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Prevalence of Antibodies Against of *Neospora caninum* in Dairy Cattle in Nuevo Leon, Mexico

J.A. Salinas-Melendez, J.J. Zarate-Ramos, R. Avalos-Ramirez, J.J. Hernandez-Escareno, G. Hernandez-Vidal, C.S. Gonzalez-Hernandez and V.M. Riojas-Valdes
Facultad de Medicina Veterinaria and Zootecnia, Universidad Autonoma de Nuevo Leon, Francisco Villa Street, Ciencias Agropecuarias Campus, Escobedo, 66050 Nuevo Leon, Mexico

Abstract: The goal of the present epidemiological research was the detection of anti-*Neospora caninum* antibodies in order to determine the presence and prevalence of neosporosis in dairy cattle herds at 8 counties from the state of Nuevo Leon, Mexico. A total of 33 herds and 371 animals were included. Detection of *Neospora caninum* was performed using a commercial kit observed prevalence was 45%. This result allowed the conclusion that animals which are seropositive for the protozoan *Neospora caninum* do exist in Nuevo Leon. Serological evidence was found in animals at milk-production stage indicating that the presence of emerging diseases in the region is a fact therefore, sanitary measurements should be re-evaluated or reinforced in order to keep the cattle free from pathogens that have a negative impact in the production of food from animal origin used for the human consumption.

Key words: Neosporosis, dairy cattle, Nuevo Leon, animal, region, milk production, Mexico

INTRODUCTION

The first neosporosis report was made by Bjerkas *et al.* (1984) at Norway from a canine case showing clinical signs related to encephalitis and myocarditis caused by a protozoan. Dubey *et al.* (1988a) were the first to use the name *Neosopora caninum* and were able to prove the Koch postulates in this animal species. That same year neosporosis was experimentally reproduced in canines (Dubey *et al.*, 1988b).

The involvement of *Neospora caninum* as abortion cause in bovines was reported by Thilsted (1989). On the other hand, Bjerkas and Presthus (1988) found that the strains isolated from canines were identical to those isolated from bovines. It has been demonstrated that *Neospora caninum* has a wide range of hosts including cattle, horses, canines and deer (Garcia-Bocanegra *et al.*, 2010; Almeria *et al.*, 2010; Dubey *et al.*, 1992; Dubey and Lindsay, 1989a-c). McAllister *et al.* (1998) were able to identify the dog as the definitive host by showing the presence of ooquistes in fecal matter of animals feed with infected tissue of taquizooitos of *Neospora caninum*. Wouda *et al.* (1999) suggested an association among the *Neospora caninum* infection at farms that have dogs and the cattle. In 1991, neosporosis was considered in the state of California, USA as the largest cause of abortion in cattle causing losses up to 35 million dollars (Anderson *et al.*, 1991). Evidence of natural infection has been found in canines (Cabezon *et al.*, 2010; Dubey *et al.*, 2009a; Bjerkas *et al.*, 1984; Dubey and Lindsay, 1990; Lindsay *et al.*, 1996), felines (Dubey *et al.*, 2002) bovines (Eiras *et al.*, 2011; Brickell *et al.*, 2010; Garcia-Ispierto *et al.*, 2010; Vanleeuwen *et al.*, 2010; Dubey *et al.*, 1992; Anderson *et al.*, 1991; Barr *et al.*, 1990; 1991a, b; Dubey *et al.*, 1990), ovine (Dubey and Lindsay, 1990), goats (Barr *et al.*, 1992), equines (Dubey and Porterfield, 1990), raccoons (Lindsay *et al.*, 2001), antelopes (Peters *et al.*, 2001) and deer (Dubey *et al.*, 2009a, b, 2008b; Lindsay *et al.*, 2002).

Experimental infections have been induced in mice (Dreier *et al.*, 1999; Lindsay *et al.*, 1992; Yamage *et al.*, 1996), rats (Lindsay *et al.*, 1992), canines (Dubey *et al.*, 2008a, b; Dubey and Lindsay, 1989b), foxes (Bjerkas *et al.*, 1984), goats (Lindsay *et al.*, 1995), cats (Millan *et al.*, 2009; Dubey and Lindsay, 1989a), sheep (Dubey *et al.*, 1996), coyotes (Lindsay *et al.*, 1996), porcine (Jensen *et al.*, 1998), gerbo (Cuddon *et al.*, 1992), rabbits (Dubey and Lindsay, 1996), bovines (Dubey *et al.*, 1996; Uggla *et al.*, 1998), buffalo (Guarino *et al.*, 2000) and primates (Barr *et al.*, 1994).

