Clinicopathological Findings of Partuberculosis in Camels Possible Steps for Control Strategy

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Abstract: *Mycobacterium avium* subspecies *paratuberculosis* causes paratuberculosis a chronic debilitating granulomatous enteritis of camels as well as domestic and wild ruminants. The clinical manifestation of the disease in camel is not well characterized, therefore this study was aimed to investigate the clinical and pathological pictures of camel that are suffering from partuberculosis. A total of 12 young camels that were presented to the Veterinary Teaching Hospital, King Faisal University were investigated. Clinical and pathologic examination were performed. The results revealed highly significant increase in creatinine, blood urea nitrogen, magnesium, AST and ALT in diseased camels. While glucose total protein and albumin were highly significantly decreased in diseased camels when compared to healthy ones. Post-mortem testing indicated thickening corrugation of the intestinal wall folded mucosa enlarged and oedemated ileocaecal and mesenteric lymph nodes. The microscopic findings detected short blunt and distorted intestinal villi with hyperactive goblets cells of the villi and the crypts of lieberkuhn contained mucin droplets. The lamina propria was heavily infiltrated with mononuclear cells mostly macrophages. This clinical picture of paratuberculosis may be used to initiate control strategy to limit the spread of the disease in camel herds.

Key words: Camels, *Mycobacterium paratuberculosis*, hematology, blood chemistry, post-mortem

INTRODUCTION

Paratuberculosis is a chronic debilitating granulomatous enteritis of domestic and wild ruminants mainly cattle sheep goats, camel, deer, antelope and bison. The disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (Collins, 2003; Gonzalez *et al.*, 2005). Clinically, the disease in ruminants is characterized by irreversible wasting, diarrhea, dehydration and later may be death. The infection is chronic, progressive and unresponsive to treatment (Ayele *et al.*, 2001). The clinical picture of the disease was well documented in domesticated ruminants rather than camel.

In Saudi Arabia, paratuberculosis (locally known Silag) was documented in different ruminant specie namely cattle, sheep, goat and camel (Ahmed and Towfik, 1995; Gameel *et al.*, 1994; Alluwaimi *et al.*, 1999; Al-Hajri and Alluwaimi, 2007; Alluwaimi, 2008; Zaghawa *et al.*, 2011). Since, camel population is very high in the country where they are considered valuable animals with significant social and economical impact. In the last few years, it was observed that paratuberculosis

is becoming an increasing health problem in many camel herds. The general circumstantial evidences are increased clinical cases, samples from abattoirs, owner's observations and the veterinarians examination. This increase in clinical cases number was hindered by efficient diagnostic tests, although PCR ELISA tests were attempted (Alhebbabi and Alluwaimi, 2010; Alharbi *et al.*, 2012). Consequently control and eradication program will be difficult to implement. As the clinical data of the disease in camel is very scanty, this study was conducted to investigate clinical and pathological pictures of camel that are suffering from partuberculosis.

MATERIALS AND METHODS

Animals: A total of 12 camels with age ranging from 2-4 years that were presented to the Veterinary Teaching Hospital, King Faisal University were investigated. Clinical examination was performed. The animals had clinical signs that included watery diarrhea for >2 months and signs of weakness, emaciation, fluctuating temperature and dehydration. Blood samples were taken and analyzed for hematology as well as blood chemistry.

Post-mortem examination: One of the camels with bad condition was humanely killed using a high intravenous dose of thiopental sodium. Immediately after euthanasia pathological examinations was performed. Tissue samples from ileum, colon, mesenteric and ileocaecal lymph nodes were collected. The tissues were trimmed to a smaller size fixed in 10% buffered formalin, embedded in paraffin wax sectioned at 4-5 μ m thick and stained with haematoxylin and eosin (H&E).

