

Reproductive failures associated with antibodies against caprine arthritis-encephalitis virus, *Toxoplasma gondii* and *Neospora caninum* in goats in the state of Sao Paulo, Brazil

Falhas reprodutivas associadas com a presença de do vírus da artrite-encefalite caprina, *Toxoplasma gondii* e *Neospora caninum* em caprinos no estado de São Paulo, Brasil

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Abstract

This study aimed at assessing the occurrence of antibodies against the caprine arthritis-encephalitis virus (CAEV), *Toxoplasma gondii* and *Neospora caninum*, as well as the associations between the presence of antibodies and the occurrence of reproductive failures in goats. Serum samples were collected from 923 goats of both sexes, over 3 months of age, from 17 dairy farms located in different municipalities of São Paulo State, Brazil. Infections by *T. gondii*, *N. caninum* and CAEV were evaluated by indirect methods of diagnosis based on indirect fluorescence antibody test (IFAT), *Neospora* agglutination test (NAT), and agar gel immunodiffusion (AGID), respectively. A survey was conducted on the farms to obtain information about reproduction dates (abortions, stillbirths and births of weak and premature kids) and zoosanitary management. Antibodies against CAEV, *T. gondii* and *N. caninum* was found in 37.81%, 23.62% and 17.23% respectively. There was no significant association between the presence of anti-CAEV antibodies and CAEV/*T. gondii* or CAEV/*N. caninum* co-infection, suggesting that CAEV does not predispose goats to infection by these agents. However, when CAEV/*T. gondii* ($p < 0.01$) or CAEV/*N. caninum* ($p < 0.001$) co-infection was present, the occurrence of reproductive failures was significantly higher what could indicate that CAEV-induced immunosuppression may predispose goats to develop the clinical symptoms of toxoplasmosis and neosporosis increasing the risks of the reproductive failures.

Keywords: Goat. CAEV. *Toxoplasma gondii*. *Neospora caninum*. Co-infection. Reproductive failures.

Resumo

O objetivo do presente estudo foi avaliar a ocorrência de anticorpos para o vírus da artrite-encefalite caprina (CAEV), *Toxoplasma gondii* e *Neospora caninum* e de possíveis associações entre a presença de anticorpos e a ocorrência de problemas reprodutivos em caprinos. Para tanto, foram colhidas amostras sanguíneas de 923 caprinos de ambos os sexos, acima de três meses de idade e oriundos de 17 propriedades leiteiras, de diferentes municípios do estado de São Paulo, Brasil. Os diagnósticos para *T. gondii*, *N. caninum* e CAEV foram baseados, respectivamente, na reação de imunofluorescência indireta (RIFI), teste de aglutinação para *Neospora* (NAT) e a imunodifusão em gel de ágar (IDGA). Um inquérito epidemiológico foi aplicado nas propriedades para obtenção de informações sobre dados reprodutivos (abortamentos, natimortalidade e nascimentos de filhotes fracos e prematuros) e de manejo zoossanitário. As ocorrências de anticorpos foram de 37,81% para CAEV, de 23,62% para *T. gondii* e de 17,23% para *N. caninum*. Não houve associação significativa entre a presença de anticorpos anti-CAEV e co-infecção com *T. gondii* ou *N. caninum*, sugerindo que o CAEV não predispõe os caprinos à infecção por estes agentes. Entretanto, quando havia, nas fazendas, animais com co-infecção pelo CAEV e *T. gondii* ($p < 0,01$) ou CAEV e *N. caninum* ($p < 0,001$) as ocorrências de falhas reprodutivas foram significativamente maiores, sugerindo que a imunossupressão causada pelo CAEV pode predispor os caprinos ao desenvolvimento de sintomas clínicos da toxoplasmose e neosporose, potencializando os riscos da ocorrência de problemas reprodutivos causados por estas enfermidades.

Palavras-chave: Caprino. CAEV. *Toxoplasma gondii*. *Neospora caninum*. Falhas reprodutivas.

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Introduction

Goats infected by the caprine arthritis-encephalitis virus (CAEV) are quite predisposed to other diseases. This is partly explained by the depressive effect that may be exerted by this lentivirus on immunological reserves¹. Nevertheless, associations of CAEV with other agents remain unreported.

Toxoplasmosis commonly affects small ruminants causing reproductive problems that lead to great economic losses. Goats are one of the most susceptible species to *T. gondii*^{2,3}. According to Tizard⁴, infections caused by protozoa are common in immunodepressed animals. In humans with acquired immunodeficiency syndrome (AIDS), toxoplasmosis can be aggressive and frequently fatal leading to infection of the central and visceral nervous system, as well as lymph nodes⁵. Studies conducted by Witt et al.⁶ and Lucas et al.⁷ in felines infected with feline immunodeficiency virus (FIV) showed that *T. gondii* infection is also more frequent in FIV-positive animals.

