Parasite Epidemiology and Control 3 (2019) e00085

Contents lists available at ScienceDirect

Parasite Epidemiology and Control

journal homepage: www.elsevier.com/locate/parepi

Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* infections and associated factors in sheep from Costa Rica

Rodolfo Villagra-Blanco [a,b,*], Osvaldo Barrantes-Granados [c], Danilo Montero-Caballero [d], Juan José Romero-Zúñiga [b], Gaby Dolz [b]

[a] Institute of Parasitology, Faculty of Veterinary Medicine, Justus Liebig University Giessen, Giessen 35392, Germany
[b] Programa de Investigación en Medicina Poblacional, Escuela de Medicina Veterinaria, Universidad Nacional (UNA), P.O. Box 86-3000, Heredia, Costa Rica
[c] Servicio Nacional de Salud Animal (SENASA), Ministerio de Agricultura y Ganadería, Heredia, Costa Rica
[d] Instituto Nacional de Aprendizaje (INA), San José, Costa Rica

ARTICLE INFO

Article history:
Received 31 October 2018
Received in revised form 7 January 2019
Accepted 8 January 2019

Keywords:
Abortion
Sheep
Toxoplasmosis
Neosporosis
Costa Rica

ABSTRACT

The presence of antibodies against *Toxoplasma gondii* and *Neospora caninum* were analyzed in 392 sheep sera from ten Costa Rican ovine flocks using indirect immuno-enzymatic assays. Additionally, general information about sheep management, environment, and clinical reproductive disorders was assessed through a questionnaire to inquire factors related to these apicomplexan parasites. A total of 161 (41.1%) serum samples reacted positive to *T. gondii*, 43 (10.9%) to *N. caninum* and 26 (6.63%) to both parasites. *Toxoplasma gondii* serorreactors were detected in all the analyzed flocks (100.0%), meanwhile *N. caninum* antibodies were found in nine flocks (90%), from the six Costa Rican regions. Factors associated with *T. gondii* were the co-presence of cattle (OR = 5.06; C.I.95%; 2.08–12.30; p: <0.001), grey foxes (*Urocyon cinereoargenteus*) and opossums (*Didelphis marsupialis*) (OR = 2.44; C.I.95%; 1.50–3.95; p: <0.001) inside or around the farms, and the presence of peccaries (*Tayassu* sp.) (OR = 0.35; C.I.95%; 0.16–0.74; p: 0.0058) was a variable associated with *N. caninum* seropositivity. The obtained results of *T. gondii* and *N. caninum* infections in sheep flocks from Costa Rica should be considered for the proper prevention and control strategies against these apicomplexan abortive parasites.

1. Introduction

Toxoplasma gondii and *Neospora caninum* are two closely related obligatory apicomplexan parasites associated with reproductive disorders in ruminants, including sheep (*Ovis aries*), causing embryonic reabsorption, mummification, abortion, stillbirth and neonatal losses, leading to substantial economic losses in livestock production (Dubey, 2009; Reichel et al., 2013). Particularly, *T. gondii* plays a considerable zoonotic role, especially through the consumption of ruminant-infected raw or undercooked meat or milk, which was demonstrated to infect humans (Tenter et al., 2000).

* Corresponding author at: Institute of Parasitology, Biomedical Research Center Seltersberg, Justus Liebig University Giessen, Schubertstrasse 81, 35392 Giessen, Germany.
E-mail address: Rodolfo.A.Villagra-Blanco@vetmed.uni-giessen.de. (R. Villagra-Blanco).

https://doi.org/10.1016/j.parepi.2019.e00085

Toxoplasmosis and neosporosis are both cosmopolitan diseases: seroprevalences in sheep flocks from different global areas have been reported using different diagnostic methods in numerous investigations during the last decade (Bártová et al., 2009; Rossi et al., 2011; Moreno et al., 2012; Liu et al., 2015). In Costa Rica, seroprevalences of *T. gondii* have been reported in cattle (34.4%; Arias et al., 1994), dairy goats (62.1%; Villagra-Blanco et al., 2018) and even humans (76%; Arias et al., 1996). In the same way, the presence and seroprevalences of *N. caninum* have been described in Costa Rican goats (6.1% - 7.9%; Dubey et al., 1996; Villagra-Blanco et al., 2018), dairy cattle (43.3%; Romero-Zúñiga et al., 2005) and dogs (48.4%; Palavicini et al., 2007).

