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CLINICO-BIOCHEMICAL AND POSTMORTEM INVESTIGATIONS IN 60 CAMELS (*Camelus dromedarius*) WITH JOHNE'S DISEASE

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ABSTRACT

This paper describes the clinical, haematological, biochemical and pathological findings in 60 camels (*Camelus dromedarius*) affected with *Mycobacterium paratuberculosis*. The clinical findings were long-standing diarrhoea (sometimes intermittent), weight loss and poor condition. Haematological abnormalities included decreased haematocrit value, decreased haemoglobin concentration and leukocytosis. Analysis of serum revealed hypoproteinemia, hypoalbuminemia and hyperglobulinemia. Other serum abnormalities included hypocalcemia, hypomagnesemia and elevated serum activities of aspartate aminotransferase. The activities of γ-glutamyl transpeptidase as well as the concentrations of total bilirubin, urea nitrogen, creatinine, phosphorus and glucose were normal. Antemortem diagnosis of Johne's disease in the camels depended on the clinical as well as the results of Ziehl-Neelsen staining of rectal smears and detection of acid-fast bacilli. Postmortem examination showed highly thickened intestinal mucous membranes that form folds. Granulomatous lesions were seen in the mesenteric, hepatic and mediastinal lymph nodes. Histopathological examination showed intense infiltration of epithelioid cells in the mucosa and submucosa of the intestine. These cells also infiltrated the lymph node, which also showed abscess with liquefactive necrosis. Acid-fast bacilli were also seen inside the epithelioid cells in the intestine and lymph nodes. Other pathological changes included congestion and fatty changes in the liver and degeneration of the renal tubular epithelium in the kidney.

Key words: Camels, clinicobiochemical, haematology, Johne's disease, postmortem

Johne's disease (JD) or paratuberculosis is characterised by persistent and progressive diarrhea, weight loss, debilitation and eventually death. The disease produces chronic, contagious enteritis and affects cattle, sheep, goats, farmed deer and other domestic and wild ruminants (Higgins, 1986; Wernery and Kaaden, 2002; Fowler, 2010; Al-Swailem *et al,* 2011; Alharbi *et al,* 2012). Paratuberculosis occurs worldwide. In tropical areas with intensive dairy farming, paratuberculosis presents a serious economic problem due to culling of clinical cases, reduced milk production and the costs of laboratory testing and control measures (Collins and Nordlund, 1991; Ott *et al,* 1999; Fowler, 2010). The causative agent, *Mycobacterium avium* subsp. *paratuberculosis* (Mptb) is excreted in the faeces of infected animals and hence it can be ingested with contaminated food or water. The bacteria spread to the intestinal mucosa or mesenteric lymph nodes where they can cause chronic inflammation. Mptb is also able to cross the placenta to the foetus (Alharbi *et al,* 2012).

Potential impact on consumer demand for milk associated with product safety needs to be considered as the causative organism, Mptb, may also be a cause of Johne's disease (Stott *et al,* 2005; Chamberlin and Naser, 2006). The gold standard for diagnosing paratuberculosis is bacterial isolation of Mptb from faeces or tissues. Although the organisms can be shed in milk, the fecal-oral route is the primary mechanism for transmission of Mptb and this is reflected in disease control recommendations (Clarke, 1997). These are similar in most countries and based on removal of clinical cases, identification of sub-clinical cases by objective tests, and hygienic neonates rearing (Kennedy and Benedictus, 2001; Benedictus and Kalis, 2003). Not all infected animals become clinical cases, but they remain excretors of Mptb. Following oral infection, Mptb enters the lymphatics through the tonsils and the intestinal mucosa. Peyer's patches take up the microorganisms from the intestinal lumen and transport them through the intestinal mucosa. The incubation period is generally 18 to 24 months (Wernery and Kaaden, 2002; Tharwat *et al,* 2012).

In Saudi Arabia, camels are reported to be more susceptible to paratuberculosis than most other

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ruminants leading to severe emaciation (Radwan *et al,* 1991). In Russia, similar conditions are occurring (Buchnev *et al,* 1991). The disease is known to cause intestinal and hepatic lesions in sheep, goats and cattle (Jones *et al,* 1997; Mahmoud *et al,* 2002; Tharwat *et al*, 2012). The macroscopic lesions of the disease in sheep are thickening and corrugation of the intestinal mucosa, enlargement of mesenteric lymph nodes and hepatic granuloma (Mahmoud *et al,* 2002). Diffuse epithelioid cell infiltration in the intestinal mucosa, mesenteric lymph nodes and liver are the main microscopic lesions of the disease (Clarke, 1997; Mahmoud *et al,* 2002). Remote lesions of paratuberculosis were described in cattle and include lymphocyte depletion in the thymus and arteriosclerotic lesions in the heart and aorta. Rarely, wide spread granulomatous lesions affecting the kidneys, lungs and systemic lymph nodes were described in advanced clinical cases (Clarke, 1997; Tharwat *et al,* 2012).

