

Frequency of Anti-*Neospora Caninum* Antibodies in Ovines Destined to Clandestine Slaughter in Rio de Janeiro

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RESUMO:

SILVA, A. F.; R. I. J. COSENDEY; F. B. ZANDONADI, F. C. R. OLIVEIRA, W. LILENBAUM; G. MARTINS; S. S. VENTURI; A. M. R. FERREIRA. *Frequência de anticorpos anti-Neospora caninum em ovinos destinados ao abate clandestino no Rio de Janeiro. Revista Série Ciências da Vida.* Estudos sorológicos de frequência de anticorpos anti-*N. caninum* comprovam a disseminação e importância da enfermidade em ovinos, as taxas variaram desde 1.8% no Rio Grande do Norte, Brasil, até 63% no norte da Jordânia. O presente estudo teve por objetivo determinar a frequência de anticorpos anti- *N. caninum* em ovinos abatidos clandestinamente no estado do Rio de Janeiro. Amostras de sangue de 132 ovinos foram obtidas em cinco locais que fazem abates de ovinos sem a devida fiscalização sanitária. Para detecção de anticorpos anti-*N. caninum* foi utilizado o teste de ELISA indireto para ruminantes na diluição 1:10 realizado e interpretado conforme as especificações do fabricante. Os resultados demonstraram que 12% (17/132) dos ovinos testados apresentaram anticorpos anti-*N. caninum* em seus soros. O estudo demonstrou que o *N. caninum* está presente em ovinos destinados ao abate sem fiscalização no estado do Rio de Janeiro. Sugerimos que mais pesquisas devem ser realizadas para determinação dos meios de transmissão entre os ruminantes infectados e os seres humanos.

Palavras-chave: *Neospora caninum*, ovinos, abate clandestino.

ABSTRACT:

SILVA, A. F.; R. I. J. COSENDEY; F. B. ZANDONADI, F. C. R. OLIVEIRA, W. LILENBAUM; G. MARTINS; S. S. VENTURI; A. M. R. FERREIRA. Serological studies on the frequency of against *N. caninum* prove the dissemination and important of this disease in ovine, with percentages ranging from 1.8% in Rio Grande do Norte, Brazil, to 63% in the north of Jordan. This study aimed to determine the frequency of antibodies to *N. caninum* in sheep slaughtered in properties or slaughterhouse illegally in the state of Rio de Janeiro, Brazil. Blood samples from 132 ovines were collected at five sites that are slaughtering ovine without proper sanitary inspection. In order to detect anti-*N. caninum* antibodies an indirect ELISA for ruminant animals was used, with final dilution 1:10 and performed and interpreted according to the manufacturer's specifications. The results showed that 12% (17/132) of sheep tested had antibodies *N. caninum* in their sera. The study demonstrates that *N. caninum* is present in ovine in the State of Rio de Janeiro. Further research to unravel the means of transmission between ruminants infected and humans.

Key Words: *Neospora caninum*, ovine, clandestine slaughter.

Introduction

Neospora caninum (*N. caninum*) was first reported as a parasite similar to *Toxoplasma gondii* producing clinical neurological signs in boxer dogs in Norway (BJERKAS et al., 1984). Dubey et al. (1988) reported differences in this parasite creating a new genre with a single specie *N. caninum*. Since then *N. caninum* has been reported in several species as sheep, goats and cattle (DUBEY, 2003) in many countries as New Zealand (REICHEL et al., 1998), Argentina (BASSO et al., 2001) and Italy (CRINGOLI et al., 2002).

Serological studies on the frequency of antibodies against *N. caninum* demonstrate the dissemination of neosporosis in ovine, with percentages ranging from 1.8% in Rio Grande do Norte, Brazil, (SOARES et al., 2009) to 63% in the north of Jordan (ABO-SHEHADA & ABU-HALAWEH, 2010). In Brazil, studies of the

prevalence of *N. caninum* in ovine have been conducted in São Paulo (FIGLIUOLO et al., 2004), Rondônia (AGUIAR et al., 2004), Rio Grande do Sul (VOGEL et al., 2006), Paraná (ROMANELLI et al., 2007), Distrito Federal (UENO et al., 2009), Rio Grande do Norte (SOARES et al., 2009), Mato Grosso do Sul (ANDREOTTI et al., 2009) and Alagoas (FARIA et al., 2010).

In ovine this disease was historically recognized in England for Hartley and Bridge (1975), in a lamb that died within one week of age with suspected toxoplasmosis. After fifteen years Dubey et al. (1990), published the identification of *N. caninum* after a new analysis of the tissue of this lamb. Although the anti-*N. caninum* antibodies has been reported in humans, the importance of disease in humans has not been determined (TRANAS et al., 1999, LOBATO et al., 2006, MCCANN et al., 2008, BENETTI et al., 2009). Thus people who have direct contact with the meat and products derived from this can be exposed to the infection by *N. caninum*. This risk is even greater when the slaughtering of animals is illegal and there is no security apparatus for people who handle the meat of these animals during slaughter (FREITAS et al., 2001).