Several studies worldwide have mentioned that areas with high number of oocysts contamination, communal water supplies, pasturing systems, herd size and animal feeding habits were potential risk factors linked to exposure and infection with *T. gondii* and *N. caninum* in sheep (Klun et al., 2006; Tzanidakis et al., 2012; Hamilton et al., 2014; Gazzonis et al., 2015; Liu et al., 2015; Maganga et al., 2016). In Costa Rica, factors related to neosporosis in dairy cattle included age, breed, parity of the dam and the lack of purposive sampling to diagnose abortive infectious disease (Romero et al., 2002). Moreover, the contact of goats with dogs, cats, and even wild animals has been recently reported as variables associated with toxoplasmosis or neosporosis in Costa Rican caprine flocks (Villagra-Blanco et al., 2018).

This study aimed to determine the seroprevalence of *T. gondii* and *N. caninum* in sheep flocks from Costa Rica and to identify possible variables associated with seropositivity for these abortive protozoan parasites.

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, Ley de Bienestar Animal of Costa Rica).

2.2. Study population

The analyzed flocks were used commercially to produce tropical hair breed lambs (100%) and these animals were maintained under semi-intensive conditions (80%). The sampled sheep breeds were Dorper (30%), Kathadin (30%), Pellybuey (10%) and mixes (30%) from these and other breeds including Blackbelly, Texel, Suffolk, and Santa Ines. No commercial milk production was recorded. The different flocks were registered in the database of the Small Ruminant Program, National Animal Health Service (SENASA) of Costa Rica or affiliated to independent local ovine associations.

The sample size was calculated according to data published by the National Institute of Statistics and Census (INEC) of Costa Rica in 2014, which reported a population of 35.800 sheep distributed in 1792 flocks. The estimation of the representative sample population in each region and for each pathogen was performed with Win Episcope 2.0 (Thrusfield et al., 2001). As no previous serological studies were available about the infections caused by both parasites within the Costa Rican sheep population, expected anti-*T. gondii* (40%) and anti-*N. caninum* (5%) prevalences were estimated with 95.0% confidence level using Win Episcope 2.0 (Thrusfield et al., 2001). The analyzed population consisted of 392 sheep from farms sampled nationwide as part of the surveillance program against brucellosis during 2013–2017 (Hernández-Mora et al., 2017). The Cannon and Roe's (1982) formula was used to determine the sample size to determine presence or absence of disease inside each flock (5% expected prevalence at 95.0% confidence level). This research was conducted in ten Costa Rican sheep flocks, randomly selected, willing to participate on a voluntary basis. The analyzed flocks were chosen according to proportional allocation: Central Region (three flocks), North Huetar Region (two flocks), Central Pacific Region (two flocks), and one in the other regions (Atlantic Huetar, Chorotega, and Brunca). The selection of the sampled animals inside each flock was randomly performed, but only adult animals (older than eight months) were considered.

2.3. Sample collection and survey

The 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. In the laboratory, the samples were centrifuged for 8 min at 3500 ×*g*. Serum was isolated and frozen at −20 °C until further use. A questionnaire was conducted to the farmers to assess possible factors associated with *N. caninum* and *T. gondii* serostatus. Information was asked concerning housing conditions, management, animal feeding habits, lamb husbandry, abortions, reproductive disorders, the presence of other domestic animals on the farm, and contact with surrounding wildlife animals.

2.4. Enzyme-linked Immunosorbent Assay (ELISA)

The detection of specific antibodies in the sheep serum samples against these protozoa were performed using the IDScreen® *Toxoplasma gondii* and *Neospora caninum* Indirect Multispecies ELISAs, from IDVet® (Montpellier, France). According to Proctor et al. (2008) and Reichel et al. (2008), these assays reported high sensitivity (*T. gondii*: 100%; *N. caninum*: 99.6%) and specificity (*T. gondii*: 100%; *N. caninum*: 98.8%) in ovine sera. The samples were processed according to the manufacturer's protocol. For validation, 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 using the optical density data from

the different serum samples and the average of the optical density of the positive control sera, using the 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 with 95% confidence intervals were assessed; besides, frequencies of the general characteristics and management conditions inside each sheep flock were estimated. Factors associated with *T. gondii* and *N. caninum* were evaluated by odds ratio (OR) estimation, the sheep flock served as the random variable. We used a non-conditional logistic regression for each agent in two steps; first, the univariate analysis was performed for each independent variable. Variables 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).

