



Influence of euthanasia, the intensity of inflammatory lesion and viral load in the laboratory diagnosis of rabies in cattle¹

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ABSTRACT.- Wisser C.S., Fernandes M.E.S., Melchiorretto E., Ogliari D., Gava A., Batista H.B.C.R., Mori E. & Traverso S.D. 2021. **Influence of euthanasia, the intensity of inflammatory lesion and viral load in the laboratory diagnosis of rabies in cattle.** *Pesquisa Veterinária Brasileira* 41:e06782, 2021. Centro de Ciências Agroveterinárias, Universidade do Estado de Santa Catarina, Av. Luiz de Camões 2090, Bairro Conta Dinheiro, Lages, SC 88520-000, Brazil. E-mail: claudiawisser@hotmail.com

This research reports the use of different diagnostic tests in cattle, naturally infected by *Rabies lyssavirus* (RABV), and correlates the positivity of the tests with the clinical moment of euthanasia, the intensity of the inflammatory lesion and viral load. It also highlights the possibility of euthanasia in early stages of the disease as a way to improve animal welfare. For that, samples of 34 bovine brains were collected for analysis, preserved in 10% buffered formaline and refrigerated with subsequent freezing. The samples were subjected to direct immunofluorescence antibody technique (DFAT) tests, viral isolation in cell culture (VICC), histopathology with hematoxylin and eosin staining (HE), immunohistochemistry (IHC), Shorr stained neural tissue smears (DSS), Reverse transcription polymerase chain reaction (RT-PCR) and polymerase chain reaction by quantitative reverse transcriptase (qRT-PCR). The areas used for analysis were the cerebellum, parietal telencephalon and thalamus. Samples with Negri bodies (NBs) or immunostaining in at least one of the analyzed areas were considered positive. For the study of the intensity of histological lesions, the lesions were classified into grades 0, 1, 2 and 3 and the positivity of the test in the presence or absence of NBs in one of the three areas analyzed. To verify the influence of the disease clinical evolution, 4-four groups of analysis were created according to the animal's clinical status at moment of the euthanasia, being: M1 = animal euthanized while standing, M2 = euthanized when in sternal recumbence, M3 = euthanized when in lateral recumbence, M4 = animal with natural death. Of the 34 brains evaluated, IHC was positive in 100% of cases, DFAT was positive in 97.05% of them, and in this negative sample the presence of RABV was confirmed by VICC. NBs were seen in 88.23% of the cases, and the DSS test was positive in 82.35% of them. All diagnostic techniques showed positive cases in all groups analyzed. Each case was positive in at least two diagnostic methods. All cases that contained NBs were positive for rabies in the other tests. In this study, it was observed that the variables analyzed (intensity of injury and clinical evolution at the moment of euthanasia) had an influence only

¹ Received on March 4, 2021.

Accepted for publication on April 12, 2021.

Part of the Doctoral Dissertation of the first author.

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on HE and DSS techniques, which are based on NB research to form the diagnosis, but did not interfere with the effectiveness of the diagnosis performed by detecting the viral antigen performed by DFAT and IHC. All isolated RABV samples included in the present study have a genetic lineage characteristic of hematophagous bats *Desmodus rotundus*. The evaluation of qRT-PCR showed that the amount of virus did not interfere in the positivity of the tests. This work shows that IHC and DFAT are safe diagnostic techniques. They are capable of detecting RABV even in euthanized animals in the early stages of clinical evolution with mild intensities of histological lesions.

INDEX TERMS: Euthanasia, inflammatory lesion, viral load, diagnosis, rabies, cattle, detection of RABV, positivity of the test, encephalitis.

