SEROPREVALENCE OF *Toxoplasma gondii* AND *Neospora caninum* IN SHEEP IN URUGUAY

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ABSTRACT: *The objective of this study was to estimate the true prevalence of seropositive individual sheep against Toxoplasma gondii and Neospora caninum in Uruguay using the Rogan–Gladen estimator in combination with Monte Carlo simulation. Ten herds were kept under observation in 2008. Each study herd was randomly selected at different farms recruited from the Departments of Artigas, Salto and Canelones. The required total sample size was determined by power analysis, and blood samples collected were analysed using a commercial ELISA for the detection of antibody to the two pathogens mentioned above. The overall seroprevalence of Toxoplasma gondii and Neospora caninum were estimated at 38.9% (5th percentile of 36.5%; 95th perc. of 41.4%; N = 1361) and 0.7% (5th percentile of 0.1%; 95th perc. of 1.4%; N = 1357), respectively. Establishing prevalence distributions using Monte Carlo simulation could be useful for further risk assessment.*

Key Words: epidemiology, ovine, South America

SEROPREVALENCIA DE *Toxoplasma gondii* Y *Neospora caninum* EN OVINOS DE URUGUAY

RESUMEN: *El objetivo del presente estudio fue estimar la prevalencia verdadera de ovejas seropositivas a Toxoplasma gondii y Neospora caninum en Uruguay, mediante el uso del estimador Rogan–Gladen en combinación con la simulación Monte Carlo. Diez rebaños fueron observados durante el año 2008. Cada rebaño bajo estudio fue seleccionado al azar de establecimientos pertenecientes a los Departamentos de Artigas, Salto y Canelones. El tamaño de muestra fue determinado por el poder estadístico del análisis. Las muestras de sangre fueron analizadas mediante un ELISA comercial para la detección de anticuerpos contra los dos parásitos mencionados anteriormente. La seroprevalencia global para Toxoplasma gondii y Neospora caninum fue estimada en 38.9% (36.5% para el percentil 5; 41.4% para el percentil 95; N = 1361) y 0.7% (0.1% para el percentil 5; 1.4% para el percentil 95; N = 1357), respectivamente. Establecer la distribución de prevalencia mediante la simulación de Monte Carlo podría ser de utilidad para futuros análisis de riesgo.*

Palabras clave: epidemiología, ovino, Sudamérica.

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INTRODUCTION

The quality of sheep farming in Uruguay is relatively high and the close interaction between producers, the extension services and the veterinary services acts a significant role in maintaining this condition. There is a growing concern that low reproductive performance in ovine populations may progressively obstruct the industry. This is normally recognised as the single most significant problem associated with the increasing numbers of sheep and concomitant increase in stocking rate (1).

Toxoplasma gondii is a protozoan parasite which infects humans as well as other warm-blooded animals including sheep. If ewes become infected with *Toxoplasma gondii*, they do not usually appear sick. But if they contract the disease early in gestation, resorption or mummification results; if late in gestation, abortions or perinatal deaths occur (2).

Neospora caninum is another protozoan parasite which has been confused previously with *Toxoplasma gondii*. While an early record of *Neospora caninum* congenital infection in sheep dates back earlier, *Neospora caninum* has been recently shown to be a cause of abortions of sheep (3).

Prevalence is a scale of animal disease frequency that concentrates on existing status rather than new events. Diagnostic tests are regularly used for prevalence studies and, preferably, true prevalence (TP) should be estimated from apparent prevalence or percentage of samples classified as test–positive (Pos) by adjusting for test sensitivity (Se) and specificity (Sp) (4). The objective of this study was to estimate the TP of seropositive individual sheep against *Toxoplasma gondii* and *Neospora caninum* in Uruguay using the Rogan–Gladen estimator in combination with Monte Carlo simulation.

MATERIALS AND METHODS

STUDY AREA

Uruguay is located in the south–eastern part of South America bordering the South Atlantic Ocean, between Argentina in the west and Brazil in the northeast. Seventy-seven percent of the total land area of 175,020 km^2 is occupied by permanent pasture that is suitable for livestock production (5). Beef cattle (11 million animals), dairy cattle (0.7 million animals) and sheep (10 million animals) compose the majority of the agricultural economic product. Livestock accounts for three quarters of agricultural GDP (6). Uruguay has a sheep meat production of 27,000 tonnes per year and a sheepskin production of 9,800 tonnes per year (7). The north side of the country (Artigas and Salto Departments) had the concentration of sheep population (about 31 % of the total population) (5,7). These two departments and Canelones Department north to the capital city Montevideo were selected as the study area.

