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Short Communication

Seroprevalence and factors associated with *Toxoplasma gondii*-, *Neospora caninum*- and *Coxiella burnetii*-infections in dairy goat flocks from Costa Rica

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ABSTRACT

A total of 391 goats from 13 dairy flocks from all Costa Rican regions were analyzed for *Toxoplasma gondii*-, *Neospora caninum*- and *Coxiella burnetii*-related seroprevalence by enzyme-linked immunosorbent assays (ELISA). Additionally, a risk factor analysis for these parasitic infections was performed based on a questionnaire considering several environmental and housing/management factors. A total of 62.1% (243/391) of individual serum samples revealed seropositive for *T. gondii,* 7.9% (31/391) for *N. caninum,* and 1.8% (7/391) for *C. burnetii*. At herd level, the overall seroprevalence for *T. gondii* was 100%, for *N. caninum* 69.2% and for *C. burnetii* 7.7%. However, no clinical signs related to toxoplasmosis, neosporosis or Q fever were apparent in these flocks. *T. gondii*-related risk factors were the contact with cats (OR = 3.44; CI 95%; 2.0–5.91), dogs (OR = 5.75; CI 95%; 2.84–11.66), and white-tailed deer (*Odocoileus virginianus*) (OR = 0.15; CI 95%; 0.08–0.26) within or around the farms. The presence of reproductive males in each flock (OR = 0.32; CI 95%; 0.14–0.74) and the coexistence of sheep (OR = 0.46; CI 95%; 0.2–1.08) and cattle (OR = 5.94; CI 95%; 1.70–20.78) revealed as protective and risk factors respectively for *N. caninum* infections. This study determined for the first time the seroprevalences of *N. caninum, T. gondii* and *C. burnetii* in Costa Rican goat flocks. Particularly, the high within-herd seroprevalences determined for *T. gondii* requires further surveillance to complement these findings.

1. Introduction

Toxoplasma gondii and *Neospora caninum* are two closely related apicomplexan parasites associated with reproductive disorders in ruminants, such as foetal reabsorption, mummification, abortion, stillbirth and neonatal losses, leading to substantial economic losses in livestock production (Reichel et al., 2013). *Toxoplasma gondii* also plays a considerable zoonotic role since the consumption of infected raw or undercooked meat or milk from ruminants has been demonstrated to cause human toxoplasmosis (Tenter et al., 2000). *Coxiella burnetii* is an intracellular gamma proteobacterium of the family *Coxiellaceae*, which causes reproductive disorders in small ruminants and Q fever in humans (Van den Brom et al., 2015). This pathogen can be transmitted by ticks and other arthropods, but the main source of infection for domestic animals and humans is exposure to parturient secretions by inhalation of contaminated aerosols (Woldehiwet, 2004).

In Costa Rica, seroprevalences of *T. gondii* were reported, so far, only in rodents (5% in mice and 30.4% in rats; Chinchilla, 1978), cattle (34.4%; Arias et al., 1994) and chicken (40.6%; Abrahams-Sandi and Vargas-Brenes, 2005). The presence of *Neospora*-associated abortion was firstly described in Costa Rica in a dairy goat by Dubey et al. (1996). Further studies determined a dairy herd seroprevalence of 94.7% (89/94), showing an overall individual seroprevalence of 43.3% (1185/2743) (Romero et al., 2005). Infections with *N. caninum* occur rather by vertical (64% of cases) than by horizontal transmission (22% of cases) in Costa Rica, however the latter value is much higher than reported in other countries, probably due to the ecology and biodiversity of this country (Romero and Frankena, 2003). With respect to *C. burnetii,* respective DNA was not detected in milk powder samples from Costa Rica using real-time PCR analysis (Tilburg et al., 2012). So far and to the best of our knowledge, there are no data available on *T. gondii* and *C. burnetii* seroprevalences in small ruminants from Costa

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Rica. To fill this gap, the current study aimed to determine the seroprevalence of *T. gondii, N. caninum and C. burnetii* in goat flocks from Costa Rica, and to identify risk or protective factors being associated to seropositivity for these three pathogens.

2. Materials and methods

2.1. Ethic statement

The present study was conducted under the protocols established by the Animal Welfare Board (Comisión de Bienestar Animal) of the Universidad Nacional (Heredia, Costa Rica) and adhered to the legal requirements of the Animal Welfare Law (Ley 7451 de Bienestar Animal) of Costa Rica.

2.2. Study population

In the current study, exclusively flocks with dairy goat keeping of typical breeds, such as Saanen, Toggenburg, Anglo-Nubian and Alpine were included. The different flocks were registered in the database of the Small Ruminant Program of the National Animal Health Service (SENASA) of Costa Rica or affiliated to independent local caprine associations. The majority of analyzed flocks (69.2%) kept a small number of animals (< 100 goats), mostly maintained under semi-intensive conditions (61.5%). These animals were kept together with other domestic species, such as dogs (84.6%), cattle (61.5%), horses (53.8%), pigs (53.8%), poultry (46.2%), cats (46.2%), and sheep (38.5%).