RESUMO.- [Influência da eutanásia, intensidade da lesão inflamatória e carga viral no diagnóstico laboratorial da raiva em bovinos.] Esta pesquisa relata a utilização de diferentes testes de diagnóstico em bovinos, naturalmente infectados pelo *Rabies lyssavirus* (RABV), e correlaciona a positividade dos testes com o momento clínico da eutanásia, a intensidade da lesão inflamatória, e a carga viral. Salienta também a possibilidade da eutanásia em estágios precoces da doença como forma de melhorar o bem-estar animal. Para isso amostras de 34 encéfalos bovinos foram coletados para análise, conservadas em formol tamponado 10% e sob refrigeração com posterior congelamento. As amostras foram submetidas aos testes de imunofluorescência direta (IFD), isolamento viral em cultivo de células (IVCC), histopatologia com coloração de hematoxilina e eosina (HE), imuno-histoquímica (IHQ), esfregaço direto com coloração de Shorr (EDS), reação da polimerase em cadeia por transcriptase reversa (RT-PCR) e reação da polimerase em cadeia por transcriptase reversa quantitativo (qRT-PCR). As áreas utilizadas para análise foram o cerebelo, telencéfalo parietal e tálamo. Foram consideradas positivas as amostras que apresentaram Corpúsculo de Negri (CNs) ou imuno-marcação em ao menos uma das áreas analisadas. Para o estudo da intensidade das lesões histológicas, as lesões foram classificadas em graus 0, 1, 2 e 3 e a positividade do teste na presença ou ausência de CN em uma das três áreas analisadas. Para verificar a influência da evolução clínica da doença foram criados 4 grupos de análise conforme o estado clínico do animal no momento da eutanásia, sendo: M1 = animal eutanasiado em estação, M2 = eutanasiado em decúbito esternal, M3 = eutanasiado em decúbito lateral, M4 = animal com morte natural. Dos 34 encéfalos avaliados a IHQ foi positiva em 100% dos casos, a IFD foi positiva em 97,05%, sendo que na amostra negativa a presença de RABV foi confirmada por IVCC. A histologia com HE, através da visualização das CNs, foi positiva em 88,23 % dos casos, e o teste de EDS, foi positivo em 82,35%. Todas as técnicas de diagnóstico apresentaram casos positivos em todos os grupos analisados. Cada caso foi positivo em, pelo menos, dois métodos de diagnóstico. Todos os casos que continham CN foram positivos para raiva nos demais testes. Nesse estudo observou-se que as variáveis analisadas intensidade de lesão e evolução clínica no momento da eutanásia tiveram influência somente nas técnicas de HE e EDS, que se baseiam na pesquisa do CN para formação do diagnóstico, mas não interferiram na eficácia do diagnóstico realizado através da detecção do antígeno viral realizado por IFD e IHQ. Todas as amostras RABV isoladas incluídas no presente estudo apresentam linhagem genética característica de morcegos hematófagos *Desmodus rotundus*. A avaliação de qRT-PCR demonstrou que a quantidade de vírus não interferiu na positividade dos testes.

Esse trabalho mostra que a IHQ e a IFD são técnicas seguras de diagnóstico e que mesmo em animais eutanasiados em estágios iniciais de evolução clínica com intensidades leve de lesões histológicas, são capazes de detectar o RABV.

TERMOS DE INDEXAÇÃO: Eutanásia, lesão inflamatória, carga viral, diagnóstico, raiva, bovinos, detecção de RABV, positividade do teste, encefalite.

INTRODUCTION

Rabies is an infectious disease of mandatory notification caused by RABV (*Rabies lyssavirus*), an RNA virus of the *Lyssavirus* genus that affects the Central Nervous System (CNS) of different species. This almost 100% fatal disease is a widely distributed zoonosis (Buchen-Osmond 2003, Faizee et al. 2012, Beigh et al. 2015).

Direct immunofluorescence antibody technique (DFAT) and viral isolation are the gold standard tests for diagnosing the disease, and viral isolation can be performed in cell culture (VICC) or through intracerebral inoculation in mice (Mouse intracerebral test - MIT) (WHO 2013). In addition to these strategies, other methods are applied in the laboratory routine, such as histological diagnosis through immunohistochemistry (Pedroso et al. 2008), or through the visualization of Negri bodies (NB) by hematoxylin and eosin (HE) and Seller's stains (Sharma et al. 2014).

