooked meat</i>				
Apply	16	10	62.5	0.81
Does not apply	23	14	60.9	
<i>Hand washing</i>				
Apply	39	24	61.5	
Exposure				
<i>Days in a week</i>				
Up to 3	2	1	50.0	0.74
4 to 7	37	23	62.2	

Variables	Total (n=39)	Positive (n=24)	%	p-value
<i>Years of work</i>				
1	12	7	58.3	0.85
3	9	6	66.7	
5	6	3	50.0	
10	1	0	0.0	
>10	11	8	72.7	

Regarding hygienic habits, of the 39 individuals surveyed, 57.1% of those who used masks were found positive; this value is exceeded by 64% of the seropositive operators who did not use this method of protection (Table 1). OR was 0.75, indicating that the non-use of masks is actually a protective factor (OR < 1) and not a risk factor (OR > 1).

As for disinfecting the working material, 55.9% of those who used this practice were found seropositive, while the five individuals who do not normally use this preventive measure were all positive (Table 1). However, OR was 0.11, which indicates that this variable is not a risk factor. On the other hand, regarding the alimentary variables, consumption of undercooked meat was a definite risk factor (OR = 1.07), as 62.5% of those individuals who consumed this source of contamination were positive, although 60.9% of those who did not consume it were also positive (Table 1).

Of the total respondents, 100% (39 individuals) mentioned that they performed hand washing as a standard procedure before eating; however, 61.5% of them were found positive to *T. gondii* in the serological evaluation (Table 1). Finally, of the 39 individuals surveyed, 12 have been working at the slaughter plant for one year (30.7%), and seven of them were found positive (58.3%). Of the nine operators who have worked there for three years, six resulted seropositive (66.7%). Similarly, three workers with five years of employment were found positive (50%), while 72.7% of the operators who have worked at the plant for more than 10 years resulted seropositive (Table 1).

Analyzing the results of the exposure to raw meat variable it was evident that this is the risk factor that had the greatest magnitude of association with positive results to *T. gondii* (OR = 1.64). In this sense, it was demonstrated in Table 1 that 62.2% of the workers exposed during four to seven days a week resulted positive, while only one of the two individuals with exposure of up to three days to this potential source of infection was seropositive.

DISCUSSION

The value of seroprevalence found in this study was much higher than that described by the last national survey conducted in the city of Osorno by Zamorano et al. in 1999, who found a seropositivity that ranged between 19 and 23%, using the indirect hemagglutination technique, in a population of 305 individuals without occupational risks, 160 of which (52.5%) were blood donors and 145 (47.5%) had some sexually transmitted disease (10).

Regarding seroprevalence values found in populations with occupational risks, there is only one unpublished study in the national context, conducted by Poblete in 2013, who, using the same technique, analyzed 40 serum samples from veterinarians of the colonies of Concepción and Chillán, and obtained a prevalence of 10% (11). While both studies used the same technique, the study population was different, as Poblete analyzed sera from professionals who worked at minor animal clinics in these colonies, unlike the present study, in which the selected population was from a slaughter plant where only two (5.1%) of the individuals were veterinarians.

According to studies conducted in Latin America, this seroprevalence of 61.5% is consistent with that obtained by Alfaro, Barraza, and Mestra in 2000, who found a prevalence of 68.39% in 82 meat handlers from a municipal plant in Florida Blanca, Colombia, using the indirect hemagglutination technique (12). While in 2004 Daguer et al. studied the sera of 64 workers from four plants in the region of Paraná (Brazil), including slaughter plant operators, veterinarians, and health inspection technicians, using two techniques: indirect immunofluorescence (IIF) and solid-phase immunosorbent assay (ELISA), by which they obtained prevalences of 67.2 and 84.4%, respectively (13). Both studies recorded seroprevalence values greater than 60%, which rectifies the sensitivity and specificity of the techniques used (IIF = 95 and 100%; ELISA = 97 and 100%, sensitivity and specificity, respectively). Similarly to this study, no positivity for *anti-T. gondii* IgM antibody was found.

Similar findings were demonstrated in the study carried out in Colombia by Montealegre et al. in 2009, in five meat processing plants, in a total of 400 workers in contact with animals and meat products. Samples were analyzed using ELISA, and a seroprevalence of 71.8% was obtained (7). This value does not differ significantly from the value found in the present study, if we consider the difference in population size and the technique used with sensitivity and specificity values of 90%.

One of the latest studies published on the subject is a work by Alvarado-Esquivel et al. in 2011, carried out in Durango (Mexico), in a population of 124 workers exposed to raw meat for at least six months, either in slaughter plants or in butcher's shops. Samples were analyzed using ELISA; low seroprevalence (7%) was found in comparison with the value obtained in the present study (14).

With respect to the percentages for hygienic variables, for the use of masks, the obtained values differ from those published by Alvarado-Esquivel et al. in 2011, in which of 58 individuals who used masks only three were positive (5%), while of the remaining 59 who did not use them only five resulted seropositive (8%) (14).

A similar tendency was observed in the present work, although prevalence values for use and non-use of masks were higher (57.1 and 64%, respectively). The other variables present in this study regarding hygienic habits cannot be compared, since there are no national and international publications to support it.

In 2004, Daguer et al. found a positivity of 84.1% in 37 individuals who consumed raw or undercooked meat, while 17 individuals (85%) who did not consume this source of contamination with tissue cysts of *T. gondii* resulted seropositive (13). On the other hand, in 2009 Montealegre et al. found that 93 individuals (23.3%) ate raw meat, while 307 (76.8%) did not consume meat prepared in this way, but rather undercooked. The results of both studies contrast with the findings of this investigation, which found 12.8 and 87.2% for consumption and non-consumption of raw meat, respectively (7), indicating that there is no significant difference between the groups studied.

The study by Alvarado-Esquivel et al. in 2011 used a very similar survey to the one applied in this research. They found a prevalence of 0% for individuals exposed to raw meat for less than a year; 9% for individuals exposed between one and five years, and a prevalence of 7% for those exposed for more than five years (14). The same study reviewed the exposure to meat during the working week, and it showed a prevalence of 0% in individuals exposed up to three days; this contrasts with the results of the present study, which found a seroprevalence of 50% for the same range. On the other hand, the prevalence for the range of four to seven days was 7%, which in the present study was 62.2% (14). According to the results obtained in the present study, the prevalence for an exposure of up to one year was 58.3%; for three years, 66.7%; for five years, 50%, and for more than 10 years, 72.7%.

One of the explanations for the low prevalence obtained in the study by Alvarado-Esquivel et al. was that most of the individuals who participated in the research came from butcher's shops where they only handled frozen meat, which is an effective measure to eliminate the presence of *T. gondii* tissue cysts in infected tissues. This situ-

ation is totally opposite to that described in the present study (14).

It should be noted that the study population in Mexico was workers of a meat processing plant and butchers exposed to raw meat, unlike the present study, which was conducted only with personnel working at a slaughter plant, where there existed a direct or indirect contact with raw meat.

CONCLUSIONS

It is concluded that there is a high seroprevalence of *T. gondii* in operators with occupational risks in the slaughter plant of Bío-Bío in Chile.

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