Seroprevalence of *Neospora caninum* in cattle in Sudan

Mohammed O. Hussien[1], Abdelghafar M. ElFahal[1], Khalid A. Enan[1], Mohammed S. Mohammed[2], Amira M. Ibrahim[3], Khalid M. Taha[4], AbdelRahim M. El-Hussein[5]

1. Central laboratory, Ministry of Science and Technology, P.O. Box 7099, Khartoum, Sudan;
2. Faculty of Veterinary Medicine, University of El-Butana, Tambool, Sudan;
3. Veterinary Research Institute, Animal Resources Research Corporation (ARRC);
4. Atbara Veterinary Research Laboratory (AVRL), P.O. Box 121, Atbara, Sudan;
5. Animal Resources Research Corporation, P.O. Box 8067 (Al-Amarat), Khartoum, Sudan
Corresponding author: Mohammed O. Hussien, Email: moh200432@hotmail.com
Received: 31-01-2012, Accepted: 25-02-2012, Published Online: 09-06-2012
doi: 10.5455/vetworld.2012.465-468

Abstract

Aim: This cross-sectional survey was conducted to detect anti- *N. caninum* antibodies in bovine dams' sera samples in nine States of the Sudan.

Materials and Methods: Two hundred and seventy six bovine dams' sera samples collected from nine States in the Sudan were screened for anti-*Neospora caninum* antibodies using a commercial competitive ELISA (cELISA) kits.

Results: The results showed an overall prevalence rate of 15.9% (range 6.7% - 70%) of *Neospora caninum* antibodies. The prevalence rates were high in White Nile (70%) and North Kordofan (30%), moderate in the Northern (15.6%), Red Sea (13.3%) States and was low in the South Kordofan (9.4%) and River Nile (6.7%). States with high values of percentage inhibition (PI) detected in White Nile State compared to other States. No antibodies were detected in El Gadarif, Kassala and South Darfur States. The prevalence was observed to be high in crossbred dams (70%) with statistically significant difference ($P<0.05$) compared to local ecotype (9.3%). However, no correlation was observed between infection rates in two age groups.

Conclusion: Neosporosis is prevalent in cattle in most States of Sudan. Absence of evidence of infection in three of the States investigated may reflect the small number of herds and sera tested.

Keywords: *Neospora caninum*, cELISA, Cattle, Sudan

Introduction

Neosporosis is caused by the intracellular protozoan parasite *Neospora caninum* and is known as one of the major causes of infectious abortion, stillbirth and reproductive failure in cattle worldwide [1]. *Neospora caninum* was initially reported by Bjerkas, Mohn and presthus in Norway (1984) [2] as causing encephalitis in dogs. The parasite was later isolated in tissue culture [3]. Domestic canids are the natural definitive host for *N. caninum* [4]. Infected dogs excrete unsporulated oocysts in their faeces in the environment. Under favorable environmental conditions the oocysts sporulate and infection of the intermediate hosts (cattle, sheep, goats, deer, horses etc) occurs through ingestion of the sporulated cysts. Vertical transmission has also been documented and is highly responsible for the positive animals tested [5].

Clinical manifestations of Neosporosis are seen in dogs and cattle. The main clinical manifestation in cattle infected with *N. caninum* is abortion. The diagnosis of Neosporosis in live animals can be achieved by detection of anti- *N. caninum* antibodies using different serological tests, such as the indirect fluorescence antibody test (IFAT), the *Neospora* agglutination test (NAT), enzyme-linked immunosorbent assay (ELISA) and Western blotting. ELISA is an approved serological test [6] that has been used in epidemiological studies to estimate the prevalence of *N. caninum* infections, examine the relationship between exposure to *N. caninum* and abortion, milk yields and culling in cattle [7]. In Sudan, the first record of *N. caninum* infection in cattle has recently been reported by Ibrahim *et al.* [8] in Gezira and Khartoum States.