Corresponding Author: J.A. Salinas-Melendez, Facultad de Medicina Veterinaria and Zootecnia, Universidad Autonoma de Nuevo Leon, Francisco Villa Street, Ciencias Agropecuarias Campus, Escobedo, 66050 Nuevo Leon, Mexico

In bovines, the main transmission route is vertical (congenital-transplacentary) (Mainar-Jaime *et al*., 1999; Pare *et al*., 1996) but it has also been reported the horizontal transmission and experimentally through the lactogenic route (Davison *et al*., 1999). Transplacentary transmission has been experimentally induced in dogs (Dubey and Lindsay, 1989b; Cole *et al*., 1995b), cats, (Dubey and Lindsay, 1989a), sheep (Dubey and Lindsay, 1990) bovines (Dubey *et al*., 1992; Barr *et al*., 1994) and mice (Cole *et al*., 1995a). Transplacentary infection can happen several times in the same animal (Bjerkas *et al*., 1984; Dubey *et al*., 1988b).

Clinical signs have been reported in bovines approximately 2 months old with mostly nervous system signs due to alterations of the central nervous system such as ataxia, exoftalmy, loss of conscience and coordination, ocular asymmetry and paralysis. Other possible signs are low birth weight as well as alterations of growth and flexion or extension of anterior or posterior extremities, diminution of patellar reflexes, loss of consciousness and of perception (Thilsted, 1989; Brickell *et al*., 2010; Dubey *et al*., 1992; Anderson *et al*., 1991). The only clinical sign in adult cattle is abortion, occurring mostly among the 4 and 6th month of gestation however, not all seropositive animals abort (Dubey and Lindsay, 1996). Abortion can be explosive, paused or sporadic. Fetuses dying at the uterus can be reabsorbed, mummified or autolyzed and can be born alive but with the disease or be born clinically normal but chronically infected (Dubey and Lindsey, 1996).

In 2001 it was reported in Mexico the presence of neosporosis in dairy cattle at some areas in the Holstein breed whose diagnosis was performed by serology, Immunohistochemistry (IHC) and pathological lesions (Morales *et al*., 2001). The goal of this study was the detection of antibodies anti-*Neospora caninum* in order to determine the presence and prevalence of neosporosis in milking bovine herds by the competitive ELISA test.

MATERIALS AND METHODS

Sampling: The sampling was performed at 33 ranches of 8 municipalities (counties) from the state of Nuevo Leon, Mexico. Nuevo Leon has a territorial extension of 64,742 km^2, representing the 3.3% of the country surface. Its maximum altitude is 3700 m above sea level (msnm) and its minimum is of 100 msnm. It has a hot and extreme dry climate. According with governmental data, thera are at Nuevo Leon 499,000 bovines from which only 18,312 are dairy cattle (www.campomexicano.gob.mx).

Animals: The animals sampled included 2-4 years old adult females. All thirty three herds were destined to milk production. Breeds included were 85% Holstein-Friesian, 8% Jersey and 7% Holstein-Jersey mix. Animals were sampled at random.

Blood collection: About 5 mL were extracted from each animal (n = 371) from the tail vein using Vacutainer (Becton Dickinson and Co. Franklin Lakes, NJ, USA), tubes (21×11/2) without anticoagulant and with vacuum. The samples were kept refrigerated until and after they were taken to the College of Veterinary Medicine, Nuevo Leon State University. Afterward, samples were centrifugated at 3000 rpm for 15 min in order to completely separate blood serum which was stored at -20°C until analysis.

Laboratory analysis: Diagnosis for the detection of antibodies against *Neospora caninum* was performed by the ELISA technique using the *Neospora caninum* Antibody test (HerdChek Neospora caninum Antibody Test Kit Laboratorios IDEXX, Westbrook Maine 04092, U.S.A) which detects antibodies against *Neospora caninum* in bovine and goats.

