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Seroprevalence of Bovine Paratuberculosis Specific Antibodies in Khartoum and Al-Jazeera States, Sudan

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Abstract: This study was conducted to estimate the bovine paratuberculosis seroprevalence in Khartoum and Al-Jazeera states, Sudan. The generated results showed that in Khartoum state the seroprevalence of paratuberculosis was 66.7% at the herd level and 10.2% at the individual animal level. The lowest seroprevalence (8%) was found at Kuku and the highest (18.8%) was found at El-Sealeat localities. All sera collected from El-Kadaro, Wad-Medani and El-Salama localities were found negative for Mycobacterium avium Subspecies Paratuberculosis (MAP) antibodies. Khartoum North showed the highest rate of seropositivity whereas Omdurman showed the lowest. Relationship between seroprevalence and clinical manifestation were described. The results of this study reported a widespread of bovine paratuberculosis in the Khartoum state.

Key words: Seroprevalence, bovine, paratuberculosis, ELISA, antibodies, Sudan

INTRODUCTION

Paratuberculosis (Johne's Disease, (JD) is a chronic progressive, granulomatous enteritis caused by MAP (Harris and Barletta, 2001). Paratuberculosis has been recognized as a major disease of ruminants for more than a century and has significant economic and welfare effects on livestock in all continents (Chiodini *et al*., 1984). Several animal species are known to be susceptible to infection but the primary hosts are ruminants including cattle, sheep, goats, llamas, alpacas and non domesticated hoofed stock such as bison and deer (Harris and Barletta, 2001). Paratuberculosis has also been reported in a variety of free range animal species including antelope (Dukes *et al*., 1992), bighorn sheep (Williams *et al*., 1983), white-tailed deer (Chiodini and van Kruiningen, 1983), tule elk (Cook *et al*., 1996), moose (Soltys *et al*., 1967), red deer, roe-deer, fallow deer and moufflon (Pavlik *et al*., 2000), rabbits (Angus, 1990) and earthworms (Fischer *et al*., 2003). Some of these free range animals have been implicated as potential carriers of MAP for domestic ruminants (Dukes *et al*., 1992; Chiodini and van Kruiningen, 1983; Daniels *et al*., 2001).

Infections with MAP occur in the first 6 months of life and can persist for several years with mild clinical manifestations (Merkal *et al*., 1975; Kennedy and Benedictus, 2001; Manning and Collins, 2001; Storset *et al*., 2001). Recently, MAP has received an increasingly wide interest because of a rapidly growing body of scientific evidence which suggests that human infection with MAP may cause some and possibly all, cases of Crohn's disease (Naser *et al*., 2004; Uzoigwe *et al*., 2007).

ELISA was the most widely used test for screening herds. Detection of infection by ELISA appears to be dependent upon the stage of disease (Yayo-Ayele, 2001). ELISA sensitivity for clinical cases has been reported to be 87 and 15% in subclinical cases (Sweeney *et al*., 1995). Most paratuberculosis experts recommend that ELISA positive reactors should be confirmed by faecal cultures (Yayo-Ayele *et al*., 2001).

In general, it is accepted that the sensitivity of the antibody ELISA is 50% in all infected adults and this ratio rises up to 90% in clinical cases (Sweeney *et al*., 1995). A series of reports from North America and Australia have indicated that the specificity of the test may reach 97% up to 99% or above (Cox *et al*., 1991; Reichel *et al*., 1999). ELISA tests to detect MAP antibodies in milk do not correlate well with the ELISA results on serum from the same animals. Similarly, the bulk-milk ELISA test does not accurately predict the percentage of cattle in herds that were positive by ELISA on serum samples (Hardin, 1995).

In the Sudan, paratuberculosis was first diagnosed in goats by Fawi and Obied (1964). Thereafter, 9 clinical cases at El Gurashi dairy farm were diagnosed by El Derdiri. Later it was diagnosed in kuku area and Belgravia dairy farm by Abu Buker and Elsanousi (1975). Abbas *et al*. (1986) recovered the organism from clinical

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cases of goats. About 11 isolates of MAP were isolated from one thousand of cows. The investigation of Mongash in the Sudan, revealed 23 cows were positive by Ager Gel Immunodiffusion (AGID) test where as 13 out of 830 were positive by ELISA.