Neosporosis, in turn, is known to be not so relevant in ovine and caprines, but its occurrence should also be considered as it has already been demonstrated to be associated with the birth of weak and premature kids⁸. Tarantino et al.⁹, studying a dog with *Leishmania infantum* and *Neospora caninum* co-infection suggested that the immunosuppressive effects of *Leishmania* infection or long-term steroid therapy contributed to the development of *N. caninum* in this dog. Also in dogs, Cringoli et al.⁸ and Gennari et al.¹⁰ observed a significantly higher occurrence of *N. caninum* seropositivity among dogs that were *L. infantum*-positive.

The purpose of this study was to assess the associations of CAEV antibodies with the presence of antibodies against *T. gondii* and *N. caninum*, as well as to determine whether CAEV/*T. gondii* or CAEV/*N. caninum* co-infection influence the occurrence of reproductive problems in goats.

Material and Methods

Serum samples were collected from 923 goats of both sexes, older than 3 months and originated from 17 dairy farms located in different municipalities of the State of São Paulo, Brazil. Serum samples were kept at -20°C until analyze (agar gel immunodiffusion, AGID, for CAEV, indirect fluorescence antibody test, IFAT, for *T. gondii* and *Neospora* agglutination test, NAT, for *N. caninum*).

This study was approved by the Ethics Committee (CEEA) of School of Veterinary Medicine and Animal Science (UNESP) Botucatu, São Paulo, Brazil, with protocol number 17/2005.

During blood collection, a survey was conducted to obtain information on management and reproduction dates (abortions, stillbirths, and weak offspring).

Agar Gel Immunodiffusion (AGID) was performed according to Cultip et al.¹¹. The antigen used was produced by the CAEV sample provided by Dr. Yahia Chebloures of Laboratoire Associe de Recherches Sur Lés Lentivirus Chez Lés Petits Ruminants, INRA-ENVL, France, isolated by Crawford et al.¹² and replicated within the caprine synovial membrane by Abreu et al.¹³.

Indirect Fluorescence Antibody (IFAT) was performed using fluoresceín-isothiacin-labeled anti-goat IgG provided by the Laboratory of Zoonoses of Botucatu, Veterinary and Animal Science Faculty -UNESP together with the Center for Zoonoses Control of Municipality of São Paulo, according to Camargo¹⁴. Samples with a titer ≥ 16 were considered positive¹⁵.

Anti-*N. caninum* antibodies were assayed by the *Neospora* Agglutination Test (NAT) as described by Romand et al.¹⁶. *N. caninum* strain NC-1 tachyzoites were used with a cutoff value of 1:25. This test was performed in the Animal Parasitic Diseases Laboratory, United States Department of Agriculture, Beltsville, USA.

The associations of CAEV with *T. gondii* and *N. caninum* were analyzed by Goodman's association

test and the Chi-square test^{17,18}. Associations between agents and goat reproductive failures were assessed by the non-parametric test of Mann-Whitney¹⁹ with significant level of 5%.

Results

The analysis of the 923 serum samples showed that 349 (37.81%) of the animals were positive for CAEV, 218 (23.62%) for *T. gondii* and 159 (17.23%) for *N. caninum*. Overall, 344 (37.27%) were positive for at least one of the agents, 143 (15.49%) for two of the agents and 32 (3.47%) for all three agents under investigated (Table 1).

Table 2 shows the association of *T. gondii* and *N. caninum* distributed according to CAEV positivity or negativity. There was no significant association among

the presence of coccidian parasites in the CAEV-positive or CAEV-negative goats ($p > 0.05$).

Reproductive failures had no significant association ($p > 0.05$) with positivity for either *T. gondii* or *N. caninum* alone, but was significantly associated with CAEV positivity ($p < 0.05$). However, when CAEV/*T. gondii* ($p < 0.01$) or CAEV/*N. caninum* ($p < 0.001$) co-infections were present, the occurrence of reproductive failures was significantly higher (Table 3).

Discussion

Intercurrent infections associated with human or feline lentiviruses have been often described in the literature, probably due to the fact that these immunosuppressive agents lead to secondary infections caused by opportunistic parasites such as *T. gondii*²⁰.

