Vol 15 No 2, p 163-165

THE EFFICIENCY OF BOVINE ELISA IN DETECTION OF THE *Mycobacterium avium* SUBSPECIES *Paratuberculosis* (MAP) INFECTION IN CAMEL (*Camelus dromedaries*) AT DIFFERENT AGES

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ABSTRACT

Mycobacterium avium subspecies *Paratuberculosis* infection is chronic disease that infect young animals and remains undetected for long period. The immune responses to the disease is characterised by the cell mediated immunity at the early stage while antibody response dominates the late stage of the infection. Early detection of the disease is vital to prevent its transmission to the susceptible animals. ELISA was seen one the sensitive tests in detection of the disease. The application of bovine test in detecting the disease was sought to unravel its versatility in camel. 95 serum samples [2-3 years-old (3 samples), 4-6 years-old (21 samples), 7-9 years old (24 samples) and 10-15 years-old (47 samples)] were collected from dromedary camels. The analysis of serum samples with commercial ELISA indicated only 8 positive (8.4%) and one inconclusive samples. The positive samples were restricted to older ages (7-9 years-old and 10-15 years-old). Despite the emaciation of few animals, the postmortem revealed no significant changes in intestine. The results proved that bovine ELISA is feasible in detecting anti-MAP antibodies in camel. However, the limitation of the ELISA sensitivity in detecting the infection in young animals will leave wide range of infected young animals undetected. The results encourage the application of ELISA concurrent with PCR and/or faecal culture in study of MAP infection prevalence in camel in Saudi Arabia.

Key words: Bovine ELISA, camel, *Mycobacterium avium* subspecies *Paratuberculosis*

Mycobacterium avium subspecies *Paratuberculosis* (MAP) causes John's disease in domestic and wild ruminant like cattle, sheep, goats, deer, antelope and bison (Stabel, 1997) worldwide. In Saudi Arabia, John's disease was reported in sheep, goat, dairy cattle, and camel (Ahmed and Towfik, 1999; Gameel *et al,* 1994; Alluwaimi *et al,* 1999; Al Hajri and Alluwaimi, 2007).

Long incubation period is the main characteristic feature of MAP infection. Ingestion of faecal material, milk or colostrum is the main route of infection. Infected cattle shed low amount of bacteria during the subclinical stage. However, during the clinical stage the shaded organisms in faeces increase dramatically. At the clinical stage, infected animals manifest chronic diarrhoea, emaciation, decrease milk production and infertility (Stabel, 1997).

Immunodiagnostic techniques were applied to assure the early diagnosis of MAP infection. Early diagnosis of MAP infection represents one of the major obstacles in successful control of the disease (Valentin-Weignad, 2002). During the subclinical phase, the immune responses are dominated by cell-mediated immunity whereas, humoral immune responses prevail at the clinical phase (Coussens, 2001; Valentin-Weignad, 2002). Different versions of enzyme linked immunosorbent assay (ELISA) and molecular based techniques were introduced in the last decade to overcome the impediments of detecting the subclinical MAP infection. Nevertheless, the newly advanced techniques were not sensitive enough unless they were used in suitable combination to achieve clear cut diagnosis (Collins *et al,* 1993a, b; Collins, 1996; Paolicchi *et al,* 2003; Moss *et al,* 1991).

ELISA remains one of the most applied tests for the early detection of MAP infection. Overwhelming studies examined the sensitivity and specificity of ELISA in the detection of subclinical MAP infection (Nielsen and Toft, 2006; Collins, 2002; 2005). The sensitivity of ELISA in detecting the infection at the early stage of the infection is about 15%. ELISA sensitivity in moderately shedding animals is only 47-48%. However, it scores 88% in animals with clinical signs (Whitlock *et al,* 2000). Although, ELISA was

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developed to detect anti-MAP antibodies in camelid in south America (Kramsky *et al,* 2000), there are no information on application of this newly introduced techniques and their versatility in detecting MAP infection in dromedary camels.

The main objective of this study was to assure the feasibility of bovine ELISA kit as a robust technique in detecting at the late subclinical and early clinical stages of MAP infection. The efficiency of ELISA in detecting MAP infection will assure stringent approaches in control of the disease in the country.