Although the rabies laboratory diagnosis is well established, the relationship of the virus detection between the clinical disease evolution and the intensity of histological lesions still represent a wide field of study. Several studies have compared the sensitivity and specificity of the diagnostic techniques (Faizee et al. 2012, Hananeh et al. 2015); however these do not contemplate the influence of clinical evolution on the test results. Some studies report that euthanasia does not interfere with diagnosis efficiency (Ribas et al. 2013), but these studies do not inform at what clinical stage of the disease euthanasia was performed, whether at an early stage or *in extremis*. Recent studies show that clinical evolution disease does not affect the intensity of injuries (Wisser et al. 2020), but it remains to be seen whether the intensity of the injury affects the diagnostic test's efficiency.

The assumption that pathological changes in rabies are more easily observed when tissues are harvested after the spontaneous death of the animal, as well as the lack of evidence of injuries and NBs in the case of sacrifice in the early stages of the disease, have been causing the avoidance of euthanasia of animals suspected of rabies, choosing to collect samples for diagnosis only after natural death, or euthanasia in terminal stages of the disease

Choosing to keep the animal suspected of rabies alive until its spontaneous death, to collect material, implies subjecting it to hunger and thirst unnecessarily since the terminal stage of the disease is characterized by flaccid paralysis with an inability to swallow, a situation contrary to the precepts of animal welfare that has been increasingly recommended. Based on these considerations, the present study provides information on the detection of RABV through DFAT, immunohistochemistry (IHC), and the detection of NBs by HE staining and direct smear with Shorr staining (DSS), in 34 naturally infected, naturally killed, or euthanized cattle in different clinical stages of the disease, with different intensities of histological lesions and different amounts of viral antigens in the analyzed samples.

MATERIALS AND METHODS

Thirty-four samples of the CNS were obtained during the follow-up of bovine rabies in Santa Catarina, southern Brazil. Of these, 31 samples were collected by the team from the animal pathology laboratory of CAV-UDESC and three were sent by field veterinary practitioners.

The samples were fixed in 10% buffered formalin or refrigerated with subsequent freezing. The areas used for analysis were the cerebellum, parietal telencephalon and thalamus. The samples preserved in formalin were submitted to histopathology with HE and IHC stains. For IHC, polyclonal primary antibody was used in dilution 1:1000 (No. 5199, Chemicon International Inc., Temecula/CA), using LSAB-HRP kit (DakoCytomation), and as chromogen DAB (diaminobenzidine, DakoCytomation), as described by Wisser et al. (2020). For the study of the intensity of histological lesions, the lesions were classified into grades 0, 1, 2 and 3, as described by Wisser et al. (2020) and the positivity of the test in the presence or absence of NBs in one of the three areas analyzed. For this analysis, only the brains collected by the laboratory team were selected, and 31 cases were used. For the study of samples collected in HE and DSS, the intensity of the injury was confronted with the presence of the corpuscle in the same area of analysis.

In samples preserved refrigerated, the DSS were performed on the cerebellum upon arrival at the laboratory. The remainder was frozen for DFAT testing. The samples were sent in two stages with intervals of 3 years and remained frozen in conventional freezers during this period. For DFAT, smears were made on histological slides of thalamus, cerebellum and telencephalon, fixed in acetone and submitted to DFAT, with polyclonal antinucleocapsid antirabic conjugate, produced at the Pasteur Institute of São Paulo/SP (Dean et al. 1996).

For the analysis of IHC and DFAT, samples with marking in at least one of the analyzed areas were considered positive, without taking into account the area of analysis. Results were considered negative if they did not show any mark in the three tested areas. The samples that obtained divergent results in the DFAT and IHC techniques were also subjected to VICC in murine neuroblastoma cells (Neuro-2) (Castilho et al. 2007).

The 34 samples were sent to the Pasteur Institute for molecular characterization performed using RT-PCR followed by genetic sequencing and viral amplification using qRT-PCR as described by Wisser et al. (2020).

In order to verify the influence of the clinical evolution of the disease, four groups of analysis were created according to the animal's clinical status at the moment of euthanasia, being named Moment 1 (M1) = standing euthanized animals with walking capacity and slight signs of motor incoordination, Moment 2 (M2) = euthanized when in sternal recumbence, Moment 3 (M3) = euthanized when in lateral recumbence and Moment 4 (M4) = animal with spontaneous death.

