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Serological response to *Neospora caninum* infection in goats and agreement between three diagnostic techniques to detect caprine neosporosis[1]

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ABSTRACT.- Kim P.C.P., Melo R.P.B., Almeida J.C., Silva J.G., Ribeiro-Andrade M., Porto W.J.N., Pinheiro Junior J.W. & Mota R.A. 2019. **Serological response to *Neospora caninum* infection in goats and agreement between three diagnostic techniques to detect caprine neosporosis.** *Pesquisa Veterinária Brasileira 39(1):25-31.* Laboratório de Doenças Infecto-Contagiosas dos Animais Domésticos, Departamento de Medicina Veterinária, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brazil. E-mail: rinaldo.mota@hotmail.com

The present study aimed to measure the serological response of goats infected with *Neospora caninum* by assessing the diagnostic performance and agreement between three techniques (indirect immunofluorescent antibody test, IFAT; *Neospora* agglutitation test, NAT; enzyme-linked immunosorbent assay, ELISA). The panel of sera were comprised of 500 samples of goats, and 60 reference serum samples. These reference and field serum samples were tested by ELISA, NAT, and IFAT. In the field serum samples tested, the seroprevalences of anti-*N. caninum* antibodies were 3.2%, 4.6%, and 6.4% in the NAT, IFAT and ELISA, respectively. Using the IFAT as the gold standard, the NAT and the ELISA agreement was considered weak (k=0.28) and strong (k=0.75), respectively. When the IFAT performance was used for comparison purposes, the ELISA showed 91.3% sensitivity and 97.7%, specificity with a PPV of 65.2% and a NPV of 99.6%; The NAT presented sensitivity of 26.1% and specificity of 97.9% with a PPV of 37.5% and a NPV of 96.5%. Accordingly, the IFAT should remain the assay of choice for studies about *N. caninum* infection in goats in individual serum samples. A combination of serological assays with high sensitivity and specificity is recommended in serosurveys of caprine neosporosis.

INDEX TERMS: Serology, *Neospora caninum*, infection, goats, diagnostic techniques, caprine, neosporosis, serological screening tests, parasitoses.

RESUMO.- [**Resposta sorológica à infecção por *Neospora caninum* em cabras e concordância entre três técnicas diagnósticas para detecção de neosporose caprina.**] Objetivou-se avaliar a resposta sorológica de caprinos infectados com *Neospora caninum* mediante o estudo da performance e concordância de três técnicas sorológicas (RIFI, NAT e ELISA). O painel de soros testes foi composto por 500 amostras de caprinos e ainda 60 soros classificados como de referência. Todos os soros de referência e de campo foram testados por ELISA, NAT e RIFI. Nos soros de campo, as soroprevalências de anticorpos anti-*N. caninum* foram de 3,2% no NAT, 4,6% na RIFI e 6,4% no ELISA. Utilizando a RIFI como técnica de referência, a concordância de NAT e ELISA foi considerada fraca (k=0,28) e substancial (k=0,75), respectivamente. Ainda utilizando a RIFI como comparação, foram obtidos valores de sensibilidade de 91,3% e 97,7% de especificidade no ELISA, e valores preditivos positivo de 65,2% e negativo de 99,6%; NAT apresentou resultados de sensibilidade de 26,1% e de especificidade de 97,9% com valores preditivos positivo de 37,5% e negativo de 96,5%. Com base nos resultados deste trabalho, sugerimos que a RIFI permaneça como técnica de escolha no estudo da neosporose caprina em amostras individuais, resguardando as recomendações e pontos de

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corte adotados neste estudo. Indicamos a associação de técnicas sorológicas de alta sensibilidade e especificidade.

TERMOS DE INDEXAÇÃO: Sorologia, infecção, *Neospora caninum*, caprinos, técnicas de diagnóstico, neosporose, testes de triagem sorológica, parasitoses.

INTRODUCTION

Neospora caninum is an obligate intracellular coccidian parasite that belongs to the phylum Apicomplexa and is considered an important cause of abortion in cattle around the globe (Dubey et al. 2007). This protozoal organism has a complex heteroxenous life cycle in which the domestic dog and other canids act as definitive hosts and a number of ungulates play a role as intermediate hosts in the transmission of the disease agent (Dubey & Schares 2011).