Owing to the meagre data available on

Table-1. The prevalence rate of anti-*N. caninum* antibodies in bovine dams in nine States in the Sudan during the period February–December, 2011

State	Dams tested(No)	Positive(No)	Prevalence rate (%)
El Gadarif	24	0	00.0
Kassala	23	0	00.0
North Kordofan	30	9	30.0
Red Sea	30	4	13..3
River Nile	30	2	06.7
South Darfur	45	0	00.0
South Kordofan	32	3	09.4
The Northern	32	5	15.6
White Nile	30	21	70.0
The overall prevalence rate	276	44	15.9

Table-2. Influence of breed and age groups on seroprevalence of Neospora caninum in bovine dams in nine States in the Sudan during the period February–December, 2011.

Variable	Dams tested (No)	Percentage positive	P value
Breed: crossbred	30	21(70%)	< 0.05
Local ecotypes	246	23 (9.3%)	
Age groups: 3-4 years	140	20 (14.3%)	> 0.05
4 years and above	136	24 (17.6)	

Neosporosis in most states of the Sudan, this cross-sectional survey was conducted to detect anti-*N. caninum* antibodies in bovine dams' sera samples in nine states in the Sudan during February- December, 2011.

Materials and Methods

Study area: The survey was conducted during the period from February to December 2011 in the Northern, Western and Eastern Sudan and White Nile aimed to cover most of the country. Selection of these locations was based on them being the main potential areas for livestock rearing. Selection of farms was made randomly and the formal mechanism used was lottery.

Sample collection: Five ml of blood sample from each of two hundred and seventy six dams of two age groups (3-4 years and 4years and above) were collected from nine States in Sudan, viz. El Gadarif, Kassala, North Kordofan, Red Sea, River Nile, South Darfur, South Kordofan, the Northern and White Nile States during February- December, 2011. All animals examined in this study were lactating cows and belonged to small–scale farms. The presence of farm dogs was observed in majority of selected farms. Sera were separated by centrifugation at 1500 rpm / min. for 10 minutes and kept at -20OC until tested. The investigation was carried out in compliance with the animal welfare code in Sudan.

ELISA technique: Commercial competitive ELISA

(cELISA) kits for detection of anti- *N. caninum* antibodies (IgG, IgM) were purchased from VMRD, USA. The test is based on the principal of competitive ELISA in which sample serum antibody to N. caninum inhibits binding of horseradish peroxidase (HRP)-labeled *N. caninum*-specific monoclonal antibody to N. caninum antigen coated to the plates. Binding the HRP-labeled monoclonal antibody conjugate is detected by the addition of enzyme substrate and quantified by subsequent colour product development. Weak colour development due to inhibition of the monoclonal antibody binding to the antigen coated in the plates indicates the presence of *N. caninum* antibodies in sample sera. The cutoff value of antibody percentage inhibition (PI) is > 30 i.e. all samples which have antibody PI > 30 are considered positive.

Results

Anti- *N. caninum* antibodies were detected in six (67%) out of nine States with varying prevalences. The prevalence rates of antibodies ranged from 6.7% in River Nile State to 70% in White Nile State. The prevalence rates were high in White Nile (70%) and Northern Kordofan (30%), moderate in the Northern (15.6%), Red Sea (13.3%) and low in South Darfur (9.4%) and River Nile (6.7%) States, with an overall prevalence of 15.9% (44/276) with high values of percentage inhibition (PI) detected in White Nile State compared to other States (data not shown). No anti- *N. caninum* antibodies were detected in El Gadarif, Kassala and South Darfur States (Table-1).

The prevalence was observed to be high in crossbred dams (21/30, 70%) with a significant difference (P<0.05) from local ecotypes (23/246, 9.3%) but, no correlation was observed between infection rates in the two age groups tested (Table-2).

Discussion

Neosporosis has been reported in many countries [9-15] with different prevalence rates since the disease was recognized in 1988 [3]. The prevalence of Neosporosis reported in the current study (15.9%) is higher than that reported for cattle in Canada (9%), Korea (4.1%), but resembles that reported in Poland (15.6%) [12, 14, 15].

