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TREATMENT OF PARATUBERCULOSIS IN CAMELS BY RIFAMPIN AND STREPTOMYCIN

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ABSTRACT

The response of paratuberculosis of camels (locally known as Silag) to treatment with rifampin and streptomycin was investigated. Ten camels were used in this experiment as follows: Seven clinically ill camels with severe weight loss and persistent diarrhoea, and presence of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in feces and rectal scrapings, were injected intramuscularly with a dose providing 5 mg/kg body weight of rifampin and a dose of 5 mg/kg body weight of streptomycin. Diarrhoea stopped and (MAP) disappeared from the feces and rectal scrapings of infected camels from the 5th to the 9th week of treatment. The 8th camel had clinical Silag and was kept as untreated positive control, and 9th camel was free from paratuberculosis and was kept as a negative control. The positive control camel continued shedding (MAP) in faeces until the end of the experiment. The non-infected camel (negative control) remained healthy and free from infection until the end of the experiment. The tenth camel had clinical paratuberculosis and was sacrificed to study the pathology of the disease. Postmortem examination showed that the intestinal wall was dispersed with granulomas and its mucous membrane was greatly thickened and corrugated. The mesenteric lymph nodes were granulomatous and abscessed. Granulomas were also seen in the hepatic and mediastinal lymph nodes. This is the first report on the effective treatment of paratuberculosis in camels.

Key words: Camel, paratuberculosis, rifampin, streptomycin, treatment

Paratuberculosis (Johne's Disease, JD) is a slow-developing infectious disease characterised by chronic granulomatous entero-colitis and hepatitis, regional lymphadenitis and possibly other lesions that might lead to a wasting diarrhoea (Al-Dubaib and Mahmoud, 2008; Mahmoud *et al,* 2002; Clarke, 1997; Jones *et al,* 1997). The disease is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and is of world-wide distribution, affecting domesticated and wild ruminant animals (Palling *et al,* 1988; Wernery and Kaaden, 2002). Paratuberculosis of camels in Saudi Arabia is poorly documented in the literature even though the disease is known to infected sheep and goats causing severe pathology (Mahmoud *et al,* 2002; Al-Dubaib and Mahmoud, 2008). The disease is associated with high economic losses because of severe loss of slaughter weight and reduction in milk yield (Kudahl and Neilsen, 2009; Beaudea *et al,* 2007).

There were many reports incriminating *M. avium* subsp. *paratuberculosis* in the etiology of human Crohn's disease (Uzoigwe *et al,* 2007; Saror, 2005).

Control of paratuberculosis depends on early detection of the disease followed by culling of infected animals. Therapeutic treatment of the disease is not practiced because of the long duration the of the treatment course, the need for hospitalisation of infected animals and the high cost of the drugs used. However, the bacterium is known to respond to antibacterial therapy. Slocombe (1982) successfully treated a goat with a combination of rifampin, isoniazid and streptomycin, and human Crohn's disease responded well to combined antimicrobial therapy (Borody *et al,* 2002; Sharfan *et al,* 2002).

The pathology of JD in the Arabian camel was poorly documented in the literature, but the disease was known to cause intestinal and hepatic lesions in sheep, goats and cattle (Al-Dubaib and Mahmoud, 2008; Mahmoud *et al,* 2002; Jones *et al,* 1997). 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 epithellioid 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).

The damage seen in the intestine and other organs of animals 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

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(Clarke, 1997; Perez *et al,* 1999; Tanaka *et al,* 2000). Paratuberculosis has been known to cause remote lesions in many organs such as retropharyngeal lymph nodes (Sigurddottir *et al,* 1999), kidneys and lungs (Hines *et al,* 1987; Clarke, 1997). Severe depletion of lymphocytes was reported in the thymus of infected calves (Clarke, 1997). Involvement of remote organs is probably due to spread of infection by infected macrophages from the intestine via the blood as the bacterium is known to resist destruction by macrophages (Bendixsen *et al,* 1981).

This experiment describes the therapeutic effect of rifampin and streptomycin against Johne's disease in camels as well as gives detail description of its pathology.