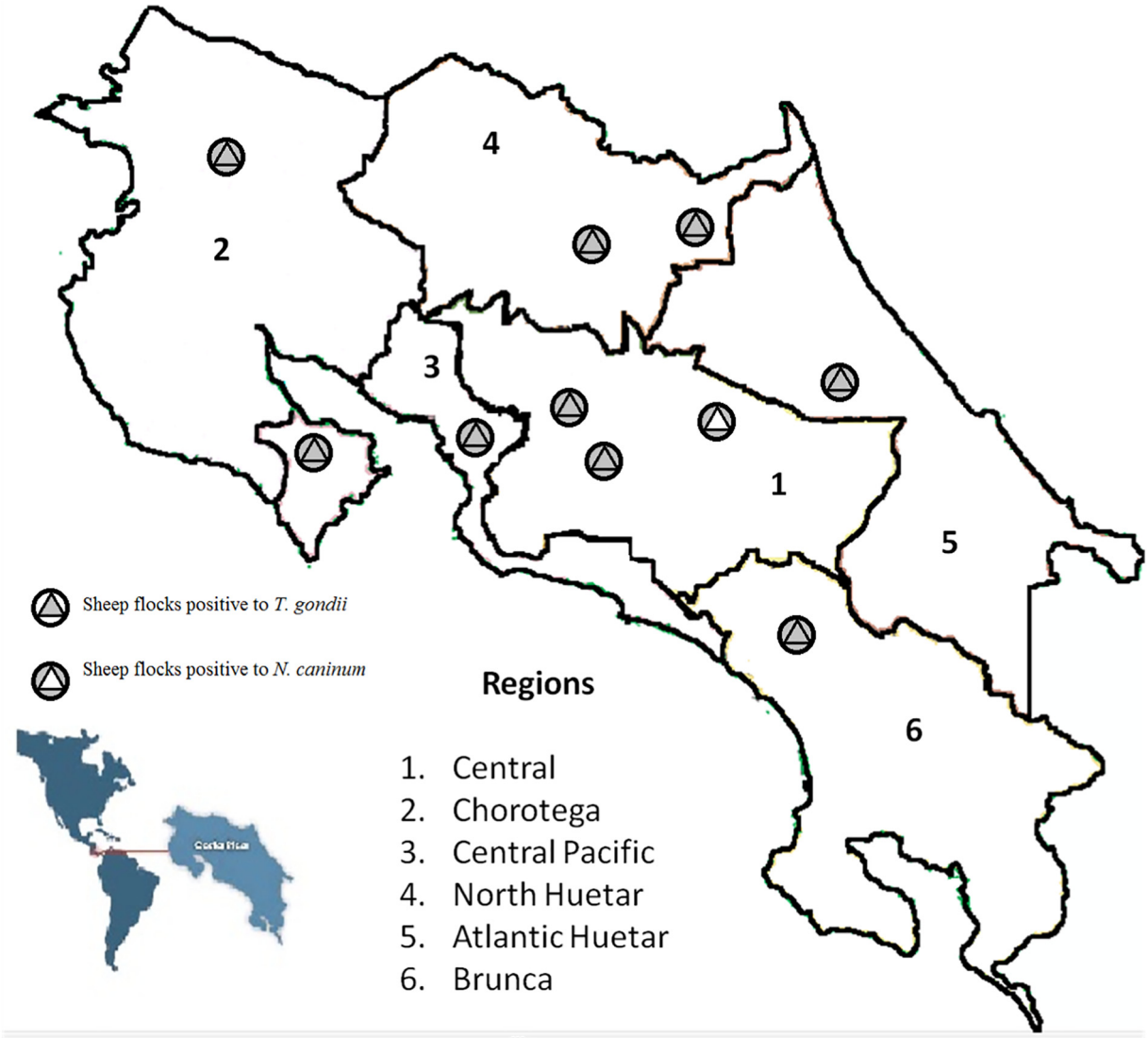

Fig. 1. Regions of Costa Rica with the location of the participating ovine flocks with *T. gondii* or (and) *N. caninum* seropositive sheep.

Table 1
Distribution of seronegative and seropositive sheep sera to *N. caninum* and *T. gondii* in Costa Rica according to flock and region.

Flock	Region	Individuals tested	Positive animals		
			N. caninum	*T. gondii*	Both parasites
1	Central	31	0	18	0
2		33	5	16	3
3		29	3	8	1
4		24	6	8	2
5	Brunca	31	7	23	7
6	Atlantic Huetar	36	4	8	1
7	Central Pacific	34	3	16	3
8		110	7	37	5
9	Chorotega	33	3	13	3
10	North Huetar	31	5	14	1
	Total	392 (100%)	43 (10.9%)	161 (41.1%)	26 (6.63%)

3. Results

From a total of 392 serum samples analyzed, 161 (41.1%) reacted positively to *T. gondii*, 43 (10.9%) to *N. caninum* and 26 (6.6%) to both parasites. Seropositive animals to *T. gondii* were detected in all flocks, meanwhile *N. caninum* antibodies were detected in 90% of the sheep farms (Fig. 1). Seropositive animals to both agents were detected in nine flocks along all regions in Costa Rica (Table 1). All the analyzed flocks reported the presence of other domestic species, such as cats (70%), dogs (70%), poultry (70%), goats (60%), horses (60%), cattle (40%) and swine (30%).