In non-pregnant animals, neosporosis is usually a latent asymptomatic infection (Buxton et al. 2002). Persistent infection throughout life is an important feature of bovine neosporosis. *N. caninum* can be transmitted vertically from the dam to the fetus in successive pregnancies resulting in repeated abortions and unthrifty, weak calves at birth. Congenitally infected animals transmit the protozoan parasite to their offspring in the next generation (Williams et al. 2000, Buxton et al. 2002). Over the years, neosporosis have been extensively studied in cows as the bovine is the most important farm animal species in economic terms (Reichel et al. 2013). Cases of neosporosis in small ruminants have also been reported worldwide, and studies on the transplacental transmission of *N. caninum* have been conducted by numerous researchers (Dubey & Schares 2011, Varaschin et al. 2012, Moreno et al. 2012, Nunes et al. 2017).

The occurrence of reproductive disorders in goats that are seropositive for *N. caninum* suggest that neosporosis is a significant cause of abortion and neonatal deaths in caprine herds (Moreno et al. 2012, Mesquita et al. 2018). *N. caninum* congenital transmission rates in goats may be as high as those reported to cattle. The prevalence of congenitally infected offspring is similarly high (Mesquita et al. 2013). Neosporosis should be included in the differential diagnosis of endemic or epidemic abortions along with other toxic and infectious causes of abortion that commonly affect farm animals (McAllister 2016). Diagnosis of abortion due to *N. caninum* infection in production animals is based on the clinical history and epidemiological data of the affected herd, serological screening of female animals, and a comprehensive diagnostic workup on aborted fetuses including necropsy, histopathology, and serology of fetal fluids (Ortega-Mora et al. 2006). The definitive diagnosis of abortion due to *N. caninum* can be tricky and relatively expensive. Asymptomatic *N. caninum* congenital infections are common. The presence of the pathogen DNA in tissues of aborted fetuses does not necessarily mean that this protozoan parasite was the cause of the abortion (Dubey & Schares 2011).

A number of serological assays which include commercially available kits have been used to detect specific anti-*N. caninum* antibodies in cattle. These ancillary tests are used primarily to distinguish between infected animals and non-infected ones. Each of these diagnostic tools has its particular features and pros and cons (Blumröder et al. 2004, Aguado-Martínez et al. 2008, Álvarez-García et al. 2013). The Indirect fluorescent antibody test (IFAT) is routinely used for the detection of specific IgM and IgG in serum samples. This assay was further optimized for the use in sera from small ruminants and other animal species (Buxton et al. 1998). Other techniques that have also been used in serological surveys of antibodies to *N. caninum* in large animals include the Enzyme-linked immunosorbent assays (ELISA) using recombinant antigens or intact tachyzoites, Immunoblotting (IB) (an immunoprotein technique), and the *Neospora* Agglutination Test (NAT). These assays should be carefully chosen according to the needs of the researcher and diagnostician (Ortega-Mora et al. 2006, Aguado-Martínez et al. 2008, Guido et al. 2016).

The evaluation of the degree of concordance (agreement) between tests to detect *N. caninum* infection and the comparative assessment of the performance of different assays used for the detection of *N. caninum* infections can be challenging, especially because a gold standard technique for the serological diagnosis of neosporosis is lacking (Ortega-Mora et al. 2007, Guido et al. 2016).

The aim of the present study was to evaluate the serological response of goats to *N. caninum* by assessing the performance and agreement between three different serological techniques used to detect specific IgG antibodies against *N. caninum* in serum samples of naturally and experimentally infected animals.

MATERIALS AND METHODS

Panel of sera and experimental design. The present survey consisted of a panel of serum samples for testing and a panel of reference (control) sera. Sample selection criteria used in this study were those available in the section about analytical and diagnostic performance characteristics of laboratory tests published in the OIE Guideline "Principles and Methods of Validation of Diagnostic Assays for Infectious Diseases" (OIE 2013). A total of 560 caprine serum samples were analyzed. Serum samples were divided into two categories: reference sera (positive controls and negative controls), and samples from naturally infected animals raised for subsistence in extensive goat farms (sera for testing) from semi-arid region of Pernambuco State, Brazil. A total of 500 field samples were collected from female goats, aged between one and three years, from different races. Four properties were chosen for convenience (ease of access). These collections were approved by the Committee on Ethics in the Use of Animals (CEUA) of the Federal University of Alagoas, under license number 78/2017. *Neospora caninum* serostatus of these caprine herds was unknown.