Statistical analysis: Data were analyzed by the General Linear Model (GLM) procedure for unequal numbers (SAS, 2002). The Least Square Mean (LSM) $\pm$ standard errors were calculated for diseased and healthy camels and tested for significances using the student t-test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Blood findings: The haematological data are shown in Table 1. The present results revealed highly significant increase in the level of granulocytes and leukocytes in diseased camels when compared to healthy ones. Moreover, there was highly significant decrease in haemoglobin, RBCS while packed cell volume was just significantly lower in diseased camels when compared to healthy ones.

The results of the biochemical analysis revealed that there was highly significant increase in creatinine

magnesium, AST and ALT in diseased camels when compared to healthy ones (Table 2). In the meantime, Blood Urea Nitrogen (BUN) was just significantly increased in diseased camels. On the other hand, both total protein and albumin showed highly significant decrease in diseased camels when compared to healthy ones. No significant difference was detected in phosphorus, glucose, URIC and Ca.

Gross findings: The macroscopic changes principally affect the ileum and colon. The most prominent gross lesions were thickening and corrugation of the intestinal wall. The mucosa was folded into transverse ridges (Fig. 1a). The ileocaecal and mesenteric lymph nodes were enlarged and oedematous (Fig. 1b). No other gross lesions were noticed.

Microscopic findings: The microscopic findings in the ileum and the colon were quite similar in most cases. The intestinal villi were short, blunt and distorted. The villi were fused to each other in some cases. The goblets cells of the villi and the crypts of lieberkuhn were hyperactive and contained mucin droplets. The lamina propria was heavy infiltrated with mononuclear cells mostly macrophages (Fig. 1c) as well as a few number of eosinophils. The peyer's patches as well as lymphoid aggregation in the ileum and the colon, respectively exhibited hyperplasia. In most cases the mesenteric and the ileocaecal lymph nodes appeared to be hyper-activated. There was a marked lymphocytic hyperplasia in lymphoid follicles and medullary cords. The most prominent findings were medullary histiocytosis (Fig. 1d). Interest in raising camels is rapidly increasing in the kingdom of Saudi Arabia. The purpose of camel raising includes showing and production of meat and milk. The growing interest has lead to significant increase in the value of camels. Unfortunately, the elevated numbers of camel herds suffering from *M. avium* subspecies *paratuberculosis* causes great impact on this industry. This impact is not only a matter of live or death but also significant loss of productivity. The fact that no reliable data on the economic value of the impact of the disease on camel industry must not underestimate the effort to research approaches to control the disease. The impact of *M. paratuberculosis* in other domestic animals may be reduced following management changes, test and culling and vaccination (Bastida and Juste, 2011).

Detecting clinically ill animals and performing fast blood profile provide early steps in identifying *M. paratuberculosis* likely affected animals. Clinical signs that are highly indicative of *M. paratuberculosis* included watery diarrhea, weakness, emaciation, fluctuating

Table 1: The basic haematological picture in control and 12 affected camels

Contents	Control		Affected		Significance
	Mean	SE	Mean	SE	
MONP	4.78	0.50	5.68	0.56	
GRNP	38.41	3.25	54.80	1.51	**
HB	15.13	0.52	12.49	0.69	**
WBC	5.01	0.51	16.05	0.78	**
LYMP	56.85	3.06	39.53	1.78	**
PCV	36.45	1.98	44.44	1.98	*
RBCS	12.42	0.56	9.20	0.67	**

Table 2: The basic biochemical findings in control and 12 affected camels

Items	Control		Affected		Significance
	Mean	SE	Mean	SE	
Creatinine	1.29	0.02	2.54	0.16	**
BUN	15.11	0.82	18.53	0.54	*
AST	48.21	2.23	131.09	2.16	**
P	3.24	0.17	3.38	0.21	
Glucose	97.87	2.20	81.03	4.31	
T. PROT	6.48	0.38	5.20	0.57	**
URIC	0.69	0.02	0.69	0.03	
ALT	10.26	0.73	32.22	4.01	**
Mg	0.73	0.07	1.47	0.16	**
Ca	10.37	0.32	10.23	0.42	
ALB	3.13	0.19	2.10	0.29	*