RESULTS AND DISCUSSION

Seroprevalence obtained at the different counties from Nuevo Leon is shown in Table 1. All 8 counties sampled had serologically positive animals to *Neospora caninum*. The county with the higher prevalence was Anahuac (78%) followed by Vallecillo (56%) and Zuazua (52%) whereas Paras and Marin had the lowest results with 20 and 30%, respectively. Total frequency for Nuevo Leon was 45%.

Results obtained in the present study on the prevalence of *Neospora caninum* in dairy herds from 8 counties of the state of Nuevo Leon, Mexico are consistent with the previously described by Morales *et al*. (2001) in which they report a prevalence of 47% in dairy cattle whereas in this study was of 45%. The ELISA technique utilized in the present study for diagnosis is according to Pare *et al*. (1996), more consistent, objective, fast, precise as well as more sensitive and specific as compared with others. There are in Nuevo Leon previous data on the serological detection of positive animals to *Neospora caninum*, in both dairy and beef cattle however, the frequency found in those studies is similar to the one observed by Morales *et al*. (2001).

Researchers have to take into account that prevalence variability is influenced by the country, region and diagnostic technique used (Dubey, 2003).

Table 1: Prevalence of antibodies against *Neospora caninum* in dairy cattle in the state of Nuevo Leon, Mexico

Country	No. of samples	No. of herds	Positive animals	%
Anahuac	18	2	14	78
Apodaca	110	8	53	48
Higueras	8	1	3	37
Marin	92	5	28	30
Paras	10	1	2	20
Sabinas	38	5	15	39
Vallecillo	64	8	36	56
Zuazua	31	3	16	52
Total	371	33	167	45

It can be mentioned that results obtained demonstrate a higher frequency in herds destined to dairy production which is similar to data reported by other researchers (Benetti *et al*., 2009; Venturini *et al*., 1999; Mainar-Jaime *et al*., 1999; Dubay and Linsay, 1996) but too high as compared to the findings of Klevar *et al*. (2010) and Kamga-Waladjo *et al*. (2010). With regard to the disease transmission, it has been recognized that the most common is the vertical (Wouda *et al*., 1992; Venturini *et al*., 1999) therefore, herds with no animals with good production traits some times import infected animals in order to increase the productivity and those animals can bring the infection into the herd through healthy carriers (Lindsay *et al*., 1995). Information about the disease is rare in Mexico due to this, the role of this parasite in the reproductive problems of dairy herds has been poorly studied. The data presented above represent a small amount of the information required for a complete understanding of the *Neospora caninum* role in the reproductive and productive problems for the country livestock. Therefore, more detailed studies are required for this goal.

CONCLUSION

Results obtained in the present study demonstrate immunological evidence which shows a high prevalence of neosporosis in dairy herds at the state.

REFERENCES

Almeria, S., R. Araujo, W. Tuo, F. Lopez-Gatius, J.P. Dubey and L.C. Gasbarre, 2010. Fetal death in cows experimentally infected with Neospora caninum at 110 days of gestation. Vet. Parasitol., 169: 304-311.

Anderson, M.L., P.C. Blanchard, B.C. Barr, J.P. Dubey, R.L. Hoffman and P.A. Conrad, 1991. Neospora-like protozoan infection as a major cause of abortion in California dairy cattle. J. Am. Vet Med. Assoc., 198: 241-244.

Barr, B.C., J.D. Rowe, K.W. Sverlow, R.H. BonDurant, A.A. Ardans, M.N. Oliver and P.A. Conrad, 1994. Experimental reproduction of bovine fetal Neospora infection and death with a bovine Neospora isolate. J. Vet. Diagn. Invest., 6: 207-215.

Barr, B.C., M.L. Anderson, J.P. Dubey and P.A. Conrad, 1991a. Neospora-like protozoal infections associated with bovine abortions. Vet. Pathol., 28: 110-116.

Barr, B.C., M.L. Anderson, L.W. Woods, J.P. Dubey and P.A. Conrad, 1992. Neospora-like protozoal infections associated with abortion in goats. J. Vet. Diagn. Invest., 4: 365-367.

Barr, B.C., M.L. Anderson, P.C. Blanchard, B.M. Daft, H. Kinde and P.A. Conrad, 1990. Bovine fetal encephalitis and myocarditis associated with protozoal infections. Vet. Pathol., 27: 354-361.