Materials and Methods

Ten camels, about 3-5 years of age and 150-180 kg body weight, were used in this experiment. The experiment was carried out during the month of October 2008. The camels were admitted to Veterinary Teaching Hospital of Qassim University and were kept in isolation at a remote pen and fed on alfa alfa (*Medicago sativa*). Water was offered freely in the pen. Seven of the camels with clinical paratuberculosis were used for the therapeutic trial. One infected camel was kept untreated as a positive control. A healthy camel free from infection was kept separate and served as a negative control. The tenth camel had clinical Silag and was sacrificed and postmortemed to study the pathology of the disease.

The 7 clinically-ill camels were injected concurrently through the intramuscular route with daily doses providing 5 mg/kg body weight rifampin and 5 mg/kg body weight streptomycin for 10 weeks. Foecal samples and rectal scrapings were taken on weekly basis. Smear were prepared and stained with ZN stain for demonstration of acid fast bacilli.

One clinically ill camel was sacrificed and postmortemed. Organs showing lesions were fixed in 10% formal saline, processed in wax, sectioned and stained with haematoxylin and eosin for routine histopathology and by ZN staining for demonstration of mycobacterium.

Results

Response to treatment

Camels infected with paratuberculosis suffered severe weight loss, anorexia and persistent diarrhoea (Fig 1). Table 1 shows the response of Silag to concurrent treatment with rifampin and streptomycin. Diarrhoea stopped one week after treatment and the camels started to gain vitality. Improvement of bodily condition was observable after three weeks of treatment. *Mycobacterium avium* subsp. *paratuberculosis* was undetectable in rectal faeces and scraping after 5-9 weeks of rifampin and streptomycin treatment while the positive control camel continued to pass the bacterium in its faeces till the end of the experiment which reveals good response to the treatment given. The negative control camel remained free of infection till the end of the experiment.

Pathological results

The intestinal wall was highly thickened and dispersed with granulomas. When sliced open, diffuse thickening, corrugation and ulceration were seen in the mucous membrane (Fig 2, 3). The mesenteric lymph nodes were highly swollen and some contain pus indicating a complicating pyogenic infection (Fig 4). Granulomas were seen in the hepatic and mediastinal lymph nodes (Fig 5).

Histopathological examination showed that the mucous membrane of the intestine is highly infiltrated with epithelioid cells (macrophages) forming a widespread granulomatous reaction. Similarly, the mesenteric lymph nodes contain widespread granulomas containing large number of epithelioid cells (Fig 6). These were shown to contain large number of acid fast bacilli when stained with ZN stain.

Discussion

The results of this experiment show clearly that paratuberculosis of camels (locally known as Silag) is a treatable disease and a concurrent daily intramuscular doses of rifampin and streptomycin

Table 1. Demonstration of acid fast organisms (Zn staining) in faeces and rectal scraping of treated camels.

Sample No.	1	2	3	4	5	6	7	Cont. + ve	Cont. - ve
0	+	+	+	+	+	+	+	+	-
1	+	+	+	+	+	+	+	+	-
2	+	+	+	+	+	+	+	+	-
3	+	+	+	+	+	+	+	+	-
4	+	+	+	+	+	+	+	+	-
5	-	+	+	+	+	+	+	+	-
6	-	+	+	+	-	-	+	+	-
7	-	+	+	+	-	-	+	+	-
8	-	-	-	+	-	-	+	+	-
9	-	-	-	-	-	-	-	+	-
10	-	-	-	-	-	-	-	+	-

Fig 1. Severe emaciation of a camel infected with paratuberculosis.