The procedures for the current study were submitted to and approved by the Ethics Committee in Experimentation of the "Centro de Ciências Agroveterinárias" (CAV) of the "Universidade do Estado de Santa Catarina" (UDESC), protocol number 1.06.15.

RESULTS

Outbreaks of bovine rabies were followed in nine municipalities, located in the Eastern, Valley, and Southern regions of the State of Santa Catarina, southern Brazil, from 2011 to 2016. The age of the affected bovines ranged from four months to 13 years, and no bovine had received rabies vaccine. Histological lesions consisted of lymphocytic and macrophage meningoencephalitis, associated with gliosis, with intensities ranging from mild to severe associated with NBs in most samples.

Of the 34 brains evaluated, IHC was positive in 100% of cases, DFAT was positive in 97.05% (33/34) of them, and in this negative sample, the presence of RABV was confirmed by VICC. Histology with HE, through the visualization of NBs, was positive in 88.23% (30/34) of the cases, and the DSS, was positive in 82.35% (28/34) of them. All diagnostic techniques showed positive cases in all groups analyzed (intensity of injury, brain area analyzed and clinical euthanasia moments). Each case was positive in at least two diagnostic methods. All cases that contained NB were positive for rabies in the other tests. The general overview of the results in each sample analyzed can be seen in Table 1.

The correlation between the intensity of the lesion and the corpuscle's in the same area of analysis by the HE and DSS techniques is shown in Figure 1. The techniques were less efficient in the ones with a lower injury intensity. The cerebellum was the best area for visualizing the NBs in the HE examination.

Of the 34 brains evaluated, six samples were collected in M1 and M2, 18 in M3 and four in M4. IHC was positive in 100% of cases, regardless of natural death or euthanasia. DFAT was positive in 97.05% of cases, this technique being negative only in an euthanized bovine at an early stage of the disease (M1), however, the presence of RABV in this sample was confirmed by VICC. The positivity of the tests was influenced by euthanasia for HE and DSS staining techniques (Fig.2).

The 34 samples analyzed belonged to the genetic lineage characteristic of hematophagous bats *Desmodus rotundus* and nine of them managed to be amplified, Bovine number 2, 3, 9, 14, 16, 26, 29, 32 and 34 (Table 1). IHC and DFAT were positive in all amplified samples. HE and DSS were negative in the second highest amplification sample (Bovine 2).

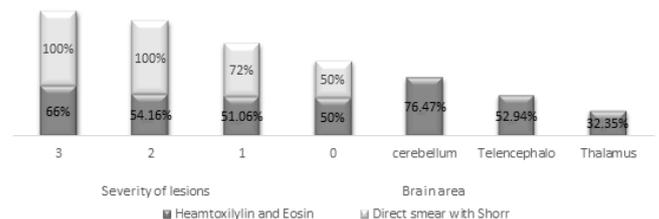


Fig.1. Percentage of Negri bodies (NB) in the different intensities of the histological lesions, for the techniques of hematoxylin and eosin (HE) and direct smear stained by Shorr (DSS) and percentage of NB in the different brain areas by the technique HE.

Table 1. Individual results for cattle, at different moments of euthanasia, diagnostic techniques, intensity of inflammatory lesion and viral load

