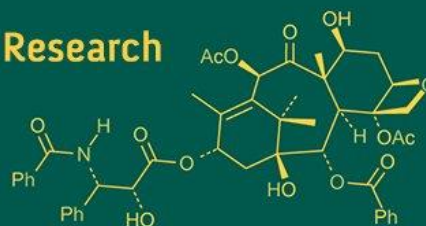


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Seroprevalence and associated risk factors of *Neospora caninum* infection among cattle in Jammu, India

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Abstract

Neosporosis caused by protozoa *Neospora caninum*, is a leading cause of abortion in cattle worldwide. The aim of the present study was to analyze the seroprevalence of *N. caninum* and associated risk factors in cattle of Jammu. A total of 180 cattle serum samples were collected from four areas of Jammu division, namely, Jammu, Akhnoor, R. S. Pura and Bishnah. The serum samples were tested using Competitive Enzyme Linked Immunosorbent Assay to detect *N. caninum* antibodies. Out of 180 samples tested, 25 were positive yielding the overall prevalence of 13.88 percent. The highest prevalence was recorded from Akhnoor (18%) followed by Jammu (14.80%), R. S. Pura (8.82%) and Bishnah (6.67%). For analysis of risk factors associated with neosporosis, eleven parameters were taken into consideration viz., type of herd, breed, age, origin of animals, method of rearing, presence of dogs, parity, history of abortion, history of repeat breeding, method of disposal of foetus and foetal membranes, hygiene and disinfection of farm. The factors that were significantly associated with *N. caninum* infection were method of disposal of foetal membranes (OR = 6.10, $p = 0.0001$) and presence of dogs (OR = 3.02, $p = 0.0139$). The overall prevalence of 13.88 percent of *N. caninum* in cattle signifies the importance of screening the cattle population of Jammu regardless of any clinical symptoms in order to learn about the exposure of the animals to *Neospora caninum*.

Keywords: Neosporosis, *Neospora caninum*, cattle, Seroprevalence, competitive ELISA

Introduction

Neosporosis is a parasitic disease caused by the protozoa *Neospora caninum* which was misdiagnosed as toxoplasmosis before it was first recognized in dogs of Norway (Bjerkas *et al.*, 1984) [4] and then it was categorized as a distinct species in 1988 (Dubey *et al.*, 1988) [10]. In livestock operations, abortions and newborn deaths are serious issues, and neosporosis is a leading cause of abortion in cattle. *N. caninum* is a coccidian parasite that belongs to the Phylum Apicomplexa, Order Eucoccidiorida and the Family Sarcocystidae. It has a heteroxenous life cycle, with dogs serving as the definitive host for sexual replication and cattle serving as the most common intermediate host for asexual replication (Basso *et al.*, 2001; Gondim, 2006; Dubey *et al.*, 2007) [3, 15, 11]. Tachyzoites, tissue cysts (including bradyzoites), and oocysts are the three infectious stages that make up the lifecycle of *N. caninum*. There are two ways for cattle to become infected: Firstly, horizontal transmission, i.e., by ingestion of eggs (oocysts) through contaminated feed or water (released by sick dogs and other canids). Secondly, vertical transmission i.e., transfer of infection from the dam to the foetus and this route is the most common mechanism of transmission. *N. caninum* can affect dogs of all ages in which majority are asymptomatic and show clinical indications usually after three weeks of age. The prominent symptom of neosporosis in dogs is severe neuromuscular dysfunction (Dubey *et al.*, 1992) [9]. Natural infection results in ascending paralysis and inflexible hind limb hyperextension, which is a hallmark of neosporosis. The hind limbs are afflicted more severely than the front legs, other symptoms include wallowing difficulties, jaw paralysis, muscle flaccidity, muscle atrophy, head tremors, forelimb ataxia, and even heart failure due to myocarditis (Dubey and Lindsay, 1993; Barber *et al.*, 1996) [8, 1]. Amongst all microorganisms of cattle, *N. caninum* is one of the most efficient transplacental transmitted parasites.

Ingestion of its oocysts (exogenous) or reactivation of infection in a chronically infected cow (endogenous) can result in transplacental transmission in post-natally acquired illnesses, and the rate of transmission varies in the above stated circumstances (Williams *et al.*, 2009) [31]. There are numerous risk factors which includes kind of herd, technique of herd management, presence of definitive hosts and reproductive issues such as repeated breeding, history of abortion and anestrus (Bruhn *et al.*, 2013) [6]. Poor water quality, the presence of dogs, unsanitary living conditions, and a history of abortions have all been identified as risk factors (Ghalmi *et al.*, 2012; Sun *et al.*, 2015) [13, 27]. Farm dog's specific feeding habits, such as eating aborted foetuses and placenta, may enhance the risk of cattle being seropositive (Van Leeuwen *et al.*, 2010) [29].

Although it is difficult to diagnose neosporosis abortion but epidemiological history and clinical results are a hallmark to make a diagnosis. Direct postmortem and indirect *in vivo* diagnostic techniques are used to diagnose bovine neosporosis (Ortega-Mora *et al.*, 2006) [24]. The detection of antibodies in the serum, body fluids and milk are used in *in-vivo* procedures. Antigens are tachyzoites, their crude or refined extracts and recombinant proteins (Conrad *et al.*, 1993; Bjorkman *et al.*, 1997; Williams *et al.*, 1997; Schares *et al.*, 2000) [7, 5, 30, 25]. The discovery of lesions and *N. caninum* in a foetus by Immunohistology and Polymerase Chain Reaction (PCR) can all help in diagnosis.