A total of 60 reference sera were divided as follows, 30 serum samples positive to *N. caninum*, and 30 serum samples negative to *N. caninum.* These sera were originally collected during a experimental *N. caninum* inoculation study previously published by Porto et al. (2016). Negative samples were collected from goats serologically negative to *N. caninum*, *Toxoplasma gondii*, Caprine Arthritis Encephalitis virus (CAEV), and *Coxiella burnetii*. These animals had no clinical history of reproductive problems, and did not seroconvert for neosporosis in three consecutive tests. Serum samples were tested by IFAT and ELISA at intervals of 30 days.

All data regarding the maintenance of *N. caninum* strains in the laboratory, selection of animals for this study, inoculation dose, sampling, and analysis of the immunological dynamics associated with *N. caninum* infection were previously published by Porto et al. (2016).

Serological tests (IFAT, ELISA, and NAT)

Antigen preparation and antigen production. The *N. caninum* Nc-Spain 7 isolate used in this study was maintained in a monolayer culture of Marc-145 cells under specific standardized laboratory conditions previously used in research conducted by Regidor-Cerrillo et al. (2008). Tachyzoites were stained with Tripan blue and resuspended in sterile PBS 1X (pH 7.4). The number of viable tachyzoites was determined with a Neubauer counting chamber. The protocol published by Álvarez-García et al. (2003) was followed for the preparation and production of the finished antigen that was used in the three serological techniques that were assessed in the present study (i.e., IFAT, ELISA, and NAT).

Indirect fluorescent antibody test (IFAT). In the present study, the IFAT was used as a reference technique (gold standard) to detect anti-*N. caninum* IgG antibodies in goat sera. The IFAT was performed according to the guidelines provided by Porto et al. (2016). The protocol established by Álvarez-García et al. (2003) was followed with some minor modifications. In summary, 10µL of the *N. caninum* tachyzoite suspension in formalin solution at a concentration of 10^7 tachyzoites/mL was inoculated into each slide well and then dried at room temperature. Slides were immersed for 10 min in acetone at -20°C in order to finish antigen fixation. A cut-off point of 1:50 was used with dilutions of caprine sera in 1X PBS (pH 7.2). Diluted sera were inoculated into each slide well, incubated at 37ºC for 30 min, and washed twice in 1X PBS for 10 min. Anti-goat IgG solution conjugated with fluorescein isothiocyanate was added to a 1:400 dilution in 0.002% Evans Blue (Sigma-Aldrich Corp., St Louis/MO, USA) and then incubated at 37°C for 30 min, followed by 2 washes with 1X PBS and 1 final wash with distilled water. After drying, slides were coverslipped using glycerin solution and visualized under a fluorescence microscope. Positive controls and negative controls were included in all the slides examined. Samples were considered positive when total peripheral fluorescence was detected in more than 50% of the tachyzoites in different fields of each well. Samples in which tachyzoites did not fluorescence or that tachyzoites displayed irregular fluorescence were interpreted as negative.

In-house ELISA. Levels of anti-*N. caninum* specific IgG antibodies were measured by an in-house ELISA technique developed by González-Warleta et al. (2014) and modified for this study by the use of lyophilized antigen of *N. caninum* in a concentration of $5x10^7$ tachyzoites/mL. For such purpose, the antigen was used in a concentration of 10^5 tachyzoites per well diluted in a carbonate-bicarbonate buffer solution (0.1M, pH=9.6). A final volume of 100µL was inoculated into each well. Elisa microplates (Greiner Bio-One GmbH, Germany) were incubated overnight at 4°C. After the plates were sensitized, each well was blocked using 300µL of a bovine serum albumin solution (Sigma-Aldrich Corp., St Louis, MO, USA) diluted 3% in 1X phosphate buffer saline (pH=7.4) containing 0.05% Tween 20 (PBS-T). This step was followed by 2 hours incubation at room temperature. Plates were washed 3 times with PBS-T. The sera used were diluted 1:100 in the blocking solution, adding 100µL of this dilution into each corresponding plate well, and incubated for 1h at 37°C. The plates were washed 3 additional times with PBS-T; 100µL of G-Biotin Protein were added as the conjugate (Sigma-Aldrich Corp., St Louis, MO, USA), diluted in 1:10,000 PBS-T, and incubated for 1h at 37°C. The plates were then washed 3 times, and 100µL of ABTS Solution substrate (Roche, Indianapolis, USA) were inoculated into these plates. The reaction was stopped after 20 min at room temperature by adding 0.3M oxalic acid solution. ELISA plate reading was performed on a spectrophotometer (Multiskan RC, Thermo Labsystems) using 405nm wavelength (OD405). Optical density values were converted to percent relative index (IRPC) using the following formula: IRPC = (OD405 sample-OD405 negative control)/(OD405 positive control-OD405 negative control)×100. An IRPC value ≥10 meant a positive result. Duplicate serum sets consisting of positive and negative controls for *N. caninum* were used for the validation of the reactions.