Bovine	Euthanasia moment	HE	DSS	IHC	DFAT	Severity inflammation and presence of Negri inclusion bodies in each region			RT-PCR
						Cerebellun	Telencéphalon	Thalamus	
1	M1	+	+	+	-	1 - NB	1	3	
2	M1	-	-	+	+	1	0	2	28,05
3	M1	+	+	+	+	1 - NB	1 - NB	2	13,074
4	M1	+	+	+	+	1 - NB	2	1	
5	M1	+	+	+	+	3	2 - NB	2 - NB	
6	M1	-	-	+	+	1 - NB	1	3	
7	M2	+	+	+	+	0 - NB	0 - NB	1 - NB	
8	M2	+	-	+	+	0 - NB	0	1 - NB	
9	M2	+	+	+	+	1 - NB	1	2	19,431
10	M2	+	+	+	+	2	1	1	
11	M2	+	+	+	+	1 - NB	1	1 - NB	
12	M2	+	+	+	+	1	0	2 - NB	
13	M2	+	+	+	+	UR-NB	UR-NB	UR	
14	M3	+	+	+	+	2 - NB	1 - NB	2 - NB	18,29
15	M3	+	+	+	+	2 - NB	1 - NB	2	19,092
16	M3	+	+	+	+	1 - NB	0 - NB	1	
17	M3	+	+	+	+	1 - NB	1	1	
18	M3	+	-	+	+	1	1	2 - NB	
19	M3	+	+	+	+	1 - NB	0	1	
20	M3	+	-	+	+	1 - NB	0 - NB	1	
21	M3	-	-	+	+	1	0	1	
22	M3	+	+	+	+	UR-NB	UR-NB	UR	
23	M3	+	+	+	+	UR-NB	UR-NB	UR	
24	M3	+	+	+	+	1	1 - NB	2	
25	M3	+	+	+	+	3 - NB	1 - NB	3	
26	M3	+	+	+	+	2 - NB	2	2	22,7
27	M3	+	+	+	+	3 - NB	1 - NB	2	
28	M3	+	+	+	+	2 - NB	1 - NB	3 - NB	
29	M3	+	+	+	+	3 - NB	1 - NB	3 - NB	32,396
30	M3	-	+	+	+	1	1	1	
31	M4	+	+	+	+	1 - NB	1 - NB	2	
32	M4	+	+	+	+	1 - NB	1	3	22,41
33	M4	+	+	+	+	2 - NB	2 - NB	3 - NB	
34	M4	+	+	+	+	2 - NB	2 - NB	3 - NB	21,38

HE = Heamtoxylin and eosin, DSS = direct smear with Shorr staining, IHC = immunohistochemistry, DFAT= direct immunofluorescence antibody technique, RT-PCR = reverse transcription polymerase chain reaction; M1 = Standing euthanized cattle's with locomotion capacity and mild signs of motor incoordination, M2 = euthanized in when sternal recumbence, M3 = euthanized when in lateral recumbence, M4 = dead naturally; (+) positive technique, (-) negative technique N+ negative; NB = presence of Negri bodies in this region, UR = unrated region.

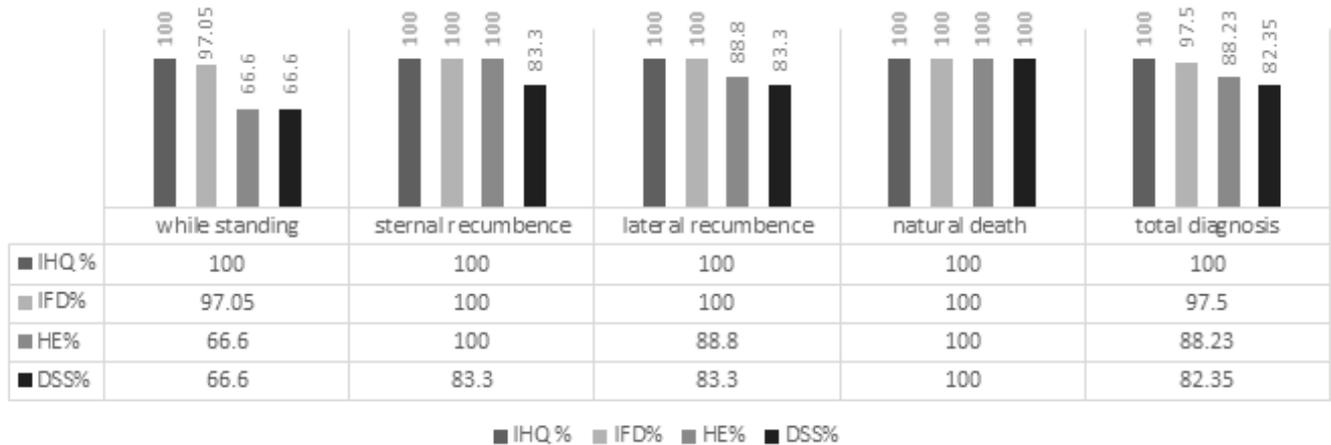


Fig.2. Percentage of total positive samples and at different moments of euthanasia, in the different diagnostic techniques used. IHQ = immunohistochemistry, IFD = direct immunofluorescence, HE = hematoxylin and eosin, DSS = Shorr stained neural tissue smears.