The risk factor analysis revealed that stillbirth and neonatal losses in the flock (OR = 3.17; C.I.95%; 1.45–7.08) represented a risk factor for *N. caninum*, meanwhile, the presence of cattle inside the ovine flocks (OR = 1.60; C.I.95%; 1.02–2.51) and the occurrence of grey foxes (*Urocyon cinereoargenteus*) and opossums (*Didelphis marsupialis*) around the farms (OR = 2.63; C.I.95%; 1.11–6.48) were found as risk factors associated with *T. gondii*. Further, the presence of wild tropical peccaries (*Tayassu* sp.) around the farms (OR = 0.31; C.I.95%; 0.14–0.68) were identified as a protective factor for *N. caninum*, meanwhile the presence of goats inside the farms (OR = 0.49; C.I.95%; 0.25–0.97/OR = 0.44; C.I.95%; 0.28–0.69) was determined as a protective factor for both parasites (Table 2a).

After the backward process, the final multivariate logistic regression model for *N. caninum* kept the presence of wild tropical peccaries (OR = 0.35; C.I.95%; 0.16–0.74; p: 0.0058) as a protective factor. Additionally, the risk variables remained for *T. gondii*: the co-existence with bovines (OR = 5.06; C.I.95%; 2.08–12.30; p: <0.001) and the presence of grey foxes and opossums around the farms (OR = 2.44; C.I.95%; 1.50–3.95; p: <0.001) (Table 2b).

4. Discussion

The seroprevalence obtained for *T. gondii* (41.1%) was higher than that published by Caballero-Ortega et al. (2008) (29.1%) in western Mexico using indirect Immunofluorescence assay (IFAT) or by Gondim et al. (1999) (18.75%) in Brazil through latex agglutination test, but lower than those reported by Hamilton et al. (2014) in sheep from Dominica (67%), Grenada (48%), Montserrat (89%) and St. Kitts and Nevis (57%) through an in house-ELISA. Despite these differences, the authors agreed, that sheep could be used as sentinels for the detection of environmental contamination of soil, water, and vegetables with infective protozoa oocysts (including *T. gondii*), particularly because of their feeding behavior. Sheep are grazers with a higher risk to ingest pathogens located close to the ground (including apicomplexan oocysts) which can be later transmitted to other intermediate or definitive hosts (Gazzonis et al., 2016).

On the other hand, the ovine seroprevalence of *N. caninum* worldwide varied from 0% to 64% depending on the serological test and the cut-off employed, but also on age, breed, gender and management of the sheep (Dubey et al., 2017). The ovine *N. caninum*

Table 2
Variables associated with *N. caninum* or (and) *T. gondii* seropositivity in sheep flocks from Costa Rica.

Variable	Category	*p*-Value	Unadjusted			Adjusted		
				C.I.95%			C.I.95%	
			OR	LL	UL	OR	LL	UL
(a) *Neospora caninum*								
Stillbirth and neonatal deaths	Yes	0.001	3.17	1.45	7.08	–	–	–
Presence of peccaries	Yes	0.001	0.31	0.14	0.68	0.35	0.16	0.74
(b) *Toxoplasma gondii*								
Presence of bovines	Yes	<0.001	1.60	1.02	2.52	5.06	2.08	12.30
Presence of grey foxes and opossums	Yes	<0.001	2.64	1.11	6.48	2.44	1.50	3.95

Codes: OR = odds ratio; IL = inferior limit; UL = upper limit; CI = confidential interval.

seroprevalence obtained in this study was 10.9%, close to the percentages detected by Sharma et al. (2015) (13%) in Grenada (Caribbean West Indies) and Panadero et al. (2010) (10.1%) in Northwest Spain using indirect and competitive ELISA respectively. In the same way, the *N. caninum* seroprevalence detected here in Costa Rican sheep were similar to that reported by Figliuolo et al. (2004) (9.2%) and Romanelli et al. (2007) (9.5%) in Brazilian sheep flocks analyzed through IFAT, but clearly different from data mentioned in other Latin American studies, e.g. Patarroyo et al. (2013) (78.6% by DOT-ELISA) in Colombia or Hecker et al. (2013) (3% by IFAT) in Argentina. The only *N. caninum* seronegative flock applied a rigorous protocol for controlling bovine neosporosis and was located in a high volcanic area of the Central Region (2270 m; average annual temperature: 13.6 °C); pointing out that altitude and temperature differences within areas might explain serological differences, since warm zones promote higher seroprevalences and oocysts sporulation than colder ones (Figliuolo et al., 2004). In any case, our findings provide the first evidence of *N. caninum* and *T. gondii* infections in sheep flocks from Costa Rica.

