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Communication

[Comunicação]

Canine distemper virus and *Toxoplasma gondii* co-infection in dogs with neurological signs

[Co-infecção pelo vírus da cinomose canina e *Toxoplasma gondii* em cães com sinais neurológicos]

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Canine distemper virus (CDV) is a highly contagious virus that occurs worldwide and causes a fatal disease in carnivores. CDV is a member of the genus *Morbillivirus* belonging to the family *Paramyxoviridae* that are responsible to infect some cell line from epithelial tissues of respiratory, gastrointestinal or nervous systems (Greene, 2006). On early stage, CDV infection shows a great diversity of signs in dogs such as respiratory, gastrointestinal, and cutaneous. In sequence or weeks later, neurological injuries occurs and in some cases may be yet the only sign presented without any other previous or concomitant signs (Amude *et al.*, 2007). In acute canine distemper encephalitis, CDV produces immunosuppression by acting directly on CD4+ cells. Therefore, dogs infected with CDV are more susceptible to develop secondary infections by *Toxoplasma gondii* and *Neospora caninum*, considered two neurotropic opportunists protozoan (Greene, 2006).

The aim of this study was to observe a possible association between CDV and *T. gondii* in dogs admitted at the Animal Infectious Diseases Service at the Universidade Estadual Paulista, Botucatu, SP, Brazil.

From 2001 to 2002, approximately 200 dogs suspected of CDV infection were attended in this

Service. From this total, 21 (12 males and nine females) dogs, with age ranging from 3 to 36 months, of different breeds and presenting classic signs of CDV were evaluated in this study.

Blood samples were obtained for detection of antibodies against *N. caninum* and *T. gondii* in serum according to Azevedo *et al.* (2005). Antibodies were detected by means of Indirect Fluorescence Antibody Test (IFAT) using tachyzoites of NC-1 strain of *N. caninum* propagated in VERO cell culture (IFAT ≥ 50) and RH strain tachyzoites of *T. gondii* propagated in Swiss mice (IFAT ≥ 64).

Even with instituted treatment (data not shown), all the selected animals died, and samples from central nervous system (CNS) were aseptically collected and evaluated by the presence of CDV RNA by RT-PCR assay. The RNA was extracted according to Boom *et al.* (1990). An aliquot of 300 μ L of suspension (20% w/v) of frozen fragments in phosphate-buffered saline (PBS pH 7.2) was evaluated. The RNA was eluted in diethylpyrocarbonate (DEPC)-treated water. The RT-PCR was carried out as previously described (Amude *et al.*, 2006a), using the set of primers designed to amplify a 287bp amplicons from the CDV nucleoprotein (NP) gene (Frisk *et al.*, 1999). Madin Darby canine kidney (MDCK)

cells infected with CDV Rockborn strain were used as positive controls. RT-PCR was performed with negative controls of the RNA extraction (sterile ultrapure water) and the reaction (clinical sample from a dog with neurocryptococcosis). The identities of the RT-PCR products were confirmed by restriction fragment length polymorphism (RFLP) with Hinf I (Invitrogen® Life Technology, USA) enzyme digestion.

The association between clinical signs, age, gender, breed, and RT-PCR for CDV and *T. gondii* and *N. caninum* serology results were evaluated using the Fisher's exact test, with confidence intervals of 95%. Analyses were done using EpiInfo 6.04.

Overall, 17 (80.9%) of the 21 fragments of the CNS examined presented CDV RNA by

RT-PCR assay. Concerning the clinical signs, only two dogs (#18 and #19) presented no neurological manifestations, however they were CDV positive by RT-PCR. From 19 dogs with neurological signs, 15 (79.0%) were CDV RT-PCR positive. The IFAT showed that eight (38.1%) of the 21 examined dogs presented antibodies anti-*T. gondii* (IFAT \geq 64), and one (4.7%) dog presented antibodies anti-*N. caninum* (IFAT=100). Only one (12.5%) *T. gondii* seropositive dog present no neurological signs (#19 dog).

Table 1 shows the results of RT-PCR, IFAT, and clinical signs of each dog. No association ($P>0.05$) was detected between age, gender, breed, clinical signs, and infection by CDV, *T. gondii*, and *N. caninum* antibodies in dogs.

Table 1. Detection of canine distemper virus (CDV) by RT-PCR assay and antibodies anti-*Toxoplasma gondii* and anti-*Neospora caninum* by indirect fluorescent antibody test in dogs with neurological symptoms

