



ELSEVIER

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

Veterinary Parasitology 114 (2003) 247–252

veterinary
parasitology

www.elsevier.com/locate/vetpar

Serological evidence of *Neospora caninum* infections in beef bulls in six counties of the Corrientes province, Argentina

D.P. Moore^a, M.G. Draghi^b, C.M. Campero^{c,*}, B. Cetrá^b,
A.C. Odeón^c, E. Alcaraz^b, E.A.J. Späth^c

^a Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

^b Instituto Nacional de Tecnología Agropecuaria (INTA), Mercedes, Argentina

^c Instituto Nacional de Tecnología Agropecuaria (INTA), CC 276, 7620 Balcarce, Argentina

Received 12 August 2002; received in revised form 9 April 2003; accepted 11 April 2003

Abstract

The purpose of the present work was to identify *Neospora caninum* infections in beef bulls belonging to 19 herds from six counties located in the Corrientes province, Argentina. The presence of antibodies to *N. caninum* was evaluated in 305 serum samples of bulls (*Bos taurus* and *Bos indicus*). Age and breed were recorded. An indirect fluorescent antibody test was used to determine specific antibodies. The number of bulls with natural *Neospora*-infection was 15 of 305 (4.9%). No association between serologic status and breed (odds ratio (OR), 0.53; 95% CI, 0.18–1.53) was found. *Neospora*-infected beef bulls were identified in the present work. The bull role in bovine neosporosis and the risk of horizontal transmission for cows should be investigated.

© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Bulls; Cattle-protozoa; Epidemiology; *Neospora caninum*

1. Introduction

The coccidian parasite *Neospora caninum* is close to *Toxoplasma gondii* (Dubey and Lindsay, 1996) and it causes abortions and economic losses in cattle (Dubey, 1999). Transplacental transmission is the main mechanism by which the parasite persists in a herd (Anderson et al., 1997). Fortunately, natural infected cattle can be identified by the specific serum antibody response (Dubey, 1999). After recognizing the dog as the definitive host of the parasite

* Corresponding author. Tel.: +54-2266-439100/439120; fax: +54-2266-439101.

E-mail address: ccampero@balcarce.inta.gov.ar (C.M. Campero).

(McAllister et al., 1998), epidemiological work established the association between the presence of dogs and the disease in cattle (Wouda et al., 1999). Although post-natal infections have been documented (Thurmond et al., 1997; Dijkstra et al., 2001), the mechanisms of horizontal transmission in cattle are still unclear.

Although rams can shed *T. gondii* tachyzoites in semen after subcutaneous inoculation of cysts, the possibility of venereal transmission from ram to ewe is minimal (Teale et al., 1982). Bulls can also shed *T. gondii* tachyzoites in seminal plasma between 14 and 84 days after experimental infection with oocysts, and venereal transmission of bovine toxoplasmosis has been suggested (Scarpelli et al., 2001). In bovine neosporosis pathological changes have been characterized in bovine females and calves (Dubey, 1999), however little is known about pathology in bulls and venereal transmission has not been investigated.

In Argentina, beef cattle breeding is carried out by natural service and several infectious diseases produce important economic losses including reproductive failures due to *N. caninum* (Venturini et al., 1995; Moore et al., 2002). The objective of the present study was to investigate the presence of antibodies to *N. caninum* in serum samples belonging to beef bulls from 19 herds in the Corrientes province, Argentina.

2. Materials and methods

2.1. Region, animals and samples

The Corrientes province has 22 counties and an extension of approximately 3.5 million ha. Cattle raising constitutes an important agricultural activity in this region with 3953 beef farms, 1.9 million cattle managed under extensive grazing, and 97,615 bulls used for natural breeding (SENASA, 2001).

The samples analyzed in this study were part of an extensive survey performed in the Corrientes province to estimate the prevalence of venereal (trichomoniasis and campylobacteriosis) and other diseases in the bull population.

In that extensive survey, a cluster sample design was used to determine the sample size ($p = 8 \pm 1.6\%$; confidence 95%, roh = medium) using a specific computer program (C survey V1.5b, Ariawan and Frerichs, 1996). The sample size was determined as a minimum of 10 bulls from 450 farms. Farms were selected using the probability proportionate to size method.