The results observed in the univariate analysis determined that ovine neosporosis was related to stillbirth and neonatal losses in the flocks. Although there is a weak relationship between abortions in ewes and ovine neosporosis, its occurrence has been strongly associated with weak offspring, stillborn births, congenital infections and reproductive failure in the flocks (Dubey et al., 1990; Corbellini et al., 2001; González-Warletta et al., 2014), situation that matches with our results. Moreover, *N. caninum* induces fetal loss in ewes, mainly through lesions in the placentas, central nervous system and skeletal muscles from the infected lambs (Dubey and Lindsay, 1990). Interestingly, the presence of peccaries was determined as a risk factor of *N. caninum* infection in sheep. In concordance with this finding, *N. caninum*-positive feral swine were reported in several areas of the United States of America (Bevins et al., 2013). Tropical peccaries, as well as feral swine, may act as a reservoirs for encysted parasites, and when being preyed upon by wild canids (coyotes, wolves), oocysts may be scattered around cattle and small ruminant farms (Bevins et al., 2013; Cerqueira-Cézar et al., 2016). However, the exact mechanisms involved in *N. caninum* transmission through wild and domestic swine, ruminants and canids are still unknown and deserve attention in further epidemiological studies.

The multivariate logistic regression model established that parasitoses caused by these apicomplexan agents were mainly influenced by the presence of bovines; despite this, *Toxoplasma gondii* infections are scarcely present in cattle; so bovine as a risk factor of ovine toxoplasmosis in Costa Rica can be explained through the ruminant sharing grazing system (Liu et al., 2015; Gazzonis et al., 2016; Villagra-Blanco et al., 2018) and the elevated contamination of the environment with *T. gondii* oocysts in mixed bovine/ovine flocks, especially under tropical conditions with continuous climate variations (Caballero-Ortega et al., 2008) and particularly considering that the oocyst transmission and its presence in raw meat from cattle and swine have been reported as the most important infection pathway of this parasite in Costa Rica (Arias et al., 1994, 1996). Moreover, other alternative routes might explain this relationship, e.g. the close contact between sheep with infected faeces from cattle and other wildlife intermediary hosts, including in our case, peccaries, grey foxes and opossums, as it has been previously described in studies conducted in North and South America (Lindsay et al., 2001; Yai et al., 2003; Aston et al., 2014).

Since toxoplasmosis represents a zoonotic disease with economic losses (Freyre et al., 1999), and neosporosis causes reproductive failure in the sheep flocks (González-Warletta et al., 2014), the improvement of the management measures in the ovine farms together with educative information for the sheep owners and further veterinary support will be necessary for the future control of infections caused by these apicomplexan parasites in the Costa Rican sheep flocks.

5. Conclusions

This study presents novel data determining seroprevalences of *T. gondii* (41.1%) and *N. caninum* (10.9%) in sheep flocks from Costa Rica. The presence of cattle, grey foxes and opossums were risk factors associated with *T. gondii*, meanwhile, the presence of peccaries was determined as a factor associated to *N. caninum* infection in Costa Rican sheep. This information pretends to be useful for the local veterinarians, human and animal health authorities as well as for farmers in order to develop and improve further control protocols against these apicomplexan parasites in Central America.

Abbreviations

°C	Celsius degree
CI	confidence interval
ELISA	Enzyme-linked Immunosorbent Assay
OD	optical density
OR	odds ratio
S/P	Sample to positive ratio

Limitations of the study

This study didn't perform confirmation of the serological results using complementary methods. Technical limitations included the actual availability of the animals and the scarce amount of serum.

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.

Funding

This work was funded by Fundación Universidad Nacional (FUNDAUNA) under the project "Presencia, prevalencia y epidemiología de agentes infecciosos en pequeños rumiantes de Costa Rica" and the Ministerio de Ciencia, Tecnología y Telecomunicaciones (MICITT-Costa Rica) through the PINN program (PND-026-15-2).