Dog Number	Gender	Age (months)	CDV	<i>T. gondii</i>	<i>N. caninum</i>	Clinical Symptom
1	F	36	+	-	-	myoclonus, weeping, and prostration
2	F	36	+	4096	-	myoclonus and paralysis of members
3	M	24	+	4096	100	weeping, prostration, and hyperesthesia
4	M	4	+	-	-	myoclonus, weeping, convulsions, cough, diarrhea, and ocular discharge
5	F	7	+	-	-	myoclonus, weeping, prostration, cough, and diarrhea
6	M	36	+	-	-	cough, nasal discharge, and ataxia
7	M	13	+	128	-	myoclonus, prostration, and convulsions
8	M	>36	+	-	-	myoclonus, weeping, and ataxia
9	M	12	+	-	-	myoclonus, convulsions, cough, diarrhea, and ocular discharge
10	M	36	-	-	-	paralysis of members and convulsion
11	M	4	-	-	-	myoclonus, paralysis of members, cough, ocular and nasal discharges
12	F	5	-	-	-	myoclonus and convulsions
13	F	>36	+	8192	-	ataxia, paddling, and walk in circles
14	F	>36	-	1024	-	myoclonus and prostration
15	F	18	+	-	-	myoclonus and prostration
16	F	>36	+	1024	-	myoclonus, paralysis of members, ocular and nasal discharges
17	M	4	+	-	-	myoclonus, prostration, hyperesthesia, and ataxia
18	M	16	+	-	-	cough, diarrhea, ocular and nasal discharges
19	M	3	+	2048	-	prostration and diarrhea
20	M	>36	+	-	-	hyperesthesia and convulsions
21	F	24	+	2048	-	myoclonus

M = Male; F = Female

Distemper is yet recognized as an important cause of neurological disease in dogs, even in animals presented without the classical form of CDV infection (Amude *et al.*, 2007). Although the widespread use of vaccination since the 1950s and 1960s markedly reduced worldwide the incidence of the disease, outbreaks still occur and neurological manifestations are considered important manifestations of distemper (Tipold *et al.*, 1992). The results showed high rate (79.0%) of CDV infection in dogs with neurological signs. In contrast, four dogs with neurologic manifestation were not positive to CDV indicating the importance of the differential diagnosis. One out of these four dogs had high antibodies titer against *T. gondii* (IFAT = 1024).

Furthermore, seven (41.1%) dogs showed simultaneously positive RT-PCR for CDV RNA and high titers (IFAT \geq 128) of antibodies to *T. gondii*, characterizing co-infection. In contrast, only one (4.7%) dog presented lower titer (IFAT = 100) against *N. caninum*.

Of the 17 dogs with CDV, 15 (88.2%) had classic and severe neurological manifestations. Others signs of CDV infection (Greene, 2006) such as prostration (87.5%) and cough (83.3%) were also recognized in those dogs. Among neurological symptoms, 14 (73.6%) dogs presented myoclonus; this sign was considered the most frequent neurological sign of distemper, being 11 (78.5%) determined in CDV positive animals. Myoclonus has been considered highly suggestive of canine distemper, although other CDV-free neurological diseases reported in dogs can also be present with myoclonic-like movements (Amude *et al.*, 2006b; Amude *et al.*,

2007). In addition, other paramyxoviruses infections and lead poisoning may also be presented with myoclonic movements (Amude *et al.*, 2006b).

Eight dogs had positive serology to *T. gondii*, being seven (87.5%) with neurological symptoms. Neurological deficits suggesting damages on nervous systems are one of some symptoms observed in *T. gondii* infections in dogs. The type and severity of clinical illness depend on the degree and localization of tissue injury, in which cell necrosis is caused by the intracellular growth of *T. gondii* (Greene, 2006). Silva *et al.* (2007) studying dogs that died due to nervous distemper, observed the presence of focal hemorrhagic and malacia in brain related to the presence of *T. gondii* cysts.

Despite a higher number of dogs suffering nervous disease showing positive RT-PCR results for CDV RNA and positive antibody titers simultaneously to *T. gondii*, these associations were not confirmed. However, the results of the present study reinforce that co-infection between these both neurovirulent infectious agents can increase the severity of neurological manifestations in dogs. On the other side *N. caninum* antibodies showed reduced association with dogs presenting neurological signs and CDV and *T. gondii* positive animals, indicative that neosporosis might play a low role in dogs with neurological manifestation in this region.

Keywords: dogs, canine distemper, toxoplasmosis, *Neospora caninum*, co-infection

RESUMO

O presente estudo relata a ocorrência de co-infecção entre o vírus da cinomose canina (CDV) e *Toxoplasma gondii* em cães com sinais neurológicos. Amostras de soro e tecido nervoso (pos-mortem) de 21 cães, suspeitos de cinomose canina foram analisadas pela Reação de Imunofluorescência indireta (RIFI) para pesquisa de anticorpos contra *T. gondii* e *N. caninum* e por RT-PCR para CDV. Dezesete (80,9%) cães foram positivos para o CDV pela RT-PCR e 8 (38,1%) foram positivos para anticorpos contra *T. gondii*. Sete cães (41,1%) apresentaram-se positivos para ambos agentes, caracterizando processo de co-infecção. Somente 1 (4,7%) cão foi soropositivo para *N. caninum* (RIFI=100), entretanto este mesmo animal foi positivo para *T. gondii* (RIFI=4096) e para CDV (RT-PCR).

Palavras-chave: cães, cinomose canina, toxoplasmose, *Neospora caninum*, co-infecção

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