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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).

Table 1
Distribution of seronegative and seropositive sera to *N. caninum*, *T. gondii* and *C. burnetii* from goats of Costa Rica according to flock and region.

Flock	Region	Individuals tested/Animals in the flock (%)	Positive animals (%)			Regional seropositivity n (%)		
			N. caninum	*T. gondii*	*C. burnetii*	*N. caninum*	*T. gondii*	*C. burnetii*
1	Central	21/23 (91.3)	3 (14.3)	5 (23.8)	0 (0.0)	7.7	54.2	0.0
2		46/145 (31.7)	4 (8.7)	9 (19.6)	0 (0.0)			
3		17/18 (94.4)	4 (23.5)	4 (23.5)	0 (0.0)			
4		38/101 (37.6)	2 (5.3)	32 (84.2)	0 (0.0)			
5		46/64 (71.9)	0 (0.0)	41 (89.1)	0 (0.0)			
6	Brunca	9/13 (69.2)	1 (11.1)	4 (44.4)	0 (0.0)	11.0	44.4	0.0
7	Atlantic Huetar	15/15 (100)	2 (13.3)	2 (13.3)	0 (0.0)	8.7	39.1	0.0
8		8/9 (88.9)	0 (0.0)	7 (87.5)	0 (0.0)			
9	Central Pacific	17/18 (94.4)	3 (17.6)	9 (52.9)	0 (0.0)	17.6	52.9	0.0
10	Chorotega	46/66 (69.7)	0 (0.0)	17 (37.0)	0 (0.0)	0.0	37.0	0.0
11	North Huetar	39/91 (42.9)	6 (15.4)	37 (94.8)	0 (0.0)	9.4	88.3	5.47
12		46/213 (21.6)	6 (13.0)	35 (76.1)	7 (15.2)			
13		43/102 (42.2)	0 (0.0)	41 (95.3)	0 (0.0)			
	TOTAL	391 (44.7)	31 (7.92)	243 (62.14)	7 (1.79)			

Since only seven goats from one single flock were positive for *C. burnetii*, no risk factor analysis could be performed.

Following the backward process, the final multivariate logistic regression model for *T. gondii* confirmed that contact with cats, dogs and white-tailed deer as risk factors to seropositivity. Meanwhile, three variables were confirmed for *N. caninum* seropositivity: the presence of more than two reproductive males (protective variable) and the co-existence with bovine (risk variable) and with sheep (protective factor) (Table 3).

4. Discussion

The current seroprevalence study provides data on *T. gondii, N. caninum*, and *C. burnetii* infections in domestic goats in Costa Rica. Thus, a high proportion of goats were detected seropositive for *T. gondii* (62.14%). In line, similar *T. gondii* seroprevalences were recently reported in four Caribbean islands (58% in Dominica, 57% in Grenada, 80% in Montserrat and 42% in Saint Kitts and Nevis) and Colombia (58%) whilst in Mexico fewer animals revealed infected (31%) (Alvarado-Esquivel et al., 2011; Cañón-Franco et al., 2014; Hamilton et al., 2014). All these authors agreed with a broad environmental contamination of *T. gondii* oocysts in soil, water, vegetables or fruits affecting small ruminant flocks.

In case of *T. gondii* infections, poor management practices (e. g. poor hygiene, lack of proper feeding, irregular vaccination, and deworming) and goat farming in close contact with other productive, companion or wild animals (univariate analysis), especially cats, dogs and white-tailed deer (multivariate analysis) were here identified as risk factors for *T. gondii* seropositivity, which is in agreement to other reports (Dubey et al., 1995; Liu et al., 2015; Bawm et al., 2016.). The most obvious risk factor is related to the co-existence of goats with cats since these may shed high numbers of oocysts which contaminate the environment. In agreement, Frenkel and Ruiz (1981) assumed that the endemicity of *T. gondii* in Costa Rica is due to the high cat population.

Table 2
Univariate analyses of risk factors associated with *N. caninum* and *T. gondii* seropositivity in 13 goat flocks from Costa Rica.