DISCUSSION

In this study, it was observed that the analyzed variables, lesion intensity, and clinical evolution at the moment of euthanasia, had an influence only on HE and DSS techniques, which are based on NB research to form the diagnosis.

It is known that the NBs observed by histology in the histochemical staining of HE, Sellers and Shorr, is pathognomonic for rabies. In this study, all tested diagnostic techniques were positive at different moments of clinical evolution, regardless of natural death or euthanasia; however, analyzing moments M1 and M4, both in the HE and DSS technique, it was found that in animals euthanized in season, the percentage of NB samples was lower (66.6%) when compared to those of spontaneous death (100%). This finding corroborates the precepts suggested by other authors, that the sacrifice of infected animals can result in cases with no NBs (Summers et al. 1995).

It is worth mentioning that all samples that contained NBs were later confirmed as rabies by the other techniques, even when the DFAT was negative. Cytology is a fast and inexpensive method that can be performed as a screening right after the animal's death. Although other tests should always reevaluate negative results, a positive result by cytology serves as an alert for the imminence of cases of rabies and streamlines prevention and control measures until the result of official methods is completed.

Likewise, the intensity of the injury has been shown to compromise the tests' positivity that requires NBs, being less effective, the lower the injury's intensity. This contradicts the precept that NBs tend to be more scarce where inflammation is more severe Maxie & Youssef (2006) and is in line with the results of Wisser et al. (2020) who observed that the region with the highest intensity of inflammatory injury, the obex, was the second most likely to detect NB. In the DSS all samples were positive at intensities 2 and 3 and in HE staining only two samples were negative at intensities 2 and 3 (Bovines 2 and 6 respectively), both euthanized while standing. Evidencing the interference of euthanasia in early clinical stages through diagnostic techniques that require detection of NBs.

Regarding the distribution of the lesion, the cerebellum was the best area for visualizing the corpuscle, similar to what was described by Wisser et al (2020), Silva et al. (2010)

and Langohr et al. (2003). It is known that rabies does not infect all brain structures equally, thus varying the presence of NB with the analyzed area and thus compromising the diagnosis efficiency.

When we compare the efficiency of the techniques that are based on the detection of the antigen, we observe the positivity of 100% of the IHC and 97.05% of the DFAT, we realize that they are superior to those found in HE and DSS, which were 88.23% and 82, 35%, respectively, which are based on the visualization of the NBs. In this study, only thalamus, cerebellum, and telencephalon were used, so for those laboratories that do not perform IHC, it is suggested the examination more areas, including the spinal cord (Bassuino et al. 2016) the trigeminal ganglion, and the stean (Wisser et al. 2020), can also contribute in this sense.

Currently, most laboratories perform the diagnosis of rabies by detecting the viral antigen, whether by DFAT and IHC, and for these techniques, the variables studied (intensity of injury and clinical moment of euthanasia) did not influence the positivity of the tests, as they did not prevent the detection of RABV regardless of the intensity of the injury or the moment of euthanasia.

IHC is widely used in the diagnostic routine in pathology laboratories and was 100% effective in diagnosing the disease. The virus's presence was detected in all samples regardless of natural death or euthanasia and even in areas with lesion intensity 1. A similar fact had been observed by other authors when evaluating samples of experimentally inoculated mice, which obtained positive results in animals with euthanasia or spontaneous death (Healy et al. 2013) and in euthanized cattle for diagnosis (Ribas et al. 2013). A critical alternative when the gold standard is not viable or the conventional histopathology with HE and DFAT cannot detect lesions or the viral antigen (Beigh et al. 2015), as occurred in this study, in addition to having the advantage of rapid virus inactivation during collection, making transportation and processing safer (Sharma et al. 2014).