The long subclinical phase, the potential for few infected animals in a herd to show clinical disease, the challenge of diagnosing early infection, and the organism's persistence in the environment represent significant challenges to prevention and control efforts (Kreeger, 1991). Because JD in camels is poorly documented in the literature, therefore, the present study was carried out to summarise the clinicobiochemical and postmortem findings in camels affected with paratuberculosis.

Materials and Methods

Animals, history and physical examination

Sixty camels were used in the present study. Animals were examined from 2007 to 2010 at Veterinary Teaching Hospital, Qassim University, Saudi Arabia. Camels were referred because of inappetance, long-standing loss of body condition and chronic watery diarrhea. According to the owners, duration of illness ranged from 1 to 12 months, and animals has treated with various medications, including oral and systemic antibiotics, corticosteroids and anthelmentics. All camels underwent a thorough physical examination (Higgins, 1986; Köhler-Rollefson *et al,* 2001), which included general behavior and condition, auscultation of the heart, lungs, rumen and intestine, measurement of heart rate, respiratory rate and rectal temperature, swinging auscultation, percussion auscultation of both sides of the abdomen and rectal examination.

Haematological and biochemical analyses

Two blood samples were collected by puncture of the jugular vein, one on EDTA and the other without anticoagulant. Haematological examinations (haematocrit, haemoglobin, total leucocyte count) were carried out on the first blood sample. After centrifugation of the second blood sample, serum samples were collected and then frozen for later analysis of clinical chemistries. In the serum, commercial kits were used to determine the concentrations of total protein, calcium, phosphorus, magnesium, glucose, total bilirubin, urea nitrogen and creatinine. The activities of aspartate aminotransferase (AST) and γ-glutamyl transpeptidase were also measured in serum samples. Automated biochemical analyser (Reflotron Plus, Roche Diagnostics, GmbH, Mannheim, Germany) was used for measurement of all serum parameters.

Postmortem and histopathological examination

Seven camels were euthanased and thoroughly examined postmortem. Organs showing lesions were fixed in 10% buffered formalin, processed in wax, sectioned and stained with hematoxylin and eosin for routine histopathology.

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA). Values are expressed as mean ± SD.

Results and Discussion

During physical examination, the most prominent clinical signs in the camels were chronic, intermittent diarrhoea, loss of body condition and inappetance. Diseased animals were examined either single or in groups (Fig 1A).

The degree of loss of body condition was either severe (Fig 1B) or moderate. The heart and respiratory rates were within normal limits, however, weak and irregular ruminal contractions were recorded. Hypothermia was observed in seventeen camels that characterised by watery diarrhea (Fig 1C). Rectal smears stained with Ziehl-Neelsen stain showed acid-fast bacilli in all diseased camels (Fig 1D).

Postmortem examination showed that the wall of the intestine was highly increased in size with granulomas visible from the external side. The mucous membrane of the intestine was increased in size forming folds (Figs 1E). The granulomatous lesions were seen in the mesenteric, hepatic and mediastinal lymph nodes. Other postmortem