Illegal slaughter is a deplorable practice that occurs in many countries, it represents one of the most serious risk factors for the exhibition to infectious agents, such as those that are transmitted to humans through contact with animals, food ingestion of suspicion sanitary quality and contamination of the environment (FREITAS et al., 2001).

No information about the occurrence of *N. caninum* in ovine for clandestine slaughter has been reported, which justifies the present study, which aimed to determine by ELISA the frequency of anti- *N. caninum* antibodies in ovine illegally slaughtered in the state of Rio de Janeiro.

Materials and Methods

Blood samples from 132 ovines were obtained at five slaughterhouses without official license, i.e., slaughtering of ovines did not have follow-up inspection either federal, state or municipal, located in state of Rio de Janeiro. The slaughter was carried out by people without knowledge about the health and hygiene rules and therefore considered illegal slaughterhouses. Blood was collected by puncturing the jugular vein of the animals using a 21G needle attached to a vacuum adapter to collect and sort vacutainer tubes. In the laboratory, the bloods were centrifuged to separate the serum which were accommodated in 2,5mL plastic tube and stored at -20 ° C until the serological test.

In order to detect anti-*N. caninum* antibodies an indirect ELISA for ruminant animals (CHEKIT*Neospora, IDEXX Switzerland AG) was used, with final dilution 1:10 and which according to the manufacturer's specifications the interpretation of results were performed using the Variable: S/P Positive Cut-off: 0.40 and Suspect Cut-off: 0.30. A recent trial of the IDEXX Neospora Ab Test demonstrated nearly 100% specificity and over 96% sensitivity in determining the serological status of ruminants from several regions across Europe (IDEXX SWITZERLAND, 2007).

The sample processing and data analysis were conducted at the Center for Advanced Research in Parasitology at the Center for Agricultural Science and Technology of Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF). To determine the risk of ovine infection by *N. caninum* metropolitan and northern regions of State of Rio de Janeiro, we used the Fisher test, with the aid of the computer program Statistical Analysis System (SAEG) version 9.1. This study was approved by The Animal Ethics Committee (CEPA), Universidade Federal Fluminense (Nº 00111/09).

Results

The overall frequency of anti-*N. caninum* antibodies in ovine from clandestine slaughtering in the state of Rio de Janeiro was 12%. This frequency and the various municipalities in the metropolitan regions and North Fluminense assessed can be seen in Table 1. When compared to the frequency of positive animals in the regions evaluated there was no statistical difference in relation to the relative risk of infection by ovine in different regions (Table 2).

Table 1. Value of ovines tested for anti-*Neospora caninum* antibodies in accordance with regions and cities of Rio de Janeiro

Region	Cities	nº of animals	anti- <i>N. caninum</i> antibodies	
			Positive	Frequency (%)
North		55	11	20
	Carapebus	30	7	23
	Quissamã	25	4	16
Metropolitana		77	6	8
	Xerém	20	1	5
	Itaboraí	18	2	11
	Cachoeira de Macacú	39	3	8
Total		132	17	12

Table 2. Frequency of anti-*Neospora caninum* antibodies in ovine slaughtered without sanitary inspection in two regions of Rio de Janeiro

Variables	Animals			Value P ^a	Relative risk (Rr)	Confidence interval (95%)
	Positive	Negative	Total			
Metropolitana	6 (5%)	71 (54%)	77 (58%)	0,0627	0,3896	0,1533 to 0,9903
North	11 (8%)	44 (33%)	55 (42%)			
Total	17 (13%)	115 (87%)	132 (100%)			

^a For the Fisher Test

Discussion

The results determined that 12% (17/132) of ovines tested had anti-*N. caninum* antibodies in their serum. No information is available in the literature about the frequency of *N. caninum* in sheep for slaughter in slaughterhouses inspected or not inspected. The percentage was higher than the 3,3% obtained by Faria et al., (2007) in goats slaughtered from the Patos city, Paraíba State, Northeast region of Brazil also slaughterhouse

under Inspection Service and lower than the 19% obtained by Boa-Morte and Oliveira (2009) from the North Fluminense region of Rio de Janeiro. However, similar of cows slaughtered in slaughterhouse under Inspection Service 14.6% (12/83) of pregnant and in 15.8% (12/76) of nonpregnant detected by Marques et al., (2011) from Paraná State. We strongly suggest that further studies should attempt to identify the frequency of neosporosis in ovine not inspected in slaughterhouses.