Barr, B.C., P.A. Conrad, K.W. Sverlow, A.F. Tarantal and A.G. Hendrickx, 1991b. Experimental fetal and transplacental Neospora infection in the nonhuman primate. Lab. Invest., 71: 236-242.

Benetti, A.H., F.B. Schein, T.R. dos Santos, G.H. Toniollo and A.J. da Costa et al., 2009. Inquiry of antibodies anti-Neospora caninum in dairy cattle, dogs and rural workers of the South-West region of Mato Grosso State. Rev. Bras. Parasitol. Vet., 1: 29-33.

Bjerkas, I. and J. Presthus, 1988. Immuno-histochemical and ultrastructural characteristics of a cyst-forming sporozoon associated with encephalomyelitis and myositis in dogs. Acta Pathol. Microbiol. Immunol. Scand., 96: 445-454.

Bjerkas, I., S.F. Mohn and J. Presthus, 1984. Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. Z. Parasitenkd., 70: 271-274.

Brickell, J.S., M.M. McGowan and D.C. Wathes, 2010. Association between Neospora caninum seropositivity and perinatal mortality in dairy heifers at first calving. Vet. Rec., 167: 82-85.

Cabezon, O, J. Millan, M. Gomis, J.P. Dubey, E. Ferroglio and S. Almeria, 2010. Kennel dogs as sentinels of Leishmania infantum, Toxoplasma gondii and Neospora caninum in Majorca Island, Spain. Parasitol. Res., 107: 1505-1508.

Cole, R.A., D.S. Lindsay, B.L. Blagburn and J.P. Dubey, 1995a. Vertical transmission of Neospora caninum in mice. J. Parasitol., 81: 730-732.

Cole, R.A., D.S. Lindsay, B.L. Blagburn, D.C. Sorjonen and J.P. Dubey, 1995b. Vertical transmission of Neospora caninum in dogs. J. Parasitol., 81: 208-211.

Cuddon, P., D.S. Lin, D.D. Bowman, D.S. Lindsay and T.K. Miller et al., 1992. Neospora caninum infection in English Springer Spaniel Littermates: Diagnostic evaluation and organism isolation. J. Vet. Int. Med., 6: 325-332.

Davison, H.C., A. Otter and A.J. Trees, 1999. Estimation of vertical and horizontal transmission parameters of Neospora caninum infections in dairy cattle. Int. J. Parasitol., 29: 1683-1689.

Dreier, K.J., L.W. Stewarter, R.L. Kerlin, D.M. Ritter and D.A. Brake, 1999. Phenotypic characterisation of a Neospora caninum temperature-sensitive strain in normal and immunodeficient mice. Int. J. Parasitol., 29: 1627-1634.

Dubey, J.P. and D.S. Lindsay, 1989a. Neospora caninum induced abortion in sheep. J. Vet. Diagn. Invest., 2: 230-233.

Dubey, J.P. and D.S. Lindsay, 1989b. Transplacental Neospora caninum infection in cats. J. Parasitol., 75: 765-771.

Dubey, J.P. and D.S. Lindsay, 1989c. Transplacental Neospora caninum infection in dogs. Am. J. Vet. Res., 50: 1578-1579.

Dubey, J.P. and D.S. Lindsay, 1990. Neosporosis in dogs. Vet. Parasitol., 36: 147-151.

Dubey, J.P. and D.S. Lindsay, 1996. A review of Neospora caninum and neosporosis. Vet. Parasitol., 67: 1-59.

Dubey, J.P. and M.L. Porterfield, 1990. Neospora caninum (Apicomplexa) in an aborted equine fetus. J. Parasitol., 76: 732-734.

Dubey, J.P., 2003. Review of Neosporosis caninum and neosporosis in animals. Korean J. Parasitol., 41: 138-138.

Dubey, J.P., A.L. Hattel, D.S. Lindsay and M.J. Topper, 1988a. Neonatal Neospora caninum infection in dogs: Isolation of the causative agent and experimental transmission. J. Am. Vet. Med. Assoc., 193: 1259-1263.

Dubey, J.P., D. Stone, O.C. Kwok and R.N. Sharma, 2008b. Toxoplasma gondii and Neospora caninum antibodies in dogs from Grenada, West Indies. J. Parasitol., 94: 750-751.