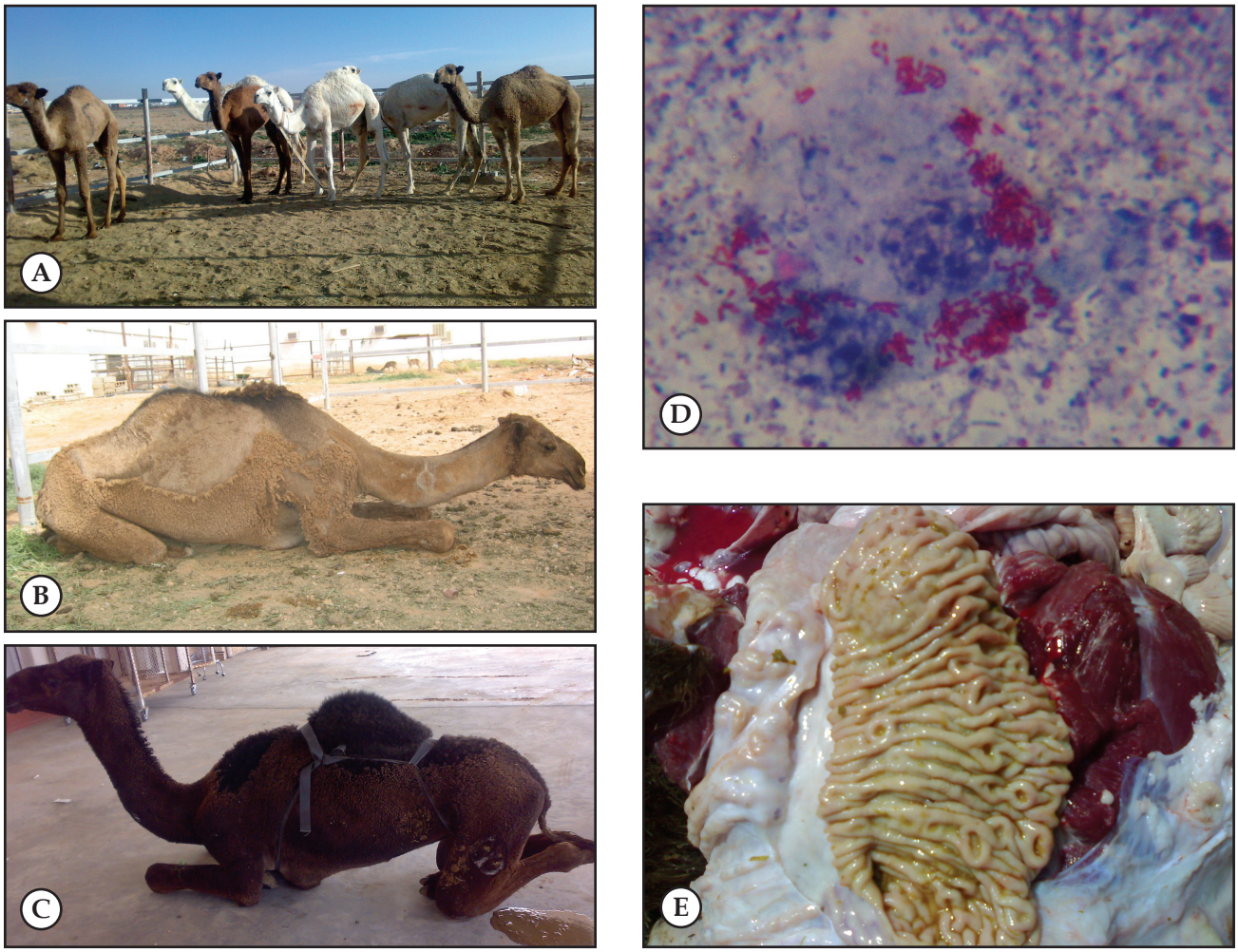

Fig 1. **A.** Diseased animals in a group **B.** Severe loss of general health **C.** Diseased animal showing watery diarrhoea **D.** Rectal smears stained with Ziehl-Neelsen stain showing acid-fast bacilli **E.** The mucous membrane of the intestine showing increased size of folds.

findings included enlarged kidneys, flabby heart and abdominal fluid.

Histopathological examination of the intestine showed intense infiltration of epithelioid cells in the mucosa and submucosa. These cells also infiltrated the lymph node, which also showed abscess with liquefactive necrosis. Acid-fast bacilli were also seen inside the epithelioid cells in the intestine and lymph nodes. Other pathological changes included congestion and fatty changes in the liver and degeneration of the renal tubular epithelium in the kidney.

In the present study, the damage seen in the intestine and other organs of camels infected with paratuberculosis could be attributed to immunological reaction due to infection as long standing cases might depress immunity and result in a wide range of lesions (Clarke, 1997; Perez *et al,* 1999; Tanaka *et al,* 2000). Paratuberculosis has been known to cause remote lesions in many organs such as retrophryngeal lymph nodes (Sigurddottir *et al,* 1999), kidneys and lungs (Hines *et al,* 1987; Clarke, 1997). Involvement of the liver and kidney in this study is probably due to spread of infection by infected macrophages from the intestine via the blood. The bacterium is known to resist destruction by macrophages (Bendixen *et al,* 1981).

Paratuberculosis is a chronic, progressive, infectious disease of ruminants characterised by intermittent diarrhea, severe weight loss, emaciation, and death (Higgins 1986; Wernery and Kaaden, 2002; Alharbi *et al,* 2012; Tharwat *et al,* 2012). In the present study, JD was suspected in camels that show a chronic, recurring, or intermittent diarrhea that does not respond to treatment. These findings may explain the reduced haematocrite value, hypoproteinemia, hypoalbuminemia and lower concentration of

serum calcium and magnesium. Other factors such as reduced appetite and emaciation as the disease progresses may contribute effectively on these serum parameters. Specific diagnosis was made on the basis of detecting Mptb from staining of rectal smears (Radostits *et al,* 2007; Al-Swailem *et al,* 2011).