Materials and Methods

Pre and post partum examination

Animals were examined before slaughter for the emaciation and diarrhoea. The ileum and mesenteric lymph nodes were examined after the slaughter.

Serum samples

A total of 95 serum samples were collected from different ages of camel at Dammam abattoir. The samples were arranged in 4 groups as follows, 2-3 years-old (3 samples), 4-6 years-old (21 samples), 7-9 years old (24 samples) and 10-15 years old (47 samples).

ELISA for the detection of the anti-MAP antibodies

The camel serum samples were analysed for the presence of anti-MAP antibodies using ID Vet ELISA kit (ID VET, Montpellier, France). After preabsorption step with *Mycobacterium phlei* to remove potential cross-reacting antibodies, 100 µl of the previously neutralised samples and controls were transferred to the coated ELISA micoroplate and incubated for 45 minutes at room temperature. After thorough washing 100 µl of 1X conjugate was then added to each well and incubated for 30 minutes at room temperature. 100 µl of substrate solution was then added to each well after rewashing and incubation in dark for 15 minutes at room temperature. Then 100 µl of stop solution was added to each well. The plate was read by ELISA reader (Thermo Labsystem, Finland) at dual wavelength 450 and 620 nm.

Results

The analysis of serum samples with ELISA

The serum analysis with ELISA revealed 8 positive (8.4%) and one doubtful (1%). The positive samples were restricted to the old animals [6 samples of 10-15 years old (12.7%) and 2 samples of 7-9 years-old (8.3%)]. Table 1 refers to the distribution of serum samples analysis according to age.

The pre and postmortem examination

Only one of the few emaciated camels appeared to be ELISA positive. The postmortem examination of ileum and mesenteric lymph nodes of the emaciated and other camels failed to reveal any significant histological changes.

Discussion

This study examined the possibility of detecting anti-MAP infection by bovine ELISA kit. It is considered the first to attempt to test the feasibility of this approach in detecting the MAP infection in camel.

MAP infection is chronic debilitating disease. Animals are infected at their early stage of life (0-4 months). However, the development of the disease is subjected to various factors like infective dose and age of the animal. Hence, three groups of animals in regard to MAP infection can be classified as affected, infectious and infected (Nielsen and Toft, 2008). The affected animals are those that manifest clinical signs such as diarrhoea, reduction of milk production and chronic weight loss. Infectious animals on the other hand, comprise those animals that shed MAP and considered a source of transmission to the susceptible animals. The third group is the infected group which lies between the affected and the infectious groups. In the infected animals MAP persists in macrophages.

In relation to the above mentioned classification, the sensitivity of ELISA is greatly influenced by the type of the group. Therefore, animals shedding less than 10 colonies per tube are more likely to be seronegative, while shedding more than 70 colonies per tube are considered strong seropositive. Hence, ELISA sensitivity in cattle naturally infected with MAP could be as low as 15% in low shedding young animals, whereas in the moderately shedding animals the sensitivity could reach to 47-48%, while the heavily shedding animals the sensitivity is 88% (Whitlock *et al,* 2000).

In view of these facts the collection of the samples was concentrated mainly on the animals above 3 years because the application of ELISA was

Table 1. The total analysis of ELISA according to age of camels.

Age	Positive	Negative	Inconclusive	Total	Age (%)
2-3	0	2	1	3	3
4-6	0	21	0	21	22
7-9	2	22	0	24	25
10-15	6	41	0	47	50
Total	**8**	**86**	**1**	**95**	100
Total (%)	**8.4**	**90.6**	**1**	100	

most likely to reveal more positive samples in old animals. In consistence with this approach the results of this study are in accordance with the previous report (Al Hajri and Alluwiami, 2007). Furthermore, the results reflect the feasibility of the MAP antigen in the ELISA kit for detecting the disease in camel as well as the practicality of the conjugated antibodies to detect the camel anti-MAP antibodies. Nevertheless, limitation of ELISA in detecting the infection in young camels will leave wide range of young infected camels (below 4 years) undetected. Hence, concurrent application of polymerase chain reaction (PCR) with ELISA is essential in increasing the detection range of the infected animals.

In general, this preliminary study provides promising insight in utilising bovine ELISA in the study of the prevalence of the MAP infection in *dromedariy camel* in Saudi Arabia.

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