For the present work, a convenience subsample was taken from the serum bank which included all samples from 19 beef farms located in six southern counties of the Corrientes province. Three hundred and five beef bulls (*Bos taurus* and *Bos indicus*) without history of clinical reproductive problems belonging to commercial beef herds located in six counties in Corrientes: Curuzú Cuatiá (three farms), General Alvear (two farms), Mercedes (four farms), Monte Caseros (three farms), Paso de los Libres (three farms) and San Martín (four farms) were evaluated (minimum: 10, maximum: 40 animals per farm).

Blood samples were obtained with the cooperation of private veterinarians, diagnostic laboratories and national research institutes, and were sent to veterinary diagnostic laboratory of INTA Mercedes-Corrientes from 1999 to 2000. Sera were separated and stored at -20°C until analysis.

The breed and age of each bull were recorded. Information about stocking rate in the farms, period of breeding, pregnancy rate, and percentage of bulls in service was also recorded.

2.2. Serological test

Indirect fluorescent antibody test (IFAT) with respective positive and negative control sera was used as previously described (Dubey et al., 1988). Slides were examined with an epifluorescence microscope (Nikon Fluophot, 40×1.3). A serological titer of 1:200 was considered positive (Reichel and Drake, 1996). The end-point titre was the last serum dilution showing distinct whole parasite fluorescence. The estimated values of the sensitivity and the specificity for the IFAT to serum dilutions between 1:64 and 1:640 are 89.7 and 87.85%, respectively (Atkinson et al., 2000).

2.3. Data analysis

The association between serologic status and breed was determined using odds ratio (OR) (Thrusfield, 1995). Data were processed by the use of Med Calc program (Med Calc, 1993). The bulls were grouped in young (<3 years), mature (3–7 years) and old (>7 years) (Turnbull, 1977) to evaluate the distribution according with serostatus and age.

3. Results

One hundred and thirty eight animals were *B. taurus* (Aberdeen Angus, Hereford), one was *B. indicus* (Brahman), and 166 composites (Brangus, Braford, Santa Gertrudis). The distribution of age was: 2 years (21 bulls), 3 years (12 bulls), 4 years (31 bulls), 5 years (48 bulls), 6 years (124 bulls), 7 years (17 bulls), 8 years (43 bulls), 9 years (seven bulls) and 10 years (two bulls). The mean age was 5.7 ± 0.1 (minimum: 2, maximum: 10).

The stocking rate in the farms was 0.6 heads per ha ± 0.2 (range 0.4–0.8). The period of breeding varied from 3 to 5 months beginning in spring or autumn. The pregnancy rate was $68.6 \pm 4.5\%$ (range 50–83). The percentage of bulls in service was $4 \pm 0.3\%$ (range 3–5.5).

Antibodies to *N. caninum* were detected in 15 (4.9%) of 305 bulls. The frequency of serologic titers at different dilutions in positive animals was as follows: 1:200 (seven bulls), 1:400 (four bulls), 1:800 (two bulls), 1:1600 (one bull), and 1:3200 (one bull). The results obtained according with the origin of each bull are shown in Table 1.

No association between breed and serostatus was found (OR, 0.53; 95% CI, 0.18–1.53). The distribution according with breed and age is shown in the Table 2.

4. Discussion

Although this study had several limitations to establish the real prevalence of neosporosis in beef bulls belonging to six counties of the Corrientes province, the low percentage of seropositive bulls (4.9%) indicate a scarce exposure to *N. caninum* in this area. Information

Table 1

Number and percentage of bulls infected with *N. caninum* from 19 beef herds located in six counties of Corrientes, Argentina

County	Beef herds	Herds with positive bulls (%)	Bulls	Positive bulls (%)
Curuzú Cuatiá	3	1 (33.3)	30	2 (6.6)
General Alvear	2	1 (50.0)	20	1 (5.0)
Mercedes	4	3 (75.0)	60	5 (8.3)
Monte Caseros	3	2 (66.6)	65	5 (7.7)
Paso de los Libres	3	1 (33.3)	76	1 (1.3)
San Martín	4	1 (25.0)	54	1 (1.9)
Total	19	9 (47.3)	305	15 (4.9)

Table 2

Number of *N. caninum* infected bulls grouped by breed and age

Breed	Years of age	Positive bulls	Negative bulls
<i>B. taurus</i>	<3	2	14
	3–7	6	94
	>7	1	21
<i>B. indicus</i> or composites	<3	0	5
	3–7	3	129
	>7	3	27

obtained in this study coincides with a low prevalence of *N. caninum* infection (4.7% of 400) detected in beef cows without signs of reproductive disease (Moore et al., 2002). The risk of postnatal exposure to *N. caninum* oocysts may be associated with high cattle-stocking density (Barling et al., 2000a). In this study, the low number of bulls seropositive to *N. caninum* could be due to extensive grazing condition on natural pastures.