It has been almost 100 years since ruminant paratuberculosis was described and the causative agent identified, and yet the disease continues to frustrate the scientific and agricultural communities. A great deal of progress has been made in development of early diagnostic techniques and control measures for minimising spread and in elimination of the disease on a herd basis. We still know very little about the pathogenesis of the infectious process, the role of the host immune response and specific cell subsets in disease development, the specific mycobacterial antigens that promote humoral and cellular immune responsiveness, and environmental factors that enhance or abrogate disease initiation (Whitlock *et al,* 1993; Smith, 2002; Radostits *et al,* 2007; Tharwat *et al,* 2012).

The haematological and biochemical findings are summarized in Table 1. Compared to the healthy control camels, the haematocrite was very low ($P < 0.05$) in sixteen of the twenty-five tested camels. Twenty-one camel had leukocytosis ($P < 0.01$). Lower levels of haemoglobin were observed in thirteen camels ($P < 0.05$). Hypoproteinemia with hypoalbuminemia was marked in twenty cases ($P < 0.05$) and hyperglobulinemia was evident in eighteen camels ($P < 0.01$). Increased concentrations of calcium and magnesium were observed in fifteen and nineteen camels, respectively ($P < 0.05$). Leukocytosis and hyperglobulinemia encountered in this study could be explained on the basis of the chronic nature of the disease. Disease chronicity and resistance to therapeutic measures have concentrated efforts on mechanisms of disease transmission, factors influencing susceptibility, serologic identification of non-clinical carrier animals, and other means of early diagnosis. During the last decade, the pathogenesis of paratuberculosis has been more closely examined. Yet with all of these combined efforts directed towards the understanding of the disease, paratuberculosis remains a significant problem to the researcher, diagnostician, and animal producer (Smith, 2002; Radostits *et al,* 2007). Most herds are initially exposed to Johne's disease by unknowingly purchasing infected carrier animals. Mptb can be transmitted in semen. The role of infected bulls in the transmission of JD to females and the fetus is not clear, but certainly can represent a significant mode of spreading the disease in the ruminant population.

Table 1. Haematological and biochemical findings in camels with Johne's disease.

Parameters	Finding at admission	Control
	(n = 25)	(n = 15)
Haematocrit (%)	22±6*	32±1.4
Haemoglobin (g/dL)	9±3*	12±3
Leukocyte count (/µL)	13914±5315**	8250±2284
Total protein (g/dL)	8.5±2.2*	7.9±0.4
Albumin (g/dL)	3.1±0.6*	4.2±0.4
Globulin (g/dL)	5.4±1.3**	3.7±0.5
Aspartate aminotransferase (U/L)	211±111*	69±44
γ-glutamyl transferase (U/L)	66±63	44±22
Total bilirubin (mg/dL)	0.5±0.4	0.8±0.3
Urea nitrogen (mg/dL)	29±9	17±10
Creatinine (mg/dL)	1.12±0.2	1.3±0.2
Calcium (mg/dL)	7.5±2.0*	8.6±0.7
Phosphorus (mg/dL)	5.4±1.1	6.7±0.3
Magnesium (mg/dL)	1.4±0.3*	2.5±0.4
Glucose (mg/dL)	43±40	61±19

*P<0.05 **P<0.01

The prognosis of camels infected with paratuberculosis is very poor. Therefore, control measures should be followed in a strict way (Radwan *et al,* 1991; Collins, 1994; Wernery and Kaaden, 2002). Clinically suspected camels should be isolated until the disease is confirmed and all infected camels should be slaughtered and carcasses properly disposed. Where possible, camel calves should be removed from their dams at birth and reared in a paratuberculosis-free environment. Appropriate sanitary measures should be applied to prevent contamination of food, water and soil; and ponds and ditches should be fenced off. In addition, newly purchased camels should be examined for paratuberculosis and vaccination should be considered.

The most important aspect of paratuberculosis control is not to buy camels with the disease if it is not present in the herd. In addition, maintaining a closed herd system is the most effective method of avoiding exposure. Purchase breeding male camels from reputable breeders who are willing to certify that their herd is free from JD. Control and eradication of

JD require careful planning, rigid sanitation, repeated testing, and persistence (Wernery and Kaaden, 2002).

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