Variable	Category	*Neospora caninum*			
		CI 95%			
		p-Value	OR	IL	UL
Number of goat kids	11–50	0.031	0.23	0.05	1.23
Number of males	> 2	0.038	0.44	0.18	1.01
Disposal of abortive materials	Burial	0.019	0.37	0.15	0.95
Co-existence with bovines	Yes	0.007	4.55	1.28	19.19
Co-existence with sheep	Yes	0.062	0.46	0.18	1.11

Variable code	Category	*Toxoplasma gondii*			
		CI 95%			
		p-Value	OR	IL	UL
Husbandry system	Extensive	< 0.001	0.09	0.01	0.44
Contact with swine	Yes	< 0.001	2.05	1.32	3.20
Co-existence of sheep	Yes	< 0.001	0.35	0.22	0.55
Contact with cats	Yes	< 0.001	5.19	3.22	8.38
Contact with dogs	Yes	< 0.001	4.39	2.42	8
Contact with coyotes	Yes	< 0.001	0.24	0.14	0.39
Contact with opossums	Yes	< 0.001	0.17	0.10	0.27
Contact with coatis	Yes	< 0.001	0.15	0.09	0.25
Contact with peccaries	Yes	< 0.001	0.15	0.09	0.24
Contact with raccoons	Yes	< 0.001	0.09	0.05	0.16
Contact with white-tailed deer	Yes	< 0.001	0.30	0.19	0.48

Codes: OR = Odds ratio; IL = Inferior limit; UL = Upper limit; CI = Confidential interval.

Table 3
Risk factors associated with *N. caninum* and *T. gondii* seropositivity in 13 goat flocks from Costa Rica according to the multivariate logistic regression model.

Variable	*N. caninum*				*T. gondii*			
	CI 95%				CI 95%			
	p-Value	OR	IL	UL	p-Value	OR	IL	UL
More than two reproductive bucks	0.008	0.32	0.14	0.74	–	–	–	–
Co-existence of bovine	0.005	5.94	1.70	20.78	–	–	–	–
Co-existence of sheep	0.076	0.46	0.20	1.08	–	–	–	–
Contact with cats	–	–	–	–	< 0.001	3.44	2.00	5.91
Contact with dogs	–	–	–	–	< 0.001	5.75	2.84	11.66
Contact with white-tailed deer	–	–	–	–	< 0.001	0.15	0.08	0.26

Codes: OR = Odds ratio; IL = Inferior limit; UL = Upper limit; CI = Confidential interval.

The unlimited contact between goats and free-roaming cats has been described as important risk factor in extensive management systems related to high herd-level seroprevalences (Czopowicz et al., 2011). In the other hand, dogs may also play an important role on the transmission of *T. gondii* since they could be affected by coprophagia when they have ingested feces of infected cats (Schares et al., 2005). Here is important to clarify, despite dogs themselves do not produce oocysts, high contaminated environments with *T. gondii* and *H. hammondi* oocysts represent a source for other animals, particularly in areas with dense populations of stray and guard dogs, who shed coprophaged/excreted oocysts after intestinal passage, which possibly explains the roll of dogs as a risk factor of *T. gondii* in the surrounding goat populations (Schares et al., 2005; Hosseininejad et al., 2011). However, further deep analysis on the mechanisms involving coprophaged/excreted oocysts by dogs and their posterior infection in small ruminants should be performed. Additionally, wildlife mammals were also identified as risk factor of toxoplasmosis, since these animals are also susceptible wild intermediate hosts of this polyxenous apicomplexan parasite, which prolong the parasite's life cycle when wild felines feed on their infected carcases, as documented by several studies (Solorio et al., 2010; Dubey et al., 2014).

In case of *N. caninum*, the lower overall seroprevalence observed in the present study (7.92%) agreed with data published in other Latin American reports, such as Moore et al., 2007 in Argentina (6.6%), Topazio et al. (2014) in Brazil (4.58%) and Sharma et al. (2015) in Grenada (5.8%). Moreover, Dubey et al. (2017) reported that worldwide caprine *N. caninum* seroprevalences range between 0 and 26%, which agree with our results. In contrast to goats, *N. caninum* infections are well established in cattle, thus a considerably higher overall *N. caninum* seroprevalence of 39.7% was found in Costa Rican dairy cattle (Romero and Frankena, 2003). The lower caprine *N. caninum* seroprevalence might be influence by the duration of the infection, the seroconversion and the applied serological tests, modifying the dynamics of the antibodies (Dubey et al., 2017). As expected, instant removal of placentas and fetuses performed by most Costa Rican goat farmers (OR = 0.37; CI 95%; 0.15–0.95) revealed as an important protective factor for caprine neosporosis, as already described by Dubey et al. (2007). However, *N. caninum* infections in Costa Rica seemed mainly to be influenced by ruminant sharing grazing (Liu et al., 2015), either through the permanence of caprine with bovine (risk variable) or/and ovine (protective variable) species. Recent literature associated pastures highly contaminated with *N. caninum* oocysts with flocks where the animal density was extremely elevated and ruminant sharing grazing practices were common, thus promoting horizontal transmission of this parasite (Haddad et al., 2005; Armengol et al., 2007).