***Neospora* agglutination test (NAT).** The NAT assay was used according to the guidelines provided by Romand et al. (1998) with some modifications. In the present study, an antigen from *N. caninum* isolate Nc-Spain 7 was used. The initial dilution of sera used was 1:25 and the final dilution was 1:50 (cut-off point); 96-well plates were used. These NAT plates had a U-shaped background. Results were interpreted as follows: samples were considered negative if a compact dot or button was formed at the bottom of the microplate well, filling more than 50% of this well. Samples were considered positive if an opaque mesh (web) was formed in at least 50% of the microplate well. Positive controls and negative controls were added to all microplates.

Data analysis. In order to compare the three serological assays performed in the present study, the IFAT was defined as the reference test (gold standard). The following parameters were calculated: Kappa coefficient (k), sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and accuracy (ACU) (Cohen 1960, Gart & Buck 1966). The k-values were interpreted according to the criteria established by Landis & Koch (1977) as follows: <0, without agreement; 0.00-0.19, poor agreement; 0.20-0.39, weak agreement; 0.40-0.59, moderate agreement; 0.60-0.79, substantial agreement; 0.80-1.00, almost perfect agreement.

RESULTS

Analysis of reference sera

The tests of all reference sera (30 positive samples and 30 negative samples) showed 100% Se, Sp, PPV, NPV, ACU, and k values equal to 1 in the ELISA and NAT, when compared to the IFAT (reference technique/gold standard) (Table 1).

Antibody search (survey) in field serum samples (seroprevalence)

Seroprevalences of anti-IgG *Neospora caninum* antibodies in the three different serological tests assessed in this study were: 3.2% (1.98-5.13) in the NAT, 4.6% (3.05-6.84) in the IFAT, and 6.4% (4.57-8.90) in the ELISA. The highest prevalence was estimated in the ELISA with a cut-off point at 1:100 whereas the lowest prevalence was estimated in the NAT with a cut-off point at 1:50.

Results of agreement (concordance) between serological tests

The results of the agreement between the three serological tests assessed in the present study and the values of Se, Sp, PPV, NVP, and ACU associated with the detection of anti-*N. caninum* IgG antibodies for the 500 serum samples tested are provided in Table 2. The agreement between the NAT and the ELISA when compared to the IFAT was considered weak and substantial, respectively, with kappa (k) coefficients of 0.28 for the NAT and 0.75 for the ELISA. When the ELISA technique was compared with the IFAT, the values of 91.3% of sensitivity and 97.7% of specificity were found, with a positive predictive value of 65.2% and a negative predictive value of 99.6%. The NAT, when compared to the IFAT, showed a sensitivity of 26.1% and a specificity of 97.9% with a positive predictive value of 37.5% and a negative predictive value of 96.5%.

Table 1. Comparative concordance between three serodiagnostic assays using reference samples of goat sera tested for anti-*Neospora caninum* antibody detection

Reference sample panel	Total (n)	Serodiagnostic tests					Sensitivity (CI 95%)	Specificity (CI 95%)	Positive predictive value (CI 95%)	Negative predictive value (CI 95%)	ACU (CI 95%)	*k* (CI 95%)
Primo-infeccion model (experimental inoculation)			IFAT[R]	Pos.	Neg.	Total						
Positive goats	30	ELISA	Pos.	30	0	30	100% (100-100)	100% (100-100)	100% (100-100)	100% (100-100)	100% (100-100)	1.0 (P) (1,000-1,000)
Negative goats	30		Neg.	0	30	30						
			Total	30	30	60						
			IFAT[R]	Pos.	Neg.	Total						
		NAT	Pos.	30	0	30	100% (100-100)	100% (100-100)	100% (100-100)	100% (100-100)	100% (100-100)	1.0 (P) (1,000-1,000)
			Neg.	0	30	30						
TOTAL	60		Total	30	30	60						

IFAT = Indirect immunofluorescence antibody test, NAT = *Neospora* agglutination test, ELISA = enzyme-linked immunosorbent assay, Pos. = positive, Neg. = negative, ACU = accuracy; [R] reference test, k = Kappa coefficient: no agreement (<0.0), p = poor (0.0-0,19), W = weak (0.20-0.39), M = moderate (0.40-0.59), S = substantial (0.60–0.79), P = almost perfect (0.80-1.00) (Landis & Koch 1977).