Required sample sizes were calculated according to data published by the National Institute of Statistics and Census (INEC) of Costa Rica in 2014, who reported a population of 12.852 goats, kept in 2.348 farms. The expected prevalence of anti-*T. gondii* (40%), anti-*N. caninum* (7%) and anti-*C. burnetii* antibodies (25%) was estimated with 95.0% confidence level and using Win Episcope 2.0 (Thrusfield et al., 2001) to determine the representative number of animals to be tested. The serological survey was conducted in 391 goats for the three abortive agents using farms sampled nationwide as part of the surveillance program against brucellosis during 2013–2017 (Hernández-Mora et al., 2017). The Cannon and Roe's formula (1982) was used to determine the sample size to be analyzed in each flock (5% expected prevalence at 95.0% confidence level). The study was conducted in 13 Costa Rican goat flocks selected by their broodstock activities, where the abortions were more frequent to happen and with owners who were willing to participate (Hernández-Mora et al., 2017). For proportional allocation, the sample flocks were present along the six regions of Costa Rica: Central (five farms), North Huetar (three farms), Atlantic Huetar (two flocks), Central Pacific (one flock), Chorotega (one flock) and Brunca (one flock).

2.3. Sample collection and survey

The selection of the animals inside each flock was randomly performed. Blood sampling was performed by bleeding from the jugular vein using BD Vacutainer® 22G × 1" needles with their respective plastic cap, adjusted to 6 ml vacuum tubes for serum (without anticoagulant). Tubes were transported in coolers keeping a temperature between 5 and 10 °C. For serum isolation, blood samples were centrifuged for 8 min at 3500 × *g*. Serum was frozen at −20 °C until further use. A questionnaire was applied to the farmers to assess possible risk factors being associated with *T. gondii, N. caninum* and *C. burnetii* serostatus. Therefore, information on housing conditions, management, animal feeding habits, goat kid husbandry, abortions and contact with other domestic/wild animals on the farm or/and the surroundings was prompted.

2.4. Enzyme-linked immunosorbent assay (ELISA)

The IDScreen® *Toxoplasma gondii, Neospora caninum* and *Coxiella burnetii* Indirect Multispecies ELISAs (IDVet®, Montpellier, France) were used to detect parasite-specific antibodies in the caprine serum samples. These assays were reported to have a high sensitivity (*T. gondii*: 100%; *N. caninum*: 99.6%; *C. burnetii*: 100%) and high specificity (*T. gondii*: 100%; *N. caninum*: 98.9%; *C. burnetii*: 100%) (Proctor et al., 2008; Álvarez-García et al., 2013; Sidibe et al., 2013; IDVET, 2016). Serum samples were processed according to the manufacturer's protocol. The sera were diluted 1:10 for the analysis of each agent. For an adequate interpretation, the average of the optical densities (OD) of the positive controls, and the difference between averages of ODs of positive and negative control sera were calculated. Serum positive percentages (S/P) were calculated according to OD data from the different serum samples and the average of OD of the positive control sera, using the following formula: S/P = (OD of sample × 100): (average OD of positive control). As recommended by the manufacturer, the serum samples with S/P percentages < 40% were considered as negative; samples with S/*P* values between 40 and 50% were scored as inconclusive (considered negative in this study) and sera with S/P values > 50% were determined as positive.

2.5. Statistical analysis

The overall and specific within-herd seroprevalences were assessed; besides, frequencies of the general characteristics and management conditions inside each goat flock were calculated. Factors associated with the agents were assessed by odds ratio (OR) estimation with the goat flock serving as the random variable. The causal variables with inferior and superior confidence intervals (CI 95%) ≤ 1 were considered as risk variable/factors, meanwhile protective variables/factors contained CI 95% ≥ 1. A non conditional logistic regression in two steps was used and first, an univariate analysis was performed for each independent variable and those ones with $p \leq 0.25$ were retained and selected for the multivariate logistic regression model performed by a step-wise backward elimination (Hosmer and Lemeshow, 2005), which was evaluated by likelihood ratio tests. The data were analyzed using EGRET for Windows version 9.2 (Cytel Software Corporation).

3. Results

From a total of 391 caprine serum samples analyzed by ELISA, 243 reacted positive to *T. gondii* (62.14%), 31 to *N. caninum* (7.92%) and 7 to *C. burnetii* (1.79%). Moreover, 20 animals were found positive for *N. caninum* and *T. gondii* (5.12%) and just two goats (0.5%) were positive for all three pathogens. *T. gondii*-specific antibodies were detected in all analyzed flocks (herd prevalence: 100%), *N. caninum*-specific antibodies in nine flocks (69.2%) from five regions, and *C. burnetii*-specific antibodies only in one flock (7.7%) in the North Huetar Region. In general, the regional seropositivity varied considerably: 39.1% - 88.3% for *T. gondii*, 0% - 17.6% for *N. caninum* and 0% - 5.5% for *C. burnetii*. Within each herd, the prevalences range from 13.3% to 95.3% for *T. gondii* and 0% to 23.5% for *N. caninum*. In the single seropositive flock, 15.2% of the goat samples contained *C. burnetii*-specific antibodies (Table 1). No clinical signs related to toxoplasmosis, neosporosis or Q fever were apparent in any flock under investigation.

The univariate analysis revealed the extensive farm management and the contact with domestic animals (pigs, sheep, dogs, cats) in the farm, as well as wild animals (coyotes, opossums, coatis, peccaries, raccoons, white-tailed deer) around the farms as risk factors for *T. gondii* seropositivity. Respective factors for *N. caninum* seropositivity were the presence of reproductive males (protective variable), a farm size with 10–50 goat kids (protective variable), adequate disposal of abortive materials (protective variable), and the co-existence of goats with bovines (risk factor) and sheep (protective variable) (Table 2).