The presence of reproductive males was found as a protective factor against caprine neosporosis; which may be based on the assumption that bucks may be less susceptible to this infection than females, due to the absence of immunosuppression periods during pregnancy (Huerta-Peña et al., 2011). Enhanced innate immune responses against *T. gondii* and *N. caninum* infections have been mentioned in caprine males than in females in numerous studies, therefore female goats demonstrated higher seropositivity (van der Puije et al., 2000; Uzêda et al., 2004; Sharma et al., 2015). Moreover, as the risk of sexual transmission of *N. caninum* is relative low (Ortega-Mora et al., 2003), we suggest that transportation of goats for mating would break the biosecurity cycle of *N. caninum*-free farms, particularly when goats are brought into flocks with reproductive bucks under different management conditions. This situation might justify the presence of males as a protective variable in the present study. However, the biological role of sex in apicomplexan infection and transmission in the caprine systems must be investigated further.

In eight flocks, *N. caninum* seropositive goats (20/31; 64.5%) were also *T. gondii* positive. These mixed infections were most probably due to management practices, abortions and significant contact with cats (*T. gondii,* OR = 3.44; CI 95%; 2.0–5.91) and dogs (*N. caninum*, OR = 5.75; CI 95%; 2.84–11.66) (Topazio et al., 2014; Gos et al., 2017).

The low seroprevalence of *C. burnetii* was similar to that determined by Gardon et al. (2001) in livestock from French Guiana (1.7%). Interestingly, the only flock with positive animals was located near to the border with Nicaragua. In this country, *C. burnetii* was recently reported to cause acute febrile illness in humans (Reller et al., 2016). In future work, *C. burnetii*-related data should be verified additionally via molecular techniques.

Finally, no clinical signs related to toxoplasmosis, neosporosis or Q fever were detected in any analyzed flocks, which can be related to several variables, such as stage of infection, pathogen strains, seroconversion and the animal immunity, as previously reported in the literature (Bezerra et al., 2013; Porto et al., 2016; Muleme et al., 2017). The absence of clinical reproductive signs (e. g. abortions and infertility) and their relationship with these mentioned variables (particularly with seroconversion) suggests also the presence of persistent or latent infected goats in these flocks, but their confirmation using immunoblot techniques are necessary to assess this hypothesis. Furthermore, the elevated cost in the importation of further diagnostic tools in Costa Rica was the principal limitation of our study that impeded the use of other detection methods as confirmation of our results. Therefore, we highly recommend the use of alternative diagnostic tools, such as PCR or immunoblot in further studies to confirm and complement the obtained data through the identification of particular pathogen-antigens.

5. Conclusions

This study determined for the first time seroprevalences of *N. caninum, T. gondii* and *C. burnetii* in goat flocks from all Costa Rican regions: all flocks were infected with *T. gondii*; *N. caninum* was found in nine flocks (69.2%), and *C. burnetii* only in one flock. Furthermore, risk and protective factors associated to seropositivity were identified: *T. gondii* infection was related with the contact of goats with cats, dogs and white-tailed deer in the farms, whereas *N. caninum* was linked to joint grazing of goats with cattle and sheep. It is important to inform the caprine producers on these results; to keep definitive hosts (dogs and cats) separated from the grazing fields, prevent them (and wild animals) to wander in pastures and feed on abortive material. It is also recommended to avoid the sharing of paddocks between cows, goats and wild ruminants, especially the maternity paddocks, and adequate management practices inside the flock to prevent transmission of apicomplexan parasites. Complementary molecular detection is necessary

to confirm the presence of *C. burnetii* in Costa Rica. Finally, further epidemiological surveillance for caprine toxoplasmosis and coxiellosis is recommended.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study were included in this published article.

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Author's contributions

RVB, GD and AES collaborated with the sample collection and survey. RVB and GD performed the ELISA analysis. RVB, HW, JJR, AW, AT, CH and GD cooperated in research design, data analysis and manuscript's review. All the authors corrected and accepted the final manuscript.

Conflict of interest statement

The authors ratified that they have no competing interests in the present study.

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