The most common neosporosis symptom is localized encephalitis, which is characterized by necrosis and nonsuppurative inflammation (Barr *et al.*, 1991) [2]. Serological tests, such as the Indirect Fluorescent Antibody Test (IFAT) and Enzyme Linked Immunosorbent Assay (ELISA) can be used to confirm the diagnosis. However, for higher sensitivity and practical reasons, ELISA is one of the best methods for qualitative research.

Materials and Methods

One hundred eighty cattle serum samples were collected in total. According to the 20th Livestock Census, the Jammu district is divided into different areas (village and ward level data, Bovine Census, DAHD Jammu 2019). It is majorly divided into four areas: Jammu, Akhnoor, R.S. Pura, and Bishnah. The samples were collected proportionately to the cattle population in each area. A commercial competitive ELISA kit (Bio X Diagnostics) was used for detection of antibodies against *N. caninum* in serum samples. Using a standardized questionnaire, data was collected on farm-level for assessment of risk factors. Risk variables such as herd history, age, abortion history, repeat breeding, placenta retention and other farm features such as farm management and hygiene were taken into consideration.

Results and Discussion

Out of 180 bovine serum samples examined, 25 were found positive for antibodies against *Neospora Caninum*, yielding an overall seroprevalence of 13.88 percent in the cattle population of Jammu. The highest prevalence was recorded in Akhnoor (18.00 percent), followed by Jammu (14.80 percent), R.S. Pura (8.82 percent) and Bishnah (6.67 percent), indicating that exposure to *N. Caninum* is widespread across the different areas surveyed.

For analysis of risk factors associated with neosporosis, eleven variables were examined, including type of herd,

breed, age, origin of animals, method of rearing, presence of dogs, parity, history of abortion, history of repeat breeding, method of disposal of foetus and foetal membranes, and hygiene and disinfection of farm. Univariable analysis indicated that seropositivity to *N. Caninum* was significantly associated with presence of dogs (OR = 3.02, 95% CI = 1.25-7.28, $p < 0.05$) and the method of disposal of foetal membranes (OR = 6.10, 95% CI = 2.44-15.24, $p < 0.05$), suggesting that environmental contamination with oocysts through improper disposal practices and contact with dogs plays an important role in transmission. In contrast, factors such as type of herd, breed, age, origin of animals, method of rearing, parity, history of abortion, repeat breeding and general farm hygiene showed higher odds of infection in certain categories but did not exhibit a statistically significant association with neosporosis in the present study. The overall seroprevalence of 13.88 percent observed in the present investigation is in line with previous reports from India. Studies conducted by Sengupta *et al.*, (2012) [26] in Karnataka and Andhra Pradesh and Himachal *et al.*, (2020) [16] in central Kerala reported seroprevalence values of 12.61 percent and 18 percent, respectively, while Mahajan *et al.*, (2019) [20] and Meenakshi *et al.*, (2008) [21] in Punjab documented prevalence rates of 10.5 percent and 9.6 percent. On a global scale, the present findings are comparable to those of Jittapalpong *et al.*, (2008) [17] in Thailand (11.7 percent), Woodbine *et al.*, (2008) [32] in England (12.9 percent), Listalves *et al.*, (2006) [19] in Venezuela (11.3 percent) and Vaclavek *et al.*, (2003) in the Czech Republic (12.6 percent), all of whom reported similar intermediate levels of seroprevalence using ELISA-based assays. These similarities suggest that the level of exposure to *N. caninum* in cattle in Jammu is broadly comparable to that seen in many other endemic regions worldwide.

Conversely, several authors have reported lower prevalence estimates, which may reflect differences in geographical conditions, husbandry practices, sample size or diagnostic approaches. Noori *et al.* (2016) [23] in Iran documented a seroprevalence of 3.8 percent, while Yadav *et al.*, (2016) [33] in Nepal's Chitwan district reported 4.84 percent, and Nasir *et al.*, (2012) [22] in South Australia and Koiwai *et al.*, (2005) [18] in Japan observed prevalence values of 2.94 percent and 5.7 percent, respectively. The variation between these reports and the present study could be attributed to differing climatic conditions, population structure, management systems, age distribution of sampled animals and the underlying regional burden of infection.

The significant association of neosporosis with the presence of dogs and with the practice of throwing foetuses and foetal membranes outside rather than disposing them by burial or burning highlights the key role of definitive hosts and environmental contamination in maintaining the infection cycle. Farms with poor sanitary practices and semi-intensive rearing methods showed higher odds of infectivity, though these associations did not reach statistical significance, suggesting that improvements in biosecurity, including controlled dog access and proper disposal of potentially infected materials, may substantially reduce the risk of *N. caninum* transmission. Overall, the observed prevalence and identified risk factors underscore the need for routine screening and targeted management interventions in cattle herds of Jammu to prevent reproductive losses associated with neosporosis

Conclusions

Since the prevalence found was significant at 13.88 percent in cattle serum samples, the study conclusively demonstrates the importance of screening the cattle population of Jammu regardless of any clinical symptoms in order to learn about the exposure of *Neospora caninum*. Findings of this study indicate that the risk factors i.e., the method of disposal and presence of dogs were statistically significant and measures should be taken for the prevention and control of *Neospora caninum* infection. Because there is a higher risk of contracting the disease, proper quarantine measures should be followed when new animals are brought into dairy farms from other farms.

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