Table 2. Comparative concordance between three serodiagnostic assays demonstrated by frequency of anti-*Neospora caninum* IgG antibodies in samples sera of 500 goats

Serodiagnostic assays					Sensitivity (CI 95%)	Specificity (CI 95%)	Positive predictive value (CI 95%)	Negative predictive value (CI 95%)	ACU (CI 95%)	*k* (CI 95%)
	IFAT[R]	Pos.	Neg.	Total						
ELISA	Pos.	21	11	32	91.3% (79.8-102.8)	97.7% (96.3-99.0)	65.2% (49.2-82.1)	99.6% (99-100.2)	97.4% (96-98.8)	0.750 (S) (0.620-0.880)
	Neg.	2	466	468						
	Total	23	477	500						
	IFAT[R]	Pos.	Neg.	Total						
NAT	Pos.	6	10	16	26.1% (8.1-44)	97.9% (96.6-99.2)	37.5% (13.8-61.2)	96.5% (94.8-98.1)	94.6% (92.6-96.6)	0.281 (W) (0.088-0.473)
	Neg.	17	467	484						
	Total	23	477	500						
	ELISA[R]	Pos.	Neg.	Total						
IFAT	Pos.	21	2	23	65.6% (49.2-82.1)	99.6% (99-100.2)	91.3% (79.8-102.8)	97.7% (96.3-99)	97.4% (96-98.8)	0.750 (S) (0.620-0.880)
	Neg.	11	466	477						
	Total	32	468	500						
	ELISA[R]	Pos.	Neg.	Total						
NAT	Pos.	12	4	16	37.5% (20.7-54.3)	99.1% (98.3-100)	75% (53.8-96.2)	95.9% (94.1-97.6)	95.2% (93.3-97.1)	0.478 (M) (0.301-0.654)
	Neg.	20	464	484						
	Total	32	468	500						
	NAT[R]	Pos.	Neg.	Total						
IFAT	Pos.	6	17	23	37.5% (13.8-61.2)	96.5% (94.8-98.1)	26.1% (8.1-44)	97.9% (96.6-99.2)	94.6% (92.6-96.6)	0.281 (W) (0.088-0.473)
	Neg.	10	467	477						
	Total	16	484	500						
	NAT[R]	Pos.	Neg.	Total						
ELISA	Pos.	12	20	32	75% (53.8-96.2)	95.9% (94.1-97.6)	37.5% (20.7-54.3)	99.1% (98.3-100)	95.2% (93.3-97.1)	0.478 (M) (0.301-0.654)
	Neg.	4	464	468						
	Total	16	484	500						

IFAT = Indirect immunofluorescence antibody test, NAT = *Neospora* agglutination test, ELISA = enzyme-linked immunosorbent assay, Pos. = positive, Neg. = negative. ACU = accuracy; [R] reference test, k = Kappa coefficient: no agreement (<0.0), p = poor (0.0-0.19), W = weak (0.20-0.39), M = moderate (0.40-0.59), S = substantial (0.60–0.79), P = almost perfect (0.80-1.00) (Landis & Koch 1977).

DISCUSSION

Experimental studies have shown that goats are susceptible to *Neopsora caninum*. Abortion occurs after pregnant does are inoculated with this protozoan parasite (Lindsay et al. 1995, Porto et al. 2016). In some countries, reports of abortion and neonatal death in naturally infected goats due *N. caninum* have been publshed (Barr et al. 1992, Dubey et al. 1996, Corbellini et al. 2001, Eleni et al. 2004, Moreno et al. 2012, Nunes et al. 2017). Abortions occur most often in seropositive goats, and culling is widely practised in eradication programs (Altbuch et al. 2012). Serodiagnosis is the first step towards initiating any surveillance or control program for neosporosis in a goat herd (Guido et al. 2016).

Our findings corroborate those of previous studies about caprine neosporosis carried out by Brazilian researchers in which low seroprevalences of *N. caninum* infection in goats were found. In Northeastern Brazil, seroprevalences of caprine neosporosis vary between 2.9% (Arraes-Santos et al. 2016) and 3.3% (Faria et al. 2007) in the IFAT assay with a cut-off point of 1:50 and 15% in the IFAT assay with a cut-off point of 1:100 (Uzêda et al. 2007). Sensitivity and specificity results of the ELISA assay for field serum samples when compared with the results of the IFAT assay were 91.3% sensitivity and 97.7% specificity.