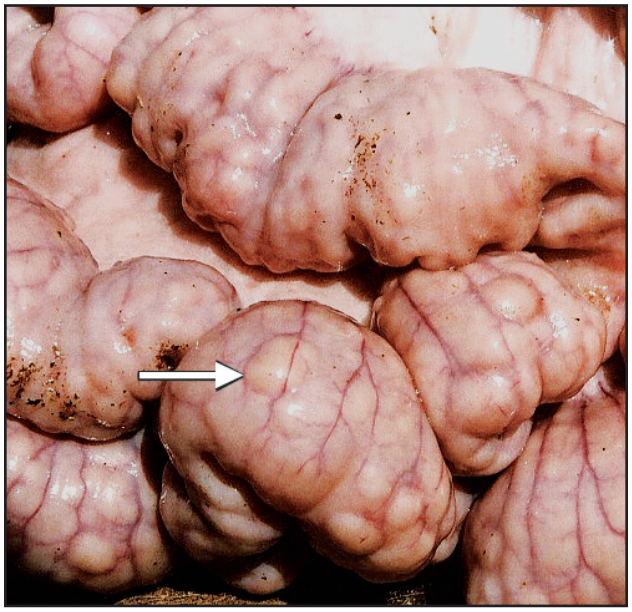

Fig 2. Granulomas in the intestinal wall.

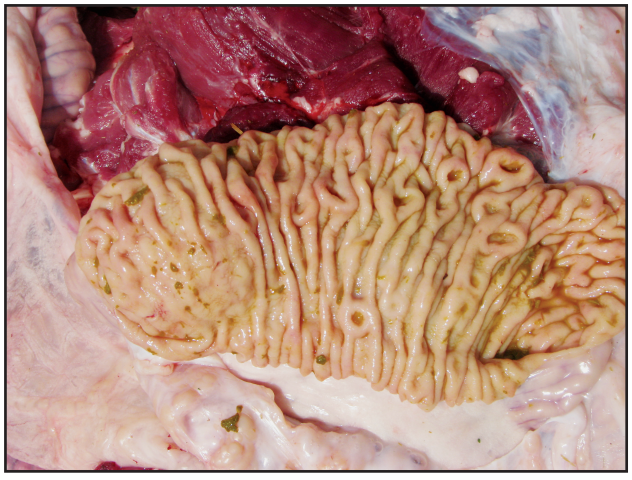

Fig 3. Intestinal mucous membrane of a camel infected with Silag showing thickening, corrugation and ulceration.

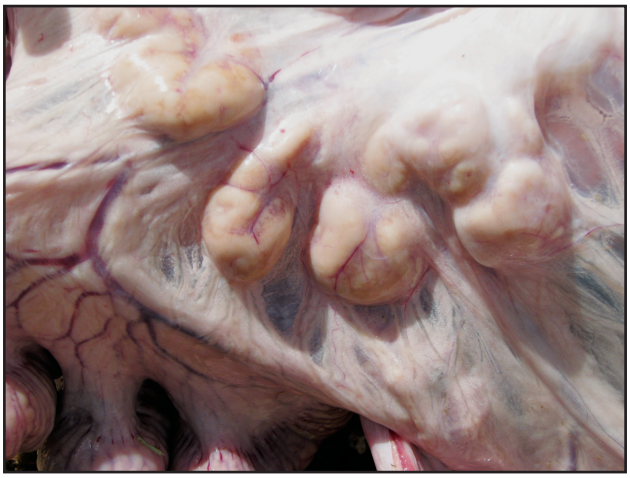

Fig 4. Granulomas in the mesenteric lymph nodes.

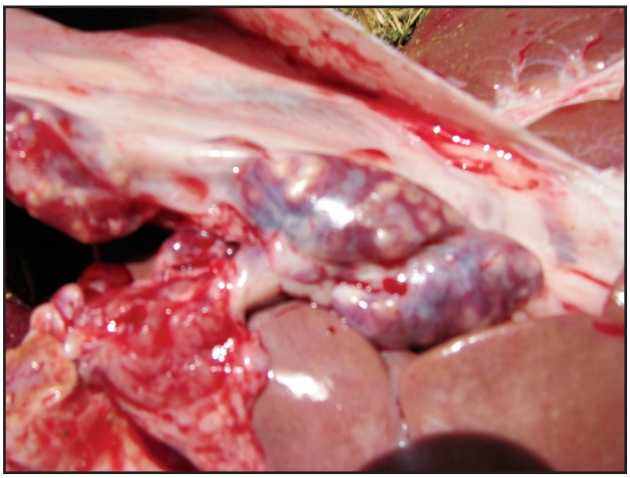

Fig 5. Hepatic lymph node containing paratuberculosis granulomas.