Screening bovine sera samples collected from nine States in Sudan for anti-*N. caninum* antibodies showed prevalence rate that ranged from 6.7% in River Nile to 70% in White Nile with no antibodies detected in El Gadarif, Kassala and South Darfur States. The difference in prevalence rates between States may be attributed to environmental differences between geographical areas and topographical reasons that affect oocysts development and survival [16] and to the limited numbers of herds tested. However, the high prevalence rate detected in White Nile State (70%) is hard to explain but it is worth mentioning that all samples (30) in this state were collected from crossbred dams from one modern dairy farm unlike all other samples which were collected from local ecotype dams. Many authors [17, 18] have shown that the presence of farm dogs (natural definitive host for *N. caninum*) is a risk factor for *N. caninum* infection in cattle. Infectious materials, like aborted fetuses, dead calves and fetal membranes could be major sources of infection to dogs. Thus the high seroprevalence of Neosporosis might be related to the presence of many stray and domestic dogs on the dairy farm premises noticed at the time of the study visit in White Nile State. Moreover, management systems are also noted to affect breed seroprevalence more than breed related susceptibility to infection [1].

The prevalence as observed here in crossbred females (70%) was significantly higher (P<0.05) than in local ecotype (9.3%). This is in disagreement with Ibrahim *et al*. [8] who reported that no significant difference between crossbred and local ecotype bovine dams was observed in Khartoum and Gezira States. This may be attributed to the fact that crossbred dams were the predominant type studied by Ibrahim *et al*. [8] in contrast to the localized small number of crossbred animals (that came from one farm) investigated in the present study.

No correlation was observed between infection rates in two age groups. Wouda *et al*. [19], Sadrebazzaz *et al*. [20] and Ibrahim *et al*. [8] reported no significant difference in seropositivity for different age groups of cattle, a fact that may suggest effective vertical transmission of the parasite.

The high values of percentage inhibition (PI) detected in White Nile State compared to other States may indicate the high intensity of infection in this state.

Conclusion

It could be concluded from this preliminary study that Neosporosis is prevalent in cattle in most States of Sudan. Absence of evidence of infection in three of the States investigated may reflect the small number of herds and sera tested. This is supported by the fact that not all herds tested in Khartoum and Gezira States of Sudan were positive for *Neospora caninum* antibodies. Therefore, further large scale epizootiological investigations on *Neospora caninum* infection including other farm animals at the country level are necessary to fully evaluate the epidemiology and economic impact of this parasite in Sudan.

Authors' contribution

Acknowledgements

Competing interests

Authors declare that they have no competing interests.

References

1. Dubey, J.P., Shares, G. and Ortega-Mora L.M. (2007): Epidemiology and control of neosporosis and *Neospora caninum*. *Clin. Microbiol. Rev.*, 20 (2): 323-67.

2. Bjerkas, I.; Mohn, S. F. and Presthus, J. (1984): Unidentified cyst-forming sporozoon causing encephalomyelitis and myositis in dogs. *Zeitschrift für Parasitenkunde.,* 70: 271–274.

3. Dubey, J. P.; Hattel, A. L.; Lindsay, D. S. and Topper, M. J. (1988): Neonatal *Neospora caninum* infection in dogs: isolation of the causative agent and experimental transmission. *J. Am. Vet. Med. Ass.,* 193: 1259–1263.

4. Basso, W.; Venturini, L.; Venturini, M. C.; Hill, D. E.; Kwok, O. C.; Shen, S. K. and Dubey, J. P. (2001): First isolation of *Neospora caninum* from the feces of a naturally infected dog. *J. Parasitol.,* 87: 612-618.

5. Dijkstra, T.; Eysker, M.; Schares, G.; Conraths, F. J.; Wouda, W. and Barkema, H. W. (2001): Dogs shed *Neospora caninum* oocysts after ingestion of naturally infected bovine placenta but not after ingestion of colostrum spiked with *Neospora caninum* tachyzoites. *J. Parasitol.,* 31: 747-752.