In the same farm 13 out of 120 were found to have acid fast bacteria in the rectal scrapings (Mongash, 1989). The aim of this study was to determine the seroprevalence rate of bovine paratuberculosis in Khartoum and Aljazeera states, Sudan.

MATERIAS AND METHODS

Sera: About 225 serum samples were collected from nine dairy herds of cross bred between Friesian and local Butana breeds. Eight herds were from Khartoum state and one from Aljazeera state. Collected sera were labelled and stored at -20°C till being used.

ELISA procedure: ELISA kits specific for detection of MAP antibodies were purchased from Institut Pourquier, Montpellier, France (Cat. No P07110). The kit procedure was similar to that described by OIE. The test was performed according to the manufacturer's instructions. Briefly, the microplate wells were coated with a protoplasmic extract of MAP.

In order to minimize cross-reactions, samples along with positive and negative controls were diluted 1:20 in a buffer containing an extract of *Mycobacterium phlei* and placed on a shaker for 15 min at 24°C. All samples and reagents were added at 100 μL well^{-1}. Diluted samples and controls were dispensed into the antigen coated wells and incubated for 1 h at 24°C. The wells were then washed manually. After washing, a peroxidase labelled monoclonal anti-ruminant IgG conjugate was added to each well and incubated for 30 min. The plates were then washed and Tetra-Methyl-Benzidine (TMB) buffer was added in each well and incubated for 10 min.

The reaction was stopped by using 0.5 M sulphuric acid and Optical Densities (OD) were read at 450 nm using a microplate reader (Digital and analog systems s.r.l, Palombara Sabina, Roma, Itlay). Each test serum and both controls were evaluated in two adjacent wells. The obtained results were interpreted according to the formula given by the manufacturer:

$$\frac{S}{P} = \frac{\text{Corrected OD 450 value of the sample}}{\text{Mean corrected OD 450 value of the positive control}} \times 100$$

- Any sample with an S/P equivalent or <60% is considered to be from an animal which has not been infected by MAP
- Any sample with an S/P is between 60 and 70% is considered to be doubtful. A second test will be necessary to confirm its status
- Any sample with an S/P equivalent or >70% is considered coming from an animal which has been infected by MAP

RESULTS AND DISCUSSION

The results of ELISA test showed that 23 out of 225 (10.2%) serum samples were positive (Table 1). This indicates that the overall prevalence of seropositive cattle was 10.2%. Paratuberculosis seropositive animals were detected in 6 herds (66.7%). Regarding localities, the lowest value of seroprevalence (8%) was found at Hilat kuku and the highest (18.8%) was found at El-Sealeat areas (Table 1). All sera collected from El-Kadaro, Wad-Medani and El-Salama were found negative for MAP antibodies. Sero-positive animals were found in all locations with different prevalence rates except for Wad-Madani. Khartoum North showed the highest rate of seropositivity while Khartoum showed the lowest (Table 1). The seroprevalence among clinical and subclinical cases were 100 and 5.6%, respectively (Table 2). In El-Salma herd although, there was a history of paratuberculosis no specific antibodies for MAP were detected.

The present study was designed to study the prevalence of MAP specific antibodies among dairy cattle in both Khartoum and Al-Jazeera states. The overall seroprevalence detected by ELISA in Khartoum (10.2) was found to be higher than that reported by Mongash (1989). This may indicate that the infection is progressing in the area of the study. In this study, the positive reactors were

Table 1: Seroprevalence of bovine paratuberculosis in Khartoum and Al-Jazeera states

State	Province	Locality	No. of test animals	No. of +ve reactors	Prevalence (%)
Khartoum	Khartoum North	El-Sealeat	16	3	18.8
		El-Bagair	50	9	18.0
		Hilat Kuku	25	2	8.0
	Khartoum	El-Kadaro	19	0	0.0
		El-Salama	33	0	0.0
		Soba	37	5	13.5
	Omdurman	Omdurman	32	4	12.5
Al-Jazeera	Wad-Medani	Wad-Medani	13	0	0.0
	Total		225	23	10.2