Author's contributions

RVB, DMC, OBG collaborated with the ovine sample collection and survey. RVB and GD performed the ELISA analysis. RVB, DMC, OBG, JJRZ, and GD cooperated in research design, data analysis, and manuscript's review. All the authors checked and accepted the final manuscript.

Conflict of interest statement

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

Acknowledgments

We acknowledge and thank all the sheep farmers who agreed to participate in this study. We also wish to thank Gabriela Hernández-Mora (DVM), Anthony Solórzano (Msc.), Marta Bonilla (DVM, Msc.) and Roberto Leiva, for their technical and professional assistance.

References

Arias, M.L., Chinchilla, M., Reyes, L., Sabah, J., Guerrero, O.M., 1994. Determination of *Toxoplasma gondii* in several organs of cattle by carbon immunoassay (CIA) testing. Vet. Parasitol. 55, 133–136.

Arias, M.L., Chinchilla, M., Reyes, L., Linder, E., 1996. Seroepidemiology of toxoplasmosis in humans: possible transmission routes in Costa Rica. Rev. Biol. Trop. 44, 377–381.

Aston, E.J., Mayor, P., Bowman, D.D., Mohammed, H.O., Liotta, J.L., Kwok, O., Dubey, J.P., 2014. Use of filter papers to determine seroprevalence of *Toxoplasma gondii* among hunted ungulates in remote Peruvian Amazon. Int. J. Parasitol. Parasites Wildl. 3 (1), 15–19.

Bártová, E., Sedlák, K., Literák, I., 2009. *Toxoplasma gondii* and *Neospora caninum* antibodies in sheep in the Czech Republic. Vet. Parasitol. 161, 131–132.

Bevins, S., Blizzard, E., Bazan, L., Whitley, P., 2013. *Neospora caninum* exposure in overlapping populations of coyotes (*Canis latrans*) and feral swine (*Sus scrofa*). J. Wildl. Dis. 49, 1028–1032.

Caballero-Ortega, H., Palma, J.M., García-Márquez, L.J., Gildo-Cárdenas, A., Correa, D., 2008. Frequency and risk factors for toxoplasmosis in ovines of various regions of the State of Colima, Mexico. Parasitology 135, 1385–1389.

Cannon, R.M., Roe, R.T., 1982. Livestock Disease Survey: A Field Manual for Veterinarians. Australian Government Publishing Service, Canberra, Australia.

Cerqueira-Cézar, C.K., Pedersen, K., Calero-Bernal, R., Kwok, O.C., Villena, I., Dubey, J.P., 2016. Seroprevalence of *Neospora caninum* in feral swine (*Sus scrofa*) in the United States. Vet. Parasitol. 226, 35–37.

Corbellini, L.G., Colodel, E.M., Driemeier, D., 2001. Granulomatous encephalitis in a neurologically impaired goat kid associated with degeneration of *Neospora caninum* tissue cysts. J. Vet. Diagn. Investig. 13, 416–419.

Dubey, J.P., 2009. Toxoplasmosis in sheep—the last 20 years. Vet. Parasitol. 163, 1–14.

Dubey, J.P., Lindsay, D., 1990. *Neospora caninum* induced abortion in sheep. J. Vet. Diagn. Investig. 2, 230–233. https://doi.org/10.1177/104063879000200316.

Dubey, J.P., Hartley, W.J., Lindsay, D.S., Topper, M.J., 1990. Fatal congenital *Neospora caninum* infection in a lamb. J. Parasitol. 76, 127–130.

Dubey, J.P., Morales, J.A., Villalobos, P., Lindsay, D.S., Blagburn, B.L., Topper, M.J., 1996. Neosporosis-associated abortion in a dairy goat. J. Am. Vet. Med. Assoc. 208, 263–265.

Dubey, J.P., Hemphill, A., Calero-Bernal, R., Schares, G., 2017. Neosporosis in Animals. CRC Press, Boca Raton Florida, USA.

Figliuolo, L.P.C., Kasai, N., Ragozo, A.M.A., de Paula, V.S.O., Dias, R.A., Souza, S.L.P., Gennari, S.M., 2004. Prevalence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in ovine from Sao Paulo State, Brazil. Vet. Parasitol. 123 (3–4), 161–166.

Freyre, A., Bonino, J., Falcón, J., Castells, D., Correa, O., Casaretto, A., 1999. The incidence and economic significance of ovine toxoplasmosis in Uruguay. Vet. Parasitol. 81, 85–88.