The association between serological status and breed or age was difficult to study due to the low number of infected bulls found in the present work. The similar distribution of seroreactors in *B. taurus* and *B. indicus* composite bulls, would suggest that all breeds are susceptible to infection by *N. caninum*. This is in agreement with previously published information (Dubey, 1999). The similar distribution of the infected bulls according with their age could suggest that seropositive bulls were congenitally infected, however this fact was not demonstrated in this work.

Experimental systemic inoculation with *N. caninum* tachyzoites in bulls caused seroconversion; however neither clinical signs nor antibodies in seminal plasma were found during 3 months after challenge (Campero et al., unpublished information). In the present work, the presence of this protozoan in semen was not investigated. Although the transmission of *T. gondii* via semen can be a possible route of infection in cattle (Scarpelli et al., 2001) and this mode of infection may be hypothesized in bovine neosporosis, it is unlikely this form of transmission would play a large role.

Because the production of milk was less in infected than in healthy cows, systemic pathological changes due to *N. caninum* infection have been suggested in dairy cattle (Thurmond

and Hietala, 1997; Hernandez et al., 2001). *N. caninum* infection contributes to dairy production inefficiency through premature culling (Thurmond and Hietala, 1996). Reduced post-weaning weight gain and carcass qualities were also found in seropositive beef calves (Barling et al., 2000b). No studies were published in this regard in beef bulls, however infected bulls could have some systemic pathological changes, which in turn could cause reduced reproductive performance. However, this factor has not been studied.

The high levels of antibodies changed over time during pregnancy in dairy cows naturally infected with *N. caninum* (Stenlund et al., 1999). In addition, it was hypothesized that antibody titres could be associated with increased estrogen concentrations in plasma at 4 months of gestation (Stenlund et al., 1999). In the present study, low antibody titres were found. This fact could be associated with the lack of recrudescence of existing infection due to different hormonal dynamics found in a bull. Hormonal studies were not performed in this work.

In conclusion, results of this study demonstrate *Neospora*-infections in beef bulls in six counties of Corrientes province, Argentina. The bull is host for *N. caninum*; however, his role in bovine neosporosis is still unknown, and the risk of horizontal transmission for cows should be investigated.

Acknowledgements

The authors want to express their appreciation for assistance provided by technicians from INTA, Mercedes and INTA, Balcarce. This work was partially funded by a Research Grant from Agencia Nacional de Promoción Científica y Tecnológica, FONCyT, PICT 0804291, Argentina.

References

- Anderson, M.L., Reynolds, J.P., Rowe, J.D., Packham, A.E., Barr, B.C., Conrad, P.A., 1997. Evidence of vertical transmission of *Neospora* sp. infection in dairy cattle. *J. Am. Vet. Med. Assoc.* 210, 1169–1172.
- Ariawan, I., Frerichs, R.R., 1996. C survey 1.5b. A cluster sampling utility program for IBM-compatible microcomputers. University of Indonesia–University of California Los Angeles (UCLA), p. 74c.
- Atkinson, R., Harper, P.A.W., Reichel, M.P., Ellis, J.T., 2000. Progress in the serodiagnosis of *Neospora caninum* infections of cattle. *Parasitol. Today*. 16, 110–114.
- Barling, K.S., Sherman, M., Peterson, M.J., Thompson, J.A., McNeill, J.W., Craig, T.M., Garry Adams, L., 2000a. Spatial associations among density of cattle, abundance of wild canids, and seroprevalence to *Neospora caninum* in a population of beef calves. *J. Am. Vet. Med. Assoc.* 217, 1361–1365.
- Barling, K.S., McNeill, J.W., Thompson, J.A., Paschal, J.C., Mccollum, T., Craig, T.M., Garry Adams, L., 2000b. Association of serologic status for *Neospora caninum* with postweaning weight gain and carcass measurements in beef calves. *J. Am. Vet. Med. Assoc.* 217, 1356–1360.
- Dijkstra, T., Barkema, H.W., Eysker, M., Wouda, W., 2001. Evidence of post-natal transmission of *Neospora caninum* in Dutch dairy herds. *Int. J. Parasitol.* 31, 209–215.
- Dubey, J.P., 1999. Recent advances in *Neospora* and neosporosis. *Vet. Parasitol.* 1589, 1–19.
- Dubey, J.P., Lindsay, D.S., 1996. A review of *Neospora caninum*. *Vet. Parasitol.* 67, 1–59.
- Dubey, J.P., Hattel, A.L., Lindsay, D.S., Topper, M.J., 1988. Neonatal *Neospora caninum* infection in dogs: isolation of the causative agent and experimental transmission. *J. Am. Vet. Med. Assoc.* 193, 1259–1263.
- Hernandez, J., Risco, C., Donovan, A., 2001. Association between exposure to *Neospora caninum* and milk production in dairy cows. *J. Am. Vet. Med. Assoc.* 219, 632–635.

