Neospora caninum in dairy cattle in Paraná State, Brazil: histological and immunohistochemical analysis in fetuses

Neospora caninum em vacas leiteiras no Estado do Paraná, Brasil: análise histológica e imuno-histoquímica em fetos[Q]

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Resumo

Trinta e quatro fetos bovinos abortados, no Estado do Paraná, foram examinados através de análise histológica e imuno-histoquímica. O *Neospora caninum* foi detectado, pela imuno-histoquímica, em oito fetos que apresentavam miocardite e encefalite não supurativa. Quatro vacas foram soropositivas para o *Neospora caninum*. Este é o primeiro relato de neosporose congênita em fetos bovinos abortados, no Estado do Paraná, Brasil.
Palavras-chave: *Neospora caninum*, aborto, rebanho leiteiro

Abstract

Thirty-four bovine abortions (fetuses) from Paraná State were examined histologically and immunohistochemically. Eight fetuses had non-suppurative encephalitis and miocarditis, and *Neospora caninum* were detected by immunohistochemistry. Four cows were seropositive to *N. caninum*. It was the first detection of congenital neosporosis in aborted bovines fetuses, in Paraná State, Brazil.
Key words: *Neospora caninum,* abortion, dairy cattle

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Introdução

Neospora caninum (*N. caninum*) is recognized as a leading cause of abortion in cattle in many countries (DUBEY; LINDSAY, 1996; MOORE, 2005). Little is known of the causes of abortion in dairy cattle in Brazil. Gondim et al. (1999) first found *N. caninum* in an aborted fetus in Brazil. Recently, CORBELLINI et al. (2002) found *N. caninum* in 39% of 46 aborted bovine fetuses from Rio Grande do Sul. We report *N. caninum* associated bovine abortion for the first time in Paraná State, Brazil.

Material e Métodos

Thirty-four aborted cattle fetuses from 22 dairy herds from Paraná State were examined histologically, immunohistochemically and serologically for the presence of *N. caninum*. The abortions occur from January 2002 to February 2003 and the fetuses were sending to Londrina University State for diagnosis. The herds were vaccinated against brucellosis, leptospirosis, BHV-1 and BVDV. Fetuses' samples from brain, myocardium, thymus, lungs, liver, spleen, kidney, muscle and placenta were collected. Tissues were fixed in 10% phospate-buffered formalin. Sections of paraffin-embedded specimens were stained with hematoxylin and eosin (HE). Specimens with histological alterations were subjected to immunohistochemical (IHC) analysis with a polyclonal antibody. Blood samples from the dams of fetuses were collected on the day of abortion, and 20 and 65 days later. The sera were analyzed by indirect immunofluorescent antibody (IFA) for the detection of anti-*N. caninum* antibodies (CONRAD et al., 1993). Reactions with titers ≥200 to *N. caninum* were considered positive (DUBEY; LINDSAY, 1996).

Resultados e Discussão

Eight fetuses (23, 5%), three to eight months old, were positive for *N. caninum* by IHC. Non suppurative inflammation and clusters of tachyzoites were observed in brain and heart of these fetuses (Table I). Gliosis and focal necrosis of cerebral cortex were also seen. Discrete to severe inflammation occurred in several organs, regardless the age of pregnancy, as exemplified by fetuses 7 and 8. These findings differ from a previous report showing that younger fetuses have more necrosis, whereas older fetuses have more inflammation (OGINO; WATANABE; WATANABE, 1992). The most consistent microscopic lesions were observed in the brain of the fetuses, a finding previously reported (DUBEY, 1999). Five of the eight fetuses that presented encephalitis also had myocardial lesions, signalizing that the heart may be useful in the diagnosis, especially when the brain is autolyzed as suggested by Cox, Reichel e Griffiths (1998).

The IHC showed positive reaction to tachyzoites and tissues from eight fetuses. We also observed tissue staining unassociated with tachyzoites in two fetuses. This tissue staining could result from the reaction of phagocyted *N. caninum* antigens (WOUDA et al., 1997). The histological analysis is used as a screening method for the IHC technique. However, in abortions caused by neosporosis, histological lesions may be absent. Therefore, it is important to complement the histological tests (OTTER et al, 1997) with serological analysis of the cows and their calves.

Four cows were seropositive to *N. caninum* in the IFA and their fetuses (1, 5, 6, and 8) were also positive in IHC. However, cows 3, 4 and 7 were seronegative for *N. caninum* in all sera samples, but their fetuses were positive in IHC (Table I). Canada et al. (2004) reported that the percentage of aborted fetuses positive to neosporosis is high in Portugal and not only from dairy herds where abortion outbreaks occurred. MOORE (2005) remembers that some studies have suggested the vertical transmission as a common fact in South America.

Table 1. Microscopic and immunohistochemical (IHC) findings in bovine fetuses and, reactivity to *Neospora caninum* of dairy cows sera by indirect immunofluorescent antibodies (IFA), Paraná State, 2003.

Fetus N° / Gestational age Age of cow		Fetuses			Cows		
		Hematoxylin-Eosin		*N. caninum* IHC	*N.caninum* IFA Serum samples		
		En*	Myo**		Day 0	Day 20	Day 65
1/ 4y	5 1/2	+	+++	Brain and heart[a]	1:400	ND	ND
2/ 4y	6	+	+++	Brain and heart[a]	ND	ND	ND
3/ 3,6y	6	+	+	Tissue staining[b]	1:50	1:50	1:50
4/ 4,6y	7	+	-	Brain[a]	1:100	1:100	1:100
5/ 3y	4	+	-	Brain[a]	1:400	1:200	1:200
6/4,8y	3	+	+	Tissue staining[b]	1:400	1:200	1:200
7/4y	7	+++	-	Brain[a]	1:100	1:100	1:100
8/3y	4	+++	+++	Brain and heart[a]	1:400	1:800	1:400

y years; * Encephalitis; ** Myocarditis; + discrete and focal; ++ moderate and multifocal; +++ intense and diffuse; – not stained; ND not done; [a] tachyzoites stained; [b] tissue staining in brain.

There is consensus among the authors that the serological test alone is not satisfactory to detect neosporosis, and that seronegative status can not exclude *N. caninum* as the cause of abortion. Negative results in serological tests must not be considered conclusive, because some infected cows or even calves may not have circulating antibodies (OTTER et al, 1997).

In conclusion, our results are in agreement with the findings of Guimarães et. al. (2004) who reported that the *N. caninum* is an important cause of the abortions in dairy cattle in Paraná State, Brazil. Additionally, the histological, immunohistochemical and serological results showed for the first time, the involvement of the *N. caninum* in the reproductive problems in dairy cattle of Paraná State.

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