DFAT and viral isolation are the gold standard techniques for the diagnosis of rabies (WHO 2013). In this research, DFAT was positive in 97% of cases (33/34), and is within the predicted values of 95-99% when performed on fresh samples (MAPA 2009). The false-negative sample belonged to the M1 group

(animals euthanized in season) and was later confirmed by viral isolation. Regarding the influence of clinical evolution in this fact, it should be considered that although the DFAT is highly sensitive and specific, negative results in this technique are often described even in those samples collected in animals with spontaneous death or euthanized *in extremis* (Langohr et al. 2003, Ferreira et al. 2012, Bassuino et al. 2016). The isolation of the virus confirmed by VICC, demonstrates the presence of this virus in the brain in sufficiently detectable quantities for diagnosis, as well as it was proven in the other diagnostic techniques performed. In addition, all other samples of the M1, M2 and M3 moments, bovines also euthanized for examination were positive. Therefore, this suggests that euthanasia did not interfere in DFAT's diagnosis or DFAT followed by viral isolation.

Similarly, the intensity of the injury and the analysis area was not the cause of the non-detection of the viral antigen by the DFAT. When analyzing Table 1, we observed that Bovine 1 (negative in DFAT) was negative in all areas of analysis. According to Bingham & van der Merwe (2002) in all species, the brain stem, especially the thalamus, is the most reliable area for analysis; for them, the thalamus most often had the most abundant antigen, making it the easiest diagnostic area for fluorescent antibodies test. This indicates that the analysis area did not influence the negative result of the DFAT, since the thalamus was included in the study. In addition, the inflammatory intensity also did not interfere with the test's positivity because the same case had different lesions intensity (3 in the thalamus and 1 in the cerebellum and telencephalon), being negative in all of them.

We know that several factors can influence the accuracy of the rabies diagnosis, among them: low viral titration examiner's (Silva et al. 2010); knowledge of the performer; base material used, the quality of the anti-rabies conjugate (WHO 2013), individual reactions; the conservation status of the samples (Germano et al. 1977).

The negative sample at DFAT was collected by the laboratory staff, being immediately refrigerated and frozen 2 hours later, remaining frozen until the samples were sent to analysis. Perhaps the freezing time and the sample's storage during transport may have interfered with the detection of RABV in this case.

Regarding the influence of qRT-PCR in the tests, the IHC and DFAT detected the antigens (RABV) in the samples with greater and lesser amplification, regardless of the moments of euthanasia and the different intensity of lesions. Although in one of the samples (Bovine 1) the HE and DSS techniques were negative, it should be noted that these diagnostic methods reacted positively in samples with greater and lesser viral amplification, Bovines 29 and 3, respectively. Thus, this indicates that the viral load, by itself, was not able to influence the negative result, for these techniques, in these analysis regions.

Intracytoplasmic inclusion bodies (NBs) in neurons have been the method of choice in the diagnosis of rabies for many years. DFAT was used for the first time in 1958, but only in 1970, it became a routine method (Woldehiwet 2005). The regularity with which inclusions are observed is quite variable. In this research, 88.23% of the samples (30/34) were positive, other authors found similar values, ranging from 68 to 87% (Langohr et al. 2003, Lima et al. 2005, Silva et al. 2010). It is

likely that the inconstancy in the appearance of the corpuscle contributed to the hypothesis that euthanasia impairs the diagnosis since before 1970, the confirmatory diagnosis depended exclusively on the visualization of the NBs.

From 1903 to the present day, more than 100 years have passed, and significant improvements have been made in equipment and diagnostic methods. Techniques such as DFAT and viral isolation have been improved, and the emergence of other methods such as IHC, direct IHC, RT-PCR that have now been incorporated into laboratory routines. This work exposes that IHC and DFAT are safe diagnostic techniques and that even in animals euthanized in the early stages of clinical evolution, with mild intensities of histological lesions, the techniques are capable of detecting RABV. Thus, early euthanasia can be performed to reduce animal suffering, without prejudice to the diagnosis.

CONCLUSION

Early euthanasia and the intensity of an inflammatory lesion do not interfere with the positivity of the immunohistochemistry (IHC) and direct immunofluorescence antibody technique (DFAT) tests, but they do interfere with the positivity of the tests that are based on the research of and Negri bodies (NBs). The viral load in the 9 samples analyzed did not affect the positivity of the tests.

Funding.- This study was funded by "Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina" (FAPESC).

Conflict of interest statement.- The author declares no conflict of interest with respect to the research, authorship and/or participation in this article.

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