Gazzonis, A.L., Veronesi, F., Di Cerbo, A.R., Zanzani, S.A., Molineri, G., Moretta, I., Moretti, A., Fioretti, D.P., Invernizzi, A., Manfredi, M.T., 2015. Toxoplasma gondii in small ruminants in Northern Italy—prevalence and risk factors. Ann. Agric. Environ. Med. 22, 62–68. https://doi.org/10.5604/12321966.1141370.

Gazzonis, A.L., Álvarez-García, G., Zanzani, S.A., Ortega Mora, L.M., Invernizzi, A., Manfredi, M.T., 2016. *Neospora caninum* infection in sheep and goats from north-eastern Italy and associated risk factors. Small Rumin. Res. 140, 7–12.

Gondim, L.F.P., Barbosa, H.V., Ribeiro, C.H.A., Saeki, H., 1999. Serological survey of antibodies to *Toxoplasma gondii* in goats, sheep, cattle, and water buffaloes in Bahia State, Brazil. Vet. Parasitol. 82, 273–276.

González-Warletta, M., Castro-Hermida, J.A., Regidor-Cerrillo, J., Benavides, J., Álvarez-García, G., Fuertes, M., Ortega-Mora, L.M., Mezo, M., 2014. *Neospora caninum* infection as a cause of reproductive failure in a sheep flock. Vet. Res. 45 (1), 88.

Hamilton, C.M., Katzer, F., Innes, E.A., Kelly, P.J., 2014. Seroprevalence of *Toxoplasma gondii* in small ruminants from four Caribbean islands. Parasit. Vectors 7, 449.

Hecker, Y.P., Moore, D.P., Manazza, J.A., Unzaga, J.M., Spath, E.J., Pardini, L.L., Venturini, M.C., Roberi, J.L., Campero, C.M., 2013. First report of seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in dairy sheep from Humid Pampa, Argentina. Trop. Anim. Health Prod. 45, 1645–1647.

Hernández-Mora, G., Bonilla-Montoya, R., Barrantes-Granados, O., Esquivel-Suárez, A., Montero-Caballero, D., González-Barrientos, R., Fallas-Monge, Z., Palacios-Alfaro, J.D., Baldi, M., Campos, E., Chanto, G., Barquero-Calvo, E., Chacón-Díaz, C., Chaves-Olarte, E., Guzmán-Verri, C., Romero-Zúñiga, J.J., Moreno, E., 2017. Brucellosis in mammals of Costa Rica: an epidemiological survey. PLoS ONE 12 (8), e0182644.

Hosmer, D.W., Lemeshow, S., 2005. Wiley series in probability and statistics. Applied Logistic Regression, Second edition John Wiley & Sons, Inc., Hoboken, NJ, USA https://doi.org/10.1002/0471722146.scard.

Klun, I., Djurković-Djaković, O., Katić-Radivojević, S., Nikolić, A., 2006. Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep and pigs in Serbia: seroprevalence and risk factors. Vet. Parasitol. 135 (2), 121–131.

Lindsay, D.S., Weston, J.L., Little, S.E., 2001. Prevalence of antibodies to *Neospora caninum* and *Toxoplasma gondii* in gray foxes (*Urocyon cinereoargenteus*) from South Carolina. Vet. Parasitol. 97, 159–164.

Liu, Z.K., Li, J.Y., Pan, H., 2015. Seroprevalence and risk factors of *Toxoplasma gondii* and *Neospora caninum* infections in small ruminants in China. Prev. Vet. Med. 118, 488–492.

Maganga, G.D., Abessolo, A.L., Mikala Okouyi, C.S., Labouba, I., Mbeang Beyeme, A.M., Mavoungou, J.F., Agossou, E., Cossic, B., Akue, J.P., 2016. Seroprevalence and risk factors of two abortive diseases, toxoplasmosis and neosporosis, in small ruminants of the Mongo County, southern Gabon. Small Rumin. Res. 144, 56–61.

Moreno, B., Collantes-Fernández, E., Villa, A., Navarro, A., Regidor-Cerrillo, J., Ortega-Mora, L.M., 2012. Occurrence of *Neospora caninum* and *Toxoplasma gondii* infections in ovine and caprine abortions. Vet. Parasitol. 187, 312–318.

Palavicini, P., Romero, J.J., Dolz, G., Jiménez, A.E., Hill, D.E., Dubey, J.P., 2007. Fecal and serological survey of *Neospora caninum* in farm dogs in Costa Rica. Vet. Parasitol. 149, 265–270.

Panadero, R., Painceira, A., Lopez, C., Vazquez, L., Paz, A., Diaz, P., Dacal, V., Cienfuegos, S., Fernandez, G., Lago, N., Díez-Baños, P., Morrondo, P., 2010. Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in wild and domestic ruminants sharing pastures in Galicia (Northwest Spain). Res. Vet. Sci. 88, 111–115.