Dubey, J.P., D.S. Lindsay and M.R. Lappin, 2009b. Toxoplasmosis and other intestinal coccidial infections in cats and dogs. Vet. Clin. North. Am. Small Anim. Pract., 39: 1009-1034.

Dubey, J.P., D.S. Lindsay, D. Hill, S. Romand and P. Thulliez *et al*., 2002. Prevalence of antibodies to Neospora caninum and Sarcocystis neurona in sera of domestic cats from Brazil. J. Parasitol., 88: 1251-1252.

Dubey, J.P., D.S. Lindsay, D.S. Adams, J.M. Gay, T.V. Baszler, B.L. Blagburn and P. Thulliez, 1996. Serologic responses of cattle and other animals infected with Neospora caninum. Am. J. Vet. Res., 57: 329-336.

Dubey, J.P., D.S. Lindsay, M.L. Anderson, S.W. Davis and S.K. Shen, 1992. Induced transplacental transmission of Neospora caninum in cattle. J. Am. Vet. Med. Assoc., 201: 709-713.

Dubey, J.P., J.L. Carpenter, C.A. Speer, M.J. Topper and A. Uggla, 1988b. Newly recognized fatal protozoan disease of dogs. J. Am. Vet. Med. Assoc., 192: 1269-1285.

Dubey, J.P., K. Mansfield, B. Hall, O.C. Kwok and P. Thulliez, 2008a. Seroprevalence of Neospora caninum and Toxoplasma gondii in black-tailed deer (Odocoileus hemionus columbianus) and mule deer (Odocoileus hemionus hemionus). Vet. Parasitol., 156: 310-313.

Dubey, J.P., M.C. Jenkins, O.C. Kwok, R.L. Zink and M.L. Michalski et al., 2009a. Seroprevalence of Neospora caninum and Toxoplasma gondii antibodies in white-tailed deer (Odocoileus virginianus) from Iowa and Minnesota using four serologic tests. Vet. Parasitol., 161: 330-334.

Dubey, J.P., S. Miller, D.S. Lindsay and M.J. Topper, 1990. Neospora caninum-associated miocarditis and encephalitis in an aborted calf. J. Vet. Diagn. Invest., 2: 66-69.

Eiras, C., I. Arnaiz, G. Alvarez-Garcia, L.M. Ortega-Mora, M.L. Sanjuanl, E. Yus and F.J. Dieguez, 2011. Neospora caninum seroprevalence in dairy and beef cattle from the Northwest region of Spain, Galicia. Prev. Vet. Med., 98: 128-132.

Garcia-Bocanegra, I., O. Cabezon, M. Pabon, F. Gomez-Guillamon and A. Arenas et al., 2010. Prevalence of Toxoplasma gondii and Neospora caninum antibodies in Spanish ibex (Capra pyrenaica hispanica). Vet. J. 10.1016/j.tvjl.2010.11.011

Garcia-Ispierto, I., C. Nogareda, J.L. Yaniz, S. Almeria and D. Martinez-Bello et al., 2010. Neospora caninum and coxiella burnetii seropositivity are related to endocrine pattern changes during gestation in lactating dairy cows. Theriogenology, 74: 212-220.

Guarino, A., G. Fusco, G. Savini, G. Di Francesco and G. Cringoli, 2000. Neosporosis in water buffalo (Bubalus bubalis) in Southern Italy. Vet. Parasitol., 91: 15-21.

Jensen, L., T.K. Jensen, P. Lind, S.A. Henriksen, A. Uggla and V. Bille-Hansen, 1998. Experimental porcine neosporosis. APMIS., 106: 475-482.

Kamga-Waladjo, A.R., O.B. Gbati, P. Kone, R.A. Lapo and G. Chatagnon et al., 2010. Seroprevalence of Neospora caninum antibodies and its consequences for reproductive parameters in dairy cows from Dakar-Senegal, West Africa. Trop. Anim. Health Prod., 42: 953-959.

Klevar, S., M. Norstrom, J. Tharaldsen, T. Clausen and C. Bjorkman, 2010. The prevalence and spatial clustering of Neospora caninum in dairy herds in Norway. Vet. Parasitol., 170: 153-157.

Lindsay, D.S., B.L. Blagburn and J.P. Dubey, 1992. Factors affecting the survival of Neospora caninum bradizoites in murine tissues. J. Parasitol., 78: 70-72.