*Significant at $p < 0.05$ between two groups; **Highly significant at $p < 0.01$ between two groups

Fig. 1: a) The colon showing mucosal corrugation similar to cerebral convolutions (arrow); b) The mesenteric lymph node showing granular surface (arrow); c) The lamina propria of the ileum showing mononuclear cells infiltration (arrow), H&E X400; d) The ileocaecal lymph node showing medullary histiocytosis (arrow), H&E X400

temperature and dehydration. Though other causes of diarrhea such as salmonella, clostridia and nutritional causes need to be differentiated. However, *M. paratuberculosis* induced diarrhea is continuous, chronic and unresponsive to treatment. The chronicity is supported by blood finding which indicated highly significant increase in WBC, GRNP, LYMP and PCV as well as highly significant decrease in HB and total protein. These findings were supported by post-mortem testing which showed thickening, corrugation of the intestinal wall folded mucosa, enlarged and oedemated ileocaecal and mesenteric lymph nodes. In the meantime. The microscopic findings detected morphologic changes including short blunt and distorted intestinal villi with hyperactive goblets cells of the villi and the crypts of lieberkuhn which were contained mucin droplets. Inflammatory changes were noticed in the lamina propria that was heavy infiltrated with mononuclear cells mostly macrophages. Finally, hyperplasia changes were manifested by lymphoid aggregation in the ileum and the colon. In most cases, the mesenteric and the ileocaecal lymph nodes appeared to be hyper-activated. There was a marked lymphocytic hyperplasia in lymphoid follicles and medullary cords. The most prominent findings were medullary histiocytosis. The post-mortem finding clearly supported the ante-mortem testing that indicated *M. paratuberculosis* is a primary cause of the disease in these camels.

Test and culling is an option to eliminate the source of infection. Testing options may include fecal culture, ELISA and PCR. Fecal culture is a sensitive method but expensive and time consuming. ELISA is highly specific but with low sensitivity (Whitlock *et al.*, 2000). In addition, ELISA is of a great choice because it is simple, inexpensive, fast, easily automated, however kits variations and inter-laboratory difference exist (Alinovi *et al.*, 2009; Garrido *et al.*, 2002; Bilbao, 2002; Dieguez *et al.*, 2009). The use of ELISA and PCR has been reported in camel herds. However, record keeping, sample handling and processing remain major obstacles (Alharbi *et al.*, 2012). Therefore, the use of clinical evaluation and hematologic and blood chemistry profile provide early and vital steps in detecting *M. paratuberculosis* affected camel and start management changes to contain the disease in camel herds. Such approach is simple, inexpensive and can be performed in the field. However, the need to train camel owners to detect clinically affected animals is crucial. Management changes may be applicable to camel herds. The changes may include limiting contact between affected and susceptible young animals. This may be achieved by separating camel calves from dam immediately after birth, feeding calves colostrum obtained from paratuberculosis known free source and milk replacement, raising replacement heifers in a separated location, prevent cross contamination and improving herd hygiene.

CONCLUSION

The findings provide an easy, convenient clinical findings that can be utilized to initiate control measurements *M. paratuberculosis* to reduce the spread of the disease in camel herds.