Serological surveys conducted around the globe have reported prevalences of antibodies against *N. caninum* in goats ranging between 2 and 23% using the IFAT, the ELISA and the NAT with different cut-off points (Dubey & Schares 2011). In the present study, field samples were tested with the three techniques routinely used for the detection of anti-*N. caninum* IgG antibodies, i.e. ELISA, NAT, and IFAT. We found a higher seroprevalence of neosposis in goats (6.4%) by using the ELISA assay. Wide variation in the seroprevalence of caprine neosporosis have been reported in the southeastern region of the country. In the State of São Paulo, southeast Brazil, a seroprevalence of 2.7% of caprine neosporosis was reported in the IFAT assay with a cut-off point of 1:50 (Santos et al. 2013) in contrast with a seroprevalence of 19.7% obtained by Modolo et al. (2008) and a seroprevalence of 17.23% found by Costa et al. (2012) when using the NAT with a cut-off point of 1:25. These variations in seropositivity may be due to a number of differences in each farm including herd management, herd hygiene, presence of definitive hosts in the area, climate variations, and environmental contamination (Dubey & Schares 2011).

The IFAT has been the assay of choice for the serological diagnosis of neosporosis in goats and sheep over the years mainly because it was the first serological test developed for such purpose (Dubey et al. 1988), and also due to the fact that cross-reactivity with other coccidian parasites is low (Dubey & Schares 2011). Therefore, the IFAT was adopted as a reference test (gold standard) in our study in order to compare its diagnostic performance with the diagnostic performance of other assays (NAT and ELISA). High background values in absorbance reading and cross-reactivity between related parasites have been reported for the ELISA assay depending on the method of antigen preparation (production) and polyclonal antibody used in the assay (Björkman et al. 1999).

In the present study, since a commercially available test and a species-specific antibody were both lacking in the market, an in-house ELISA protocol using a G protein as the conjugate, which has an affinity for both caprine and ovine immunoglobulins, was followed (Porto et al. 2017). Our findings show that the lyophilized tachyzoite-based ELISA was a highly sensitive assay which is able to detect true negative serum samples with a negative predictive value of 99.6%. Antigens based on soluble extracts contain large amounts of molecules with antigenic properties which are mainly intracellular. There are also the antigens from the membrane surface of the parasite which are preferentially recognized by the IFAT assay (Lasri et al. 2004, Silva et al. 2007). However, in the case of *Toxoplasma gondii*, the specificity and sensitivity of a technique based on the recognition of intracellular antigens have been challenged (Lasri et al. 2004). We do not know whether the variation in the preparation/production of the antigen used in the in-house ELISA has influenced the sensitivity and specificity values in the field samples. Thus, it is important to compare this assay with another assay in which soluble antigens are also used.

The low agreement between the NAT for field samples (k=0.281) and the reference technique (k=1.0) may have occurred due to a difference in the time course of infection and also because of variations in the amount of specific circulating antibodies, especially because these are samples from experimental inoculations. There are fluctuations in the levels of antibodies in chronic natural *N. caninum* infections (Packham et al. 1998). However, these fluctuations did not interfere in the identification of negative animals due to the high values of specificity that were obtained with this technique (Sp=97.9%).

The sensitivity and specificity of a particular serological test can vary according to the different cut-off points that are chosen (Dubey 2003, Lasri et al. 2004, Silva et al. 2007). Divergences in prevalences using different techniques and different cut-off points have also been reported in seroepidemiological studies about *T. gondii* infections in horses. Aroussi et al. (2015) reported that when the MAT (modified agglutination test) and the ELISA were both used, the seroprevalence had significant variations ranging between 13% and 90%. Dubey et al. (1990) concluded that the assessment of the prevalence would not be possible until additional studies were conducted to determine the sensitivity and specificity of serological tests for equine toxoplasmosis. Few studies on the seroprevalence of *N. caninum* infections in goats have used the NAT assay. This assay should be better evaluated in this animal species in additional studies to be conducted in the future.

CONCLUSIONS

The need for a careful interpretation of the serological test chosen by the investigator is essential during the diagnostic assessment of a herd and should include the analysis of individual serum samples.

We suggest that the IFAT should remain the assay of choice in the study of caprine neosporosis in individual serum samples. The cut-off points and guidelines provided in the present study should be followed.

We also recommend that, whenever possible, a combination of serological assays with high sensitivity and specificity is used in seroepidemiological surveys of caprine neosporosis.

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