SAMPLE COLLECTION

Ten herds of sheep were included in the study. Each study herd was randomly selected at different farms recruited from the Artigas (no. of the study herds = 1), Salto (no. of the study herds = 8) and Canelones (no. of the study herds = 1) Departments. To our knowledge, nothing has been reported on seroprevalence of a *Toxoplasma gondii* / *Neospora caninum* infection for at least a year. None of the sheep had been vaccinated against *Toxoplasma gondii* / *Neospora caninum* prior to sampling. The required total sample size of 1067 from a sheep population of 10 million was sufficient to produce a 95 % confidence interval with a desired precision of ±3 % when the percentage of samples classified as test–positive was 50 % (8). The sample size in each of the herds was proportionally allocated (10 % each of the total number of sheep at study herds) by the accessible financial, human and material means. The field investigation was conducted in 2008, consisted of data collection through questionnaire interviews for each farm selected, in conjunction with blood sample collections for each sheep (questionnaire results were not dealt with hereinafter).

LABORATORY EXAMINATIONS

Blood samples collected were used for diagnostic investigations. Sera were analysed using a commercial ELISA for the detection of antibody to *Toxoplasma gondii* and *Neospora caninum* in sheep serum (CHEKIT® *Toxoplasma gondii* Antibody Test Kit and *Neospora caninum* Antibody Test Kit, IDEXX Laboratories, Liebefeld–Bern, Switzerland). Positive and negative controls were included for each series of samples analysed. Absorbance was read on an ELISA reader at 450 nm. On the basis of the instruction manual of the ELISA kits, each serum sample was determined as positive, suspect or negative.

DATA ANALYSIS

Data were entered into a database using the spreadsheet software Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA). Seroprevalence estimates based on the use of an imperfect test, which is a nature of ELISA tests, must be corrected to take account of test performance. The calculations per study herd were done with the following distributions for Pos, Se and Sp:

$Pos \sim Beta (d_h + 1, n_h - d_h + 1)$,

$Se \sim Beta (d + 1, n - d + 1)$,

$Sp \sim Beta (d + 1, n - d + 1)$

where d = the number of desired (positive or negative) outcomes, n = the number of samples

tested per study herd, and the subscript 'h' indicates a herd-specific value. Based on the published ELISA sensitivity (93.4%) and specificity (100 %) values for *Toxoplasma gondii* (2), and the published ELISA sensitivity (96.6%) and specificity (99.5 %) values for *Neospora caninum* (9), estimated TP of antibodies among study sheep at each herd were calculated. TPs for each herd were derived from the percentage of samples classified as test–positive using the Rogan–Gladen estimator (10) and information about the Se and Sp:

$$TP = (Pos + Sp - 1) / (Se + Sp - 1)$$

For estimation of TP above, the Microsoft Excel 2007 in combination with the Monte Carlo simulation add-in software ModelRisk Standard version 4.0.0.2 (Vose Software, Gent, Belgium) with 10,000 iterations were used.

RESULTS

The 1361 (for testing *Toxoplasma gondii*) and 1357 (for testing *Neospora caninum*) sheep studied accounted for about 7.9 % of the study sheep population and 0.01 % of the total sheep population in Uruguay then. Of all, 0.5 % (95 % CI: 0.2–1.1 %) of the samples had test–positive against both *Toxoplasma gondii* and *Neospora caninum*. The proportion of all for test–negative against both the two disease pathogens was 63.2 % (95 % CI: 60.5–65.7 %). Table 1 displays the estimated seroprevalence against *Toxoplasma gondii* among the study sheep categorised by

Table 1. Estimated seroprevalence against *Toxoplasma gondii* among sheep in Uruguay
Tabla 1. Seroprevalencia estimada en ovejas de Uruguay contra *Toxoplasma gondii*

Herd ID	No. of sheep sampled	% of test–positive	True seroprevalence (%)	Percentiles (%)	
				5th	95th
A	245	46.9	50.3	44.6	56.0
B	55	14.5	16.8	9.1	26.1
C	43	11.6	14.1	6.3	23.7
D	100	15.0	16.7	10.8	23.5
E	29	34.5	37.9	23.1	53.5
F	368	56.0	59.9	55.2	64.6
G	84	34.5	37.3	28.4	46.6
H	82	54.9	58.7	49.1	68.1
I	49	53.1	56.9	44.7	69.2
J	306	11.8	12.7	9.6	16.2
Total	1361	36.4	38.9	36.5	41.4

Table 2. Estimated seroprevalence against *Neospora caninum* among sheep in Uruguay
Tabla 2. Seroprevalencia estimada en ovejas de Uruguay contra *Neospora caninum*