- McAllister, M.M., Dubey, J.P., Lindsay, D.S., Jolley, W.R., Wills, R.A., McGuire, A.M., 1998. Dogs are definitive hosts of *Neospora caninum*. *Int. J. Parasitol.* 28, 1473–1478.
- Med Calc, 1993. Windows 95[®], Version 4.16b.
- Moore, D.P., Campero, C.M., Odeón, A.C., Poso, M.A., Cano, D., Leunda, M.R., Basso, W., Venturini, M.C., Späth, E., 2002. Seroepidemiology of beef and dairy herds and fetal study of *Neospora caninum* in Argentina. *Vet. Parasitol.* 107, 303–316.
- Reichel, M.P., Drake, J.M., 1996. The diagnosis of *Neospora* abortions in cattle. *New Zealand Vet. J.* 44, 151–154.
- Scarpelli, L.C., Costa, A.J., Migani, M.B., Varandas, N.P., Ferro, M.I.T., Esper, C.R., 2001. Venereal transmission viability of *Toxoplasma gondii* in bovines. In: Proceedings of the XVIII International Conference of the World Association for the Advancement of Veterinary Parasitology, vol. B15p, 26–30 August, Stresa, Italy, p. 22.
- SENASA, 2001. Censo Ganadero del Servicio Nacional de Sanidad Animal (SENASA), Argentina.
- Stenlund, S., Kindahl, H., Magnusson, U., Ugglä, A., Björkman, C., 1999. Serum antibody profile and reproductive performance during two consecutive pregnancies of cows naturally infected with *Neospora caninum*. *Vet. Parasitol.* 85, 227–234.
- Teale, A.J., Blewett, D.A., Miller, J.K., 1982. Experimentally induced toxoplasmosis in young rams: the clinical syndrome and semen secretion of toxoplasma. *Vet. Rec.* 111, 53–55.
- Thrusfield, M., 1995. *Veterinary Epidemiology*, 2nd ed. Blackwell Scientific Publications, UK, 280 pp.
- Thurmond, M.C., Hietala, S., 1996. Culling associated with *Neospora caninum* infection in dairy cows. *Am. J. Vet. Res.* 57, 1559–1562.
- Thurmond, M.C., Hietala, S., 1997. Effect of *Neospora caninum* infection on milk production in first-lactation dairy. *J. Am. Vet. Med. Assoc.* 210, 672–674.
- Thurmond, M.C., Hietala, S., Blanchard, P.C., 1997. Herd-based diagnosis of *Neospora caninum*-induced endemic and epidemic abortion in cows and evidence for congenital and postnatal transmission. *J. Vet. Diagn. Invest.* 9, 44–49.
- Turnbull, P.A., 1977. An abattoir survey of bull genitalia. *Aus. Vet. J.* 53, 274–279.
- Venturini, L., Di Lorenzo, C., Venturini, M.C., Romero, J., 1995. Anticuerpos anti *Neospora* sp en vacas que abortaron. *Vet. Arg.* 12, 167–170.
- Wouda, W., Dijkstra, T.H., Kramer, A.M.H., Van Maanem, C., Brinkhof, J.M.A., 1999. Seroepidemiological evidence for a relationship between *Neospora caninum* infections in dogs and cattle. *Int. J. Parasitol.* 29, 168–1677.