Table 2: Seroprevalence of bovine paratuberculosis in relation to clinical signs

Clinical signs	Animals tested (No)	ELISA positive (No)	Prevalence (%)
Clinical cases	8	8	100.0
Subclinical cases	217	15	6.9
Total	225	23	10.2%

found at Hilat kuku, Soba and El-Bagair areas, a finding similar to that reported by Mongash (1989). These findings was direct evidence that these areas were endemic with bovine paratuberculosis and they may constitute a continuous source of shedding the infection. Although, there is a history of the disease in El-Salma area, no MAP antibodies were detected. This result might be attributed to the good husbandry management, culling and disposable of the clinical and heavy fecal shedders by the animal owners in the area as was explained previously by Whitlock *et al.* (2000).

The source of bovine paratuberculosis in Sudan was suggested to be through the importation of foreign dairy cattle from European countries (Mongash, 1989). In the study, it is reported the prevalence of MAP specific antibodies in crossbred cattle between Friesian and Butana breeds, since all the sera were collected from these cattle.

Seroprevalence in clinical cases was higher (100%) than in subclinical cases (6%), a finding similar to that reported by Sweeney and colleagues in 1995.

CONCLUSION

The results of this survey indicated that MAP antibodies are widely distributed in cattle in the Khartoum North. This may be attributed to the crowdness of the animals in this area without clear partitions between the farms which might increase the potential for exposure to contaminated faeces. This area requires special attention of veterinarians and producers in order to establish an efficient control programme with regular revaluation.

It needs to apply seroprevalence of bovine paratuberculosis as nationwide to provide information used in control programme and eradication. Due to its low cost, accuracy and ease of sample collection and shipment, it suggest ELISA can be used for this purpose as a screening test on herd or individual levels followed by other methods to confirm animals which were positive for antibodies specific for MAP by ELISA.

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REFERENCES

Abbas, B., S.E.O. Idris and A. Burhan, 1986. Isolation of *Mycobacterium paratuberculosis* from goats in Sudan. Sudan J. Vet. Sci. Anim. Husb., 25: 41-42.

Abu Buker, M.I. and S.M. Elsanousi, 1975. A survey of Johne's disease in Khartoum. Vet. Rec., 18: 94-95.

Angus, K.W., 1990. Intestinal lesions resembling paratuberculosis in a wild rabbit (*Oryctolagus cuniculus*). J. Comp. Pathol., 103: 101-105.

Chiodini, R.J., J.J. van Kruiningen and R.S. Merkal, 1984. Ruminant paratuberculosis (Johne's disease): The current status and future prospects. Cornell. Vet., 74: 218-262.

Chiodini, R.Y. and H.J. van Kruiningen, 1983. Eastern white-tailed deer as a reservoir of ruminant paratuberculosis. J. Am. Vet. Med. Assoc., 182: 168-169.

Cook, W.E., T.E. Cornish, S. Shideler, B. Lasky and M.T. Collins, 1996. Radiometric culture of *Mycobacterium paratuberculosis* from feces of tule elk. J. Wildl. Dis., 33: 635-637.

Cox, J.C., D.P. Drane, S.L. Jones, R. Ridge and A.R. Milner, 1991. Development and evaluation of a rapid absorbed enzyme immunoassay test for the diagnosis of Johne's disease in cattle. Aust. Vet. J., 68: 157-160.

Daniels, M.J., N. Ball, M.R. Hutchings and A. Greig, 2001. The grazing response to pasture contaminated with rabbit feces and implications for the transmission of paratuberculosis. Vet. J., 161: 306-313.

Dukes, T.W., G.J. Glover, B.W. Brooks, J.R. Duncan and M. Swendroski, 1992. Paratuberculosis in saiga antelope (*Saiga tatarica*) and experimental transmission to domestic sheep. J. Wildl. Dis., 28: 161-170.

