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# Neospora caninum seropositivity and association with abortions in dairy cows in Mexico

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#### **Abstract**

The seroprevalence of *Neospora caninum* infection was estimated from a sample of 813 cattle from 20 dairy herds in five regions in Mexico. The true prevalence of infection was estimated to be 42% (95% CI: 39, 46). Seropositivity was associated with abortion (odds ratio (OR) = 2.0) and was higher among cows raised on-farm (41%), than among replacement cattle purchased outside the farm (28%). The ORs relating abortion to seropositivity in individual herds ranged from 1.3 to 10. Overall, 26% of the abortions were attributed to *N. caninum*.

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#### 1. Introduction

*Neospora caninum* infection in dairy cattle has been associated with abortion, culling and low milk production (Dubey, 1999a,b). Congenital and postnatal transmission of the infection have been demonstrated transmission.

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strated in several herds, which may be the main means by which infection is maintained in cattle herds (Björkman et al., 1996; Jensen et al., 1999; Davison et al., 1999). *Neospora caninum* is an important agent of bovine abortion in the USA and some other countries (Dubey, 1999a,b). Dogs are the definitive host of the parasite (McAllister et al., 1988). This suggests that the organism could be spread by contamination of feed and the environment with fecal oocysts discharged from dogs.

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In Mexico, the government is attempting to improve dairy farming to meet the increasing national milk demand. Currently, high milk production herds are comprised mainly of purebred Holsteins, which are either raised locally or imported from the USA, Canada or other countries. These cattle are distributed mainly to the center and northeast region of Mexico, where the government promotes dairy farming. The dairy farms in these areas use modern technology and apply federal government required animal-health programs, such as brucellosis and tuberculosis control. However, it is a possibility that agents such as *N. caninum* are introduced with imported animals and spread locally through trading.

World-wide distribution of *N. caninum* has been reported by Dubey (1999a,b). Nevertheless, little information is available on neosporosis in Mexico, beyond demonstrating *N. caninum* infection has been reported in cows and aborted fetuses (Morales et al., 2001a,b). The herd-level prevalence reported in Mexico ranged from 10–100% (Morales et al., 2001a,b; Garcia-Vazquez et al., 2002). However, the reports did not provide estimates of the association of *N. caninum* infection with abortion in the main dairy regions in Mexico.

Our objectives of this study were to estimate the seroprevalence of *N. caninum* in dairy cattle and herds from five regions of Mexico, to estimate the association between *N. caninum* and abortions in individual herds and to identify management-related factors associated with seropostivity of antibodies to *N. caninum*.

#### 2. Material and methods

#### 2.1. Situation of the herds and animals

The study was conducted in the five main dairy regions of Mexico, (in the northern states of Coahuila and Chihuahua, and the central states of Hidalgo, Queretaro, and Jalisco). Stratified random sampling was used to select 20 dairy herds, four from each of the five states, between July 2002 and September 2003.

A random sample of lactating females  $\geq 2$  years old, was selected for testing from each herd such that the number of cows from each herd was sufficient to estimate the herd prevalence with 5% or less error and

95% confidence interval (CI), conservatively assuming the variance to be 25% and the seroprevalence 50%.

#### 2.2. Blood sampling

Blood samples were collected from the 813 Holstein cows selected for the study. Blood was collected in plain vacutainer from the coccygeal or jugular vein and transported to CENID-Parasitologia Veterinaria laboratory in Jiutepec, Morelos for testing. After centrifugation at  $1000 \times g$  for 15 min, serum was removed and stored at -20 °C until analysis.

#### 2.3. Serologic tests

Serum samples were tested with the ELISA test (IDEXX Laboratories) for antibodies against *N. caninum*. Each sample was tested in replicated. Sera with absorbance values above the cut-off level of 0.20 absorbance were considered as positive in accordance with the manufacture's instructions.

#### 2.4. Data analyses

The true prevalence (TP) was calculated as TP = (P + Sp-1)/(Se + Sp-1) where P = seroprevalence, Sp = specificity (0.965) and Se = sensitivity(0.886) (Mainar-Jaime et al., 1999). Information about individual-cow seropositivity was used to examine its association with abortion over all herds and for each herd, individually, second, to look for evidence of postnatal-infection by testing whether seropositivity increased with cow age, and third to test the association between purchasing replacement cattle and within herd prevalence. The first and third hypotheses were examined by the chi-square test or the Fisher exact test, and odd ratios (ORs) were calculated as a measure of the strength of the associations. The estimated attributable fraction (AF) for N. caninum abortion, the proportion of aborted N. caninum seropositive cows whose abortions were due to N. caninum, was calculated as (OR - 1)/OR (Martin et al., 1987). The population attributable factor (PAF), which estimated the proportion of the abortions in the population studied that were attributable to N. caninum was calculated as  $PAF = (AF \times N_N)/N_T$ , where  $N_N$  is the number of

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