These results are similar, but larger, of those reported in epidemiological studies with ovines in other regions of Brazil such as São Paulo 12,8% and 9,2% (LANGONI et al., 2011; FIGLIUOLO et al., 2004), Paraná 9,51% (ROMANELLI et al., 2007), Distrito Federal 8,81% (UENO et al., 2009) and ALAGOAS 9,6% (FARIA et al., 2010). Surveys conducted in other states in Brazil showed a lower frequency from that observed in the present work, such as those in Rio Grande do Norte and Rio Grande do Sul, where Soares et al. (2009) and Vogel et al. (2006) detected through the tests of RIFI and ELISA 1,8% (7/409) and 3,2% (2/62) of positive animals, respectively. Moreover in Mato Grosso do Sul Andreotti et al. (2009) had superior results by ELISA to this research, 32% (141/441), which shows a variation of the frequency of anti-*N. caninum* antibodies ovine in Brazil.

These percentage differences are explained by the use of different serological tests, survey periods, sample sizes and cutoff values, furthermore, climatic factors may also affect the abundance of viable parasitic stages in the environment for definitive and intermediate hosts and influence the overall prevalence (FARIA et al., 2007). In addition, Vogel et al. (2006) the probability of detecting a high prevalence of *N. caninum* in herds with reproductive problems is much higher as compared to herds with no history of reproductive problems. In this research, in ovines of metropolitan and northern regions of Rio de Janeiro was not possible to obtain information on the epidemiological data of animals because of the clandestine slaughter that has no proper veterinary and animal origin.

Observed, also, changes of the frequency according to the regions studied (Table 1). In the north of Rio de Janeiro was detected 20% while in the metropolitan region was 8%, no statistical difference between them. In other studies it is observed that the prevalence of ovine neosporosis can be quite variable, ranging from 0,45% (3/660) in England and Wales in the RIFI assay (HELMICK et al., 2002) to 63% of 339 ovines tested by ELISA in northern Jordan (ABO-SHEHADA & ABU-HALAWEH, 2010). According to Georgieva et al. (2006) the differences between regions within a country or world can be related to different climatic conditions which could interfere in the maintenance and viability of oocysts in the environment, as well as different types of sanitary engineering and animal exploration. The no difference between the regions studied may be related to the close similarity between these factors with the properties where they collected the samples.

In humans to Tranas et al. (1999) in California detected anti-*N. caninum* antibodies in 6.7% human serum analyzed by RIFI. Lobato et al. (2006) received 6% of seropositivity in healthy individuals of the State of Minas Gerais and Benetti et al. (2009) observed that the frequency was 10.5% (07/67) in male workers in the state of Mato Grosso. According to McCann et al. (2008) human beings infect with *N. caninum* through accidental ingestion of oocysts shed in the feces of the canids or directly when they are present in contaminated food or water. The consumption of raw or undercooked meat containing cysts of the parasite is also another form of infection to humans (MCCANN et al., 2008). Moreover Petersen et al. (1999) analyzed serum samples from 76 women with a history of abortions for infection by *N. caninum* and failed to detect antibodies against the parasite by ELISA and Western Blot.

Although there are no inferences about the importance of *N. caninum* in public health, since so far there is no record of human infection with *N. caninum*, it is proved that the human being comes into contact with the parasite and in accordance with Freitas et al. (2001) risk factors such as constant exposure and contact with

animals, organs, carcasses and offal distribution and consumption of meat from them, are more present in illegal slaughter. Furthermore, in relation to humans should give importance to milk intake, as is already proved the presence of colostral antibodies to *N. caninum* in cattle (CARDOSO et al., 2008), which may suggest possible contamination to human beings. Are rare the studies that show the epidemiological risk of consumption of slaughtered animals without adequate oversight and milk infected with *N. caninum*, which suggests further studies in relation to human contamination.

Therefore while there are no development programs with small farmers to resettle them in the formal market, shares of consumer education and enlightenment, for surveillance and health surveillance to ensure food security of the population, will not eradicate the problem of illegal trade of animal products in Brazil (ABRAHÃO et al., 2005) and consequently the spread of diseases in humans and animals.

Based on data obtained in this study we can consider a good indication of the current situation with reference to spread of the epidemiological chain of neosporosis, and being the ovine for illegal slaughter in the state of Rio de Janeiro as an intermediate host of *N. caninum*.

Conclusion

The study demonstrates that neosporosis is present in ovines in the State of Rio de Janeiro and that due to the slaughter of animals without proper sanitary surveillance, increases the risk of infection by the parasite to humans, which configures a major threat to the spread of disease in livestock and even to man, although the disease is not of public health importance. Further research to unravel the means of transmission between ruminants infected with *N. caninum* and humans.

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