Lindsay, D.S., E.J. Kelly, R. McKown, F.J. Stein and J. Plozer et al., 1996. Prevalence of Neospora caninum and Toxoplasma gondii antibodies in coyotes (Canis latrans) and experimental infections of coyotes with Neospora caninum. J. Parasitol., 82: 657-659.

Lindsay, D.S., J. Spencer and C. Rupprecht, 2001. Prevalence of agglutinating antibodies to Neospora caninum in raccoons, Procyon lotor. J. Parasitol., 87: 1197-1198.

Lindsay, D.S., N.S. Rippey, T.A. Powe, E.A. Sartin, J.P. Dubey and B.L. Blagburn, 1995. Abortions, fetal death and stillbirths in pregnant pygmy goats inoculated with tachizoites of Neospora caninum. Am. J. Vet. Res., 56: 1176-1180.

Lindsay, D.S., S.E. Little and W.R. Davidson, 2002. Prevalence of antibodies to Neospora caninum in white-tailed deer, Odocoileus virginianus, from the Southeastern United States. J. Parasitol., 88: 415-417.

Mainar-Jaime, R.C., M.C. Thurmond, B. Berzal-Herranz and S.K. Hietala, 1999. Seroprevalence of Neospora caninum and abortion in dairy cows in Northern Spain. Vet. Rec., 145: 72-75.

McAllister, M., J.P. Dubey, D. Lindsay, W. Jolley, R. Wills and A. McGuire, 1998. Dogs are definitive hosts of Neospora caninum. Int. J. Parasitol., 28: 1473-1478.

Millan, J., O. Cabezon, M. Pabon, J.P. Dubey and S. Almeria, 2009. Seroprevalence of Toxoplasma gondii and Neospora caninum in feral cats (Felis silvestris catus) in Majorca, Balearic Islands, Spain. Vet. Parasitol., 165: 323-326.

Morales, S.E., T.F. Trigo, F. Ibarra, C.E. Puente and M. Santacruz, 2001. Seroprevalence study of bovine neosporosis in Mexico. J. Vet. Diagn. Invest., 13: 413-415.

Pare, J., M.C. Thurmond and S.K. Hietala, 1996. Congenital Neospora caninum infection in dairy cattle and associated calfhood mortality. Can. J. Vet. Res., 60: 133-139.

Peters, M., P. Wohlsein, A. Knieriem and G. Schares, 2001. Neospora caninum infection associated with stillbirths in captive antelopes (Tragelaphus imberbis). Vet. Parasitol., 97: 153-157.

Thilsted, J.P., 1989. Neosporosis-like abortions in a herd of dairy cattle. J. Vet. Diagn. Invest., 1: 205-209.

Uggla, A., S. Stenlund, O.J.M. Holmdahl, E.B. Jakubek, P. Thebo, H. Kindahl and C. Bjorkman, 1998. Oral Neospora caninum inoculation of neonatal calves. Int. J. Parasitol., 28: 1467-1472.

Vanleeuwen, J.A., J.P. Haddad, I.R. Dohoo, G.P. Keefe, A. Tiwari and H.M. Scott, 2010. Risk factors associated with Neospora caninum seropositivity in randomly sampled Canadian dairy cows and herds. Prev. Vet. Med., 93: 129-138.

Venturini, M.C., L. Venturini, D. Bacigalupe, M. Machuca and I. Echaide et al., 1999. Neospora caninum infections in bovine foetuses and dairy cows with abortions in Argentina. Int. J. Parasitol., 29: 1705-1708.

Wouda, W., T. Dijkstra, A.M.H. Kramer, C. Van Maanen and J.M.A. Brinkhof, 1999. Seroepidemiological evidence for a relationship between Neospora caninum infections in dogs and cattle. Int. J. Parasitol., 29: 1677-1682.

Wouda, W., T.S.G.A.M. van den Ingh, F. van Knapen, F.J.H. Sluyter, J.P. Koeman and J.P. Dubey, 1992. Neospora abortus bij het rund in Nederland. Tijdschr Diergeneeskd, 117: 559-602.

Yamage, M., O. Flechtner and B. Gottstein, 1996. Neospora caninum: Specific oligonucleotide primers for the detection of brain cyst DNA of experimentally infected nude mice by the Polimerace Chain Reaction (PCR). J. Parasitol., 82: 272-279.