Fawi, M.T. and H.M. Obied, 1964. A note of Johne's disease among cattle in Sudan. Bull. Epizoot. Dis. Afri., 12: 437-437.

Fischer, O.A., L. Matlova, J. Bartl, L. Dvorska and P. Svastova *et al*., 2003. Earthworms (*Oligochaeta*, *Lumbricidae*) and mycobacterium. Vet. Microbiol., 91: 325-338.

Hardin, L.E., 1995. Comparison of milk with serum ELISA for the detection of paratuberculosis in dairy cows. M.Sc. Thesis, University of Missouri, Columbia.

Harris, B.N. and R.G. Barletta, 2001. *Mycobacterium avium* subsp. paratuberculosis in veterinary medicine. Clin. Microbiol. Rev., 14: 489-512.

Kennedy, D.J. and G. Benedictus, 2001. Control of *Mycobacterium avium* subsp paratuberculosis infection in agricultural species. Rev. Sci. Tech., 20: 151-179.

Manning, E.J. and M.T. Collins, 2001. *Mycobacterium avium* subsp. paratuberculosis: Pathogen, pathogenesis and diagnosis. Rev. Sci. Tech., 20: 133-150.

Merkal, R.S., A.B. Larsen and G.D. Booth, 1975. Analysis of the effect of inapparent bovine paratuberculosis. Am. J. Vet. Res., 36: 837-838.

Mongash, B.M., 1989. Diagnosis of Johne's disease in cattle. M.Sc. Thesis, Department of Medicine, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum, Sudan.

Naser, S.A., G. Ghobrial, C. Romero and J.F. Valentine, 2004. Culture of *Mycobacterium avium* subspecies paratuberculosis from the blood of patients with Crohn's disease. Lancet, 364: 1039-1044.

Pavlik, I., Z. Rozsypalova, T. Vesely, J. Bartl and L. Matlova *et al*., 2000. Control of paratuberculosis in five cattle farms by serological tests and faecal culture during the period 1990-1999. Vet. Med. Czech., 45: 61-70.

Reichel, M.P., R. Kittelberger, M.E. Penrose, R.M. Meynell and D. Cousins *et al*., 1999. Comparison of serological tests and faecal culture for the detection of *Mycobacterium avium* subspecies paratuberculosis infection in cattle and analysis of the antigens involved. Vet. Microbiol., 66: 135-150.

Soltys, M.A., C.E. Andress and A.L. Fletch, 1967. Johne's disease in a moose (Aclesacles). Bull. Wildl. Dis. Assoc., 3: 183-184.

Storset, A.K., H.J. Hasvold, M. Valheim, H. Brun-Hansen and G. Berntsen *et al*., 2001. Subclinical paratuberculosis in goats following experimental infection: An immunological and microbiological study. Vet. Immunol. Unopathol., 80: 271-287.

Sweeney, R.W., R.H. Whitlock, C.L. Buckley and P.A. Spencer, 1995. Evaluation of a ommercial enzyme-linked immunosorbent assay for the diagnosis of paratuberculosis in dairy cattle. J. Vet. Diagn. Invest., 7: 488-493.

Uzoigwe, J.C., M.L. Khaitsa and P.S. Gibbs, 2007. Epidemiological evidence for *Mycobacterium avium* subspecies paratuberculosis as a cause of Crohn's disease. Epidemiol. Infect., 135: 1057-1068.

Whitlock, R.H., S.J. Wells, R.W. Sweeney and J.V. Tiem, 2000. ELISA and faecal culture for paratuberculosis (Johne's disease): Sensitivity and specificity of each method. Vet. Microbiol., 77: 387-398.

Williams, E.S., S.P. Snyder and K.L. Martin, 1983. Pathology of spontaneous and experimental infection of North American wild ruminants with *Mycobacterium paratuberculosis*. Vet. Pathol., 20: 274-291.

Yayo-Ayele, W., M. Machackova and I. Pavlik, 2001. The transmission and impact of berculosis infection in domestic and wild ruminants. Vet. Med. Czech., 7-8: 205-224.