Table 1 - Frequency (%) of antibodies anti-caprine arthritis-encephalitis virus (CAEV), *Toxoplasma gondii* (*T. gondii*) and *Neospora caninum* (*N. caninum*) in 923 goats from the state of São Paulo - Brazil

CAEV ¹	<i>T. gondii</i> ²	<i>N. caninum</i> ³	Goats	
			Positive	%
-	-	-	404	43.77
-	-	+	42	4.55
-	+	-	78	8.45
-	+	+	50	5.42
+	-	-	224	24.27
+	-	+	35	3.79
+	+	-	58	6.28
+	+	+	32	3.47

¹Agar Gel Immunodiffusion ²Indirect Fluorescence Antibody ³*Neospora* Agglutination Test

Table 2 - Associations among the presence of antibodies anti-CAEV, *T. gondii* and *N. caninum* in 923 goats from the state of São Paulo - Brazil

CAEV	<i>T. gondii</i> and <i>N. caninum</i> Associations				P
	T ¹ (-) N ² (-)	T(-) N (+)	T(+) N (-)	T(+) N (+)	
-	404 (70,38%) ^{AA}	42 (7,32%) ^{AC}	78 (13,59%) ^{AB}	50 (8,71%) ^{AA}	<0.001
+	224 (64,18%) ^{AA}	35 (10,03%) ^{AC}	58 (16,62%) ^{AB}	32 (9,17%) ^{AA}	<0.001
P value	>0.05	>0.05	>0.05	>0.05	

Same capital letters in the line – $P > 0.05$; same lower letters in the column – $P > 0.05$

¹*Toxoplasma gondii* ²*Neospora caninum*

Table 3 - Descriptive measurements of the occurrence of the infectious agents investigated according to reproductive failures - São Paulo - 2006

Infectious agent	Reproductive Failure		P value
	Present	Absent	
CAE	57,82 ^a (14.67; 69.84)	20.00 ^b (0.00; 49,15)	<0.05
<i>T. gondii</i>	13,01 ^a (4.76; 77.14)	6.41 ^a (0.00; 35,90)	>0.05
<i>N. caninum</i>	17.10 ^a (0.00; 81.25)	5.13 ^a (0.00; 78,21)	>0.05
CAEV \ <i>T. gondii</i>	9.23 ^a (0.00; 56.25)	0.00 ^b (0.00; 6,06)	<0.01
CAEV \ <i>N. caninum</i>	7.27 ^a (1.33; 44.29)	0.00 ^b (0.00; 1,69)	<0.001

Same letter in the line - P >0.05; Median (minimum value; maximum value)

However, reports on the goat lentivirus (CAEV) are still lacking.

In this study, antibodies against *T. gondii* or *N. caninum* were similarly observed in both CAEV-positive and CAEV-negative goats. These findings do not agree with those obtained in other studies of feline lentivirus/*T. gondii* co-infection^{6,7,21} where the frequency of *T. gondii*-seropositive cats was higher in the animals with FIV than in those without this viral infection. According to Heidel et al.²⁰, FIV infection induces changes in the defense mechanism that may reactivate latent infections in these animals.

The present study, no significant association was observed between positivity for *T. gondii* and/or *N. caninum* and reproductive failures such as abortion, stillbirth and weak and debilitated kids. The occurrence of anti-*T. gondii* antibodies not associated with clinical problems is commonly observed in goats^{15,22,23}. Similarly, clinical toxoplasmosis is relatively rare in humans, occurring in 10- 20% of the cases of infection^{24,25}. Nevertheless, symptoms of *T. gondii* infection are very common in HIV-infected individuals, and toxoplasmosis is an important cause of mortality and morbidity among them²⁶.

This is in agreement with Modolo et al.²⁷ who found no significant association ($p > 0.05$) between the presence of anti-CAEV antibodies and the occurrence of reproductive failures. However, when CAEV/*T. gondii* ($p < 0.01$) or CAEV/*N. caninum* ($p < 0.001$) co-in-

fections were detected in the goats, the occurrence of reproductive failures was significantly higher, indicating that the viral infection could be responsible for the reactivation of infection by *T. gondii* or *N. caninum* and clinical signs including reproductive failures.

Reports of *N. caninum*-positivity associated with concomitant viral infections in goats are not found in the literature. Yet, studies using bovines have shown that bovine herpesvirus type 1 (BHV1) and bovine viral diarrhea (BVD) were not considered a risk factor for the occurrence of *N. caninum*²⁸ the same was observed by Bartels et al.²⁹ who also found no association between abortion and bacterial or viral agents. Cringoli et al.⁸, in Italy, reported a higher occurrence of infection with *N. caninum* in dogs positive for *Leishmania infantum*. In addition, Gennari et al.¹⁰, in Araçatuba, SP, Brazil, found that the occurrence of anti-*N. caninum* antibodies (32.6%) was 8.6-fold higher ($p \leq 0.05$) in dogs naturally infected by *L. chagasi* than in those negative for this agent (3.8%). On the other hand, similar studies with both *L. chagasi* positive- and negative dogs in Campo Grande, failed to demonstrate any association with seropositivity for *N. caninum*³⁰.

Our results suggest that CAEV infection may predispose goats to develop the clinical symptoms of infection by *T. gondii* and *N. caninum*, increasing the risks of the reproductive failures that may follow these diseases.

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