6. Von Blumroder, D.; Schares, G.; Norton, R.; Williams, D.J.; Esteban-Redondo, I.; Wright, S.; Bjorkman, C.; Frossling, J.; Risco-Castillo, V.; Fernandez-Garcia, A.; Ortega-Mora, L.M.; Sager, H.; Hemphill, A.; van Maanen, C.; Wouda, W. and Conraths, F.J. (2004): Comparison and standardisation of serological methods for the diagnosis of *Neospora caninum* infection in bovines. *Vet. Parasitol.,* 120: 11-22.

7. Hernandez, J.; Risco, C. and Donovan, A. (2002): Risk of abortion associated with *Neospora caninum* during different lactations and evidence of congenital transmission in dairy cows. *J. Am. Vet. Med. Ass.,* 221: 1742-1746.

8. Ibrahim, A. M.; Elfahal, A.M. and El Hussein, A.M. (2011): First report of *Neospora caninum* infection in cattle in Sudan. *Trop. Anim. Health Prod.* Published online 07 September 2011. DOI: 10.1007/s11250-011-9963-5.

9. Buxton, D.; Caldow, G. L.; Moley, S. W.; Marks, J. and Innes, E. A. (1997): Neosporosis and bovine abortion in Scotland. *Vet. Rec.,* 141: 649-651.

10. Campero, C. M.; Anderson, M. L.; Conosciuto, G.; Odriozola, H.; Bretschneider, G. and Poso, M. A. (1998): *Neospora caninum* associated abortion in a dairy herd in Argentina. *Vet. Rec.,* 143: 228-229.

11. Perez, E.; Gonzales, O.; Dolz, G.; Moraks, J. A.; Bair, B. and Conrad, P. A. (1998): First report of bovine neosporosis in dairy cattle in Costa Rica. *Vet. Rec.,* 142: 520-521.

12. Cabaj, W.; Chromanski, L.; Rodgers, S.; Moskwa, B. and Malczewski, A. (2000): *Neospora caninum* infections in aborting dairy cows in Poland. *Acta Parasitol.,* 45: 113-114.

13. Dijkstra, T. H.; Barkema, H. W.; Eysker, M. and Wounda, W. (2001): Evidence of post-natal transmission of *Neospora caninum* in Dutch dairy herds. *Int. J. Parasitol.,* 31: 209-215.

14. Waldner, C. L.; Henderson, J.; Wu, J. T. Y.; Coupland, R. and Chow, E. Y. W. (2001): Seroprevalence of *Neospora caninum* in beef cattle in northern Alberta. *Can. Vet. J.,* 42: 130-132.

15. Kim, J. H.; Lee, J. K.; Hwang, E. K. and Kim, D. Y. (2002): Prevalence of antibodies to *Neospora caninum* in Korean beef cattle. *J. Vet. Med. Sci.,* 64: 941-943.

16. Sanderson, M.W.; Gay, J.M. and Baszler, T.V. (2000): *Neospora caninum* sero-prevalence and associated risk factors in beef cattle in the northwestern United States. *Vet Parasitol.,* 90: 15–24.

17. Pare, J.; Fecteau, G.; Fortin, M. and Marsolais, G. (1998): Seroepidemiologic study of *Neospora caninum* in dairy herds. *J. Am. Vet. Med. Assoc.,* 213: 1595-1598.

18. Lindsay, D. S.; Dubey, J. P. and Duncan, R. B. (1999): Confirmation that the dog is a definitive host for *Neospora caninum. Vet. Parasitol.,* 82: 327-333.

19. Wouda, W.; Moen, A.R. and Schukken, Y.H. (1998): Abortion risk in progeny of cows after a *Neospora caninum* epidemic. *Theriogenol.,* 49: 1311-1316.

20. Sadrebazzaz, A.; Haddadzadeh, H.; Esmailnia, K.; Habibi, G.; Vojgani, M. and Hashemifesharaki, R. (2004): Serological prevalence of *Neospora caninum* in healthy and aborted dairy cattle in Mashhad, Iran. *Vet. Parasitol.,* 124: 201–204.

****