Patarroyo, J.H., Vargas, M.I., Cardona, J.A., Blanco, R.D., Gómez, V.E., 2013. Frecuencia serológica de infección por *Neospora caninum* en ovinos en el departamento de Córdoba, Colombia. Rev. MVZ Córdoba 18 (3), 3886–3890.

Proctor, A.F., O'Donovan, J., Marques, P.X., Gutiérrez, J., Sammin, D., Brady, C., Worrall, S., Nally, J.E., Bassett, H., Markey, B.K., 2008. Detection of Antibodies to *Toxoplasma gondii* in Serum From Experimentally Infected Pregnant Ewes. University College Dublin (2008).

Reichel, M.P., Ross, G.P., McAllister, M.M., 2008. Evaluation of an enzyme-linked immunosorbent assay for the diagnosis of *Neospora caninum* infection in sheep and determination of the apparent prevalence of infection in New Zealand. Vet. Parasitol. 151, 323–326.

Reichel, M.P., Ayanegui-Alcerreca, M.A., Gondim, L.F., Ellis, J.T., 2013. What is the global economic impact of *Neospora caninum* in cattle – the billion dollar question. Int. J. Parasitol. 43, 133–142.

Romanelli, P.R., Freire, R.L., Vidotto, O., Marana, E.R.M., Ogawa, L., de Paula, V.S.O., Garcia, J.L., Navarro, I.T., 2007. Prevalence of *Neospora caninum* and *Toxoplasma gondii* in sheep and dogs from Guarapuava farms, Paraná State, Brazil. Res. Vet. Sci. 82, 202–207.

Romero, J.J., Perez, E., Dolz, G., Frankena, K., 2002. Factors associated with *Neospora caninum* serostatus in cattle of 20 specialized Costa Rican dairy herds. Prev. Vet. Med. 53, 263–273.

Romero-Zúñiga, J.J., van Breda, S., Vargas, B., Dolz, G., Frankena, K., 2005. Effect of neosporosis on productive and reproductive performance of dairy cattle in Costa Rica. Theriogenology 64 (9), 1928–1939.

Rossi, G.F., Cabral, D.D., Ribeiro, D.P., Pajuaba, A.C.A.M., Corrêa, R.R., Moreira, R.Q., Mineo, T.W.P., Mineo, J.R., Silva, D.A.O., 2011. Evaluation of *Toxoplasma gondii* and *Neospora caninum* infections in sheep from Uberlândia, Minas Gerais State Brazil, by different serological methods. Vet. Parasitol. 175, 252–259.

Sharma, R.N., Bush, J., Tiwari, K., Chikweto, A., Bhaiyat, M.I., 2015. Seroprevalence of *Neospora caninum* in sheep and goats from Grenada, West Indies. Open J. Vet. Med. 5, 219–223.

Tenter, A.M., Heckeroth, A.R., Weiss, L.M., 2000. *Toxoplasma gondii*: from animals to humans. Int. J. Parasitol. 30 (12−13), 1217–1258.

Thrusfield, M., Ortega, C., de Blas, I., Noordhuizen, J.P., Frankena, K., 2001. WIN EPISCOPE 2.0: improved epidemiological software for veterinary medicine. Vet. Rec. 148, 567–572.

Tzanidakis, N., Maksimov, P., Conraths, F.J., Kiossis, E., Brozos, C., Sotiraki, S., Schares, G., 2012. *Toxoplasma gondii* in sheep and goats: seroprevalence and potential risk factors under dairy husbandry practices. Vet. Parasitol. 190, 340–348.

Villagra-Blanco, R., Esquivel-Suárez, A., Wagner, H., Romero-Zúñiga, J.J., Taubert, A., Wehrend, A., Hermosilla, C., Dolz, G., 2018. Seroprevalence and factors associated with *Toxoplasma gondii*-, *Neospora caninum*- and *Coxiella burnetii*-infections in dairy goat flocks from Costa Rica. Vet. Parasitol. Reg. Stud. Rep. 14, 79–84.

Yai, L.E.O., Cañón-Franco, W.A., Geraldi, V.C., Summa, M.E.L., Camargo, M.C.G.O., Dubey, J.P., Gennari, S.M., 2003. Seroprevalence of *Neospora caninum* and *Toxoplasma gondii* antibodies in the South American opossum (*Didelphis marsupialis*) from the city of Sao Paulo, Brazil. J. Parasitol. 89, 870–871.