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REFERENCES

- Ahmed, S.A. and A. Towfik, 1995. Johne's disease among sheep and goat of Qassiem area. Proceedings of the 16th Annual Conference of the IEEE Engineering in Medicine and Biology Society, March 21-23, 1995, Riyadh, Saudi Arabia.
- Al-Hajri, S.M. and A.M. Alluwaimi, 2007. The efficiency of ELISA and PCR in detecting subclinical paratuberculosis in the Saudi dairy herds. Pak. J. Biol. Sci., 10: 1906-1909.
- Alharbi, K.B., A. Al-Swailem, M.A. Al-Dubaib, E. Al-Yamani and A. Al-Naeem *et al.*, 2012. Pathology and molecular diagnosis of paratuberculosis of camels. Trop. Anim. Health Prod., 44: 173-177.
- Alhebbabi, A.M. and A.M. Alluwaimi, 2010. Paratuberculosis in camel (*Camelus dromedarius*): The diagnostic efficiency of ELISA and PCR. Open Vet. Sci. J., 4: 41-44.
- Alinovi, C.A., M.P. Ward, T.L. Lin and C.C. Wu, 2009. Sample handling substantially affects Johne's ELISA. Prev. Vet. Med., 90: 278-283.
- Alluwaimi, A.M., 2008. The efficiency of bovine ELISA in detection of the *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection in camel (*Camelus dromedaries*) at different ages. J. Camel Practice Res., 15: 163-165.
- Alluwaimi, A.M., M.E. Hatem and J.M. Almousa, 1999. The efficacy of gel immunodiffusion and fecal smear tests for diagnosis of ovine paratuberculosis in sheep in Saudi Arabia. Egypt J. Immunol., 7: 29-32.
- Ayele, W.Y., M. Machackova and I. Pavlik, 2001. The transmission and impact of paratuberculosis infection in domestic and wild ruminants. A review. Vet. Med. Czech, 46: 205-224.
- Bastida, F. and R.A. Juste, 2011. Paratuberculosis control: A review with a focus on vaccination. J. Immun. Based Ther. Vaccines, vol, 9, 10.1186/1476-8518-9-8
- Collins, M.T., 2003. Update on paratuberculosis: 1. Epidemiology of Johne's disease and the biology of *Mycobacterium paratuberculosis*. Irish Vet. J., 56: 565-574.
- Dieguez, F.J., A.M. Gonzalez, S. Menendez, M.J. Vilar, M.L. Sanjuan, E. Yus and I. Arnaiz, 2009. Evaluation of four commercial serum ELISAs for detection of *Mycobacterium avium* subsp. *paratuberculosis* infection in dairy cows. Vet. J., 180: 231-235.
- Gameel, A.A., A.S. Ali, S.A. Razig, J. Brown and A. Elhendi, 1994. A clinico-pathological study on spontaneous paratuberculosis in camels (*Camel dromedaries*). Pak. Vet. J., 14: 15-19.
- Garrido, J.M., G. Aduriz, M.V. Geijo, I. Sevilla and R.A. Juste, 2002. Comparison of different indirect ELISA methods on reference cattle. Proceedings of 7th International Colloquium on Paratuberculosis, June 11-14, 2002, International Association for Paratuberculosis, Bilbao, Spain, pp: 52-53.
- Gonzalez, J., M.V. Geijo, C. Garcia-Pariente, A. Verna and J.M. Corpa *et al.*, 2005. Histopathological classification of lesions associated with natural paratuberculosis infection in cattle. J. Comp. Pathol., 133: 184-196.
- SAS., 2002. Statistical Analysis System, Users Guide, SAS/STAT Version. 8th Edn., SAS Institute, Inc., Cary NC., USA.
- Steel, R.G.D. and J.H. Torrie, 1960. Principles and Procedure of Statistics. McGraw-Hill Book Co. Inc., New York.
- Whitlock, R.H., S.J. Wells, R.W. Sweeney and J.V. Tiem, 2000. ELISA and fecal culture for paratuberculosis (John's disease): Sensitivity and specificity of each method. Vet. Microbiol., 77: 387-398.
- Zaghawa, A., F.M. Housawi, A. Al-Naeem and M. Hammoda, 2011. Clinical investigations on paratuberculosis in camels in Al-Has, KSA. Proceedings of the 11ICP Public Health and *Mycobacterium avium* subsp. *paratuberculosis* in the Environment, July 6-8, 2011, Attica, Greece.