Herd ID	No. of sheep sampled	% of test–positive	True seroprevalence (%)	Percentiles (%)		% of simulated data removed*
				5th	95th	
A	245	5.7	5.7	3.1	8.6	0
B	54	0	1.9	0.1	5.5	31
C	43	0	2.3	0.1	6.9	25
D	98	0	1.0	0.1	3.1	47
E	28	0	3.5	0.2	10.3	18
F	368	0	0.3	0.0	0.9	86
G	82	1.2	2.1	0.2	5.4	13
H	81	1.2	2.1	0.2	5.5	12
I	50	0	2.0	0.1	5.9	29
J	308	0	0.3	0.0	1.0	81
Total	1357	1.2	0.7	0.1	1.4	13

*Data less than zero were not included for the estimation of true seroprevalence.

herds. The numbers of sheep sampled between the 10 study herds were varied from 29 to 368. All the 10 herds had percentage of samples classified as test–positive of greater than 11%, between 11.6 % and 56.0 %. All the point estimates of TP were greater than 12 %, between 12.7 % and 59.9 %. All the point estimates of TP in each herd were consistently greater than the percentages of test–positive samples. All the values for the percentage of test–positive were well within the interval of 5th and 95th percentiles of the estimated TPs. The simulating means for the Se and Sp, estimated from the study, were 93.2 % (5th percentile of 91.4 %; 95th percentile of 94.8 %) and 99.8 % (5th percentile of 99.5 %; 95th percentile of 99.9 %), respectively. Table 2 shows the estimated seroprevalence against *Neospora caninum* among the study sheep categorised by herds. The numbers of sheep sampled between the 10 study herds were varied from 28 to 368 (similar to Table 1). Only three out of the 10 herds had percentage of test–positive of greater than 0 %, between 1.2 % and 5.7 %. All the point estimates of TP were greater than 0 %, between 0.3 % and 5.7 %. All the percentages of test–positive in each herd were adjusted greater by Monte Carlo simulations. The simulating means for the Se and Sp, estimated from the study, were 95.7% (5th percentile of 91.7%; 95th percentile of 98.5%) and 99.3 % (5th percentile of 98.5 %; 95th percentile of 99.8 %), respectively. Out of the 10,000 simulations for the current *Neospora caninum* seroprevalence study, 12 to 86 % of the data (a value of < 0) for each study herd except Herd A were removed prior to estimating the TPs. The respective statistical precisions for *Toxoplasma gondii* and *Neospora caninum* were improved from ±3 % to ±2.6 % and 0.6 %, because of the eventual total number of samples of 1361 and 1357 (larger than planned) and the overall percentage of samples classified as test–positive of 36 % and 1 % (smaller than expected).

DISCUSSION

The present studies in Uruguay have investigated the seroprevalence estimates of *Toxoplasma gondii* and *Neospora caninum*. Prevalence of *Toxoplasma gondii* in sheep were reported at 28.7–38.5 % in Uruguay (11) and 51.5 % in Brazil (12). Workers observed prevalence of *Neospora caninum* in sheep at 0.625 % in New Zealand (3) and 9.5 % in Brazil (12). Detection of these two disease infections can be limited by the sample size used during herd surveys. If the survey programme includes fewer than 30 samples per herd, the risk of missing the detection of the first infected animal (first index case) is increased (13). Early detection of a recent infection is dependent on the frequency of sampling, sample size and the Se of the test. Also, there is not a serological test that has both 100 % Se and 100 % Sp available for *Toxoplasma gondii* and *Neospora caninum*. To commence a periodical monitoring programme in an unknown situation, the necessary initial step should be to determine the prevalence of the *Toxoplasma gondii* and *Neospora caninum* in each herd, farm or region. Except for the smallest sample size of 29 for *Toxoplasma gondii* and 28 for *Neospora caninum* (the Herd E), the sample sizes in this study met the expectations above. Therefore, almost all the TPs estimated in this study can be used to know the situation in each farm. The authors used a Rogan–Gladen estimation in combination with Monte Carlo simulation. The Rogan–Gladen approach has the advantage that it is widely known and also can be used as a simple deterministic function (entering fixed values for Pos, Se and Sp) which is not needed any specific software for calculation. On the other hand, there is a disadvantage of Rogan–Gladen estimator that can yield negative results (< 0) in the case of certain combinations of Pos, Se and Sp, even if Monte Carlo simulation is used simultaneously. Using Bayesian inference based on Markov chain Monte Carlo methods, that can conducted in the software WinBUGS (http://www.mrc-bsu.cam.ac.uk/bugs), improves the disadvantage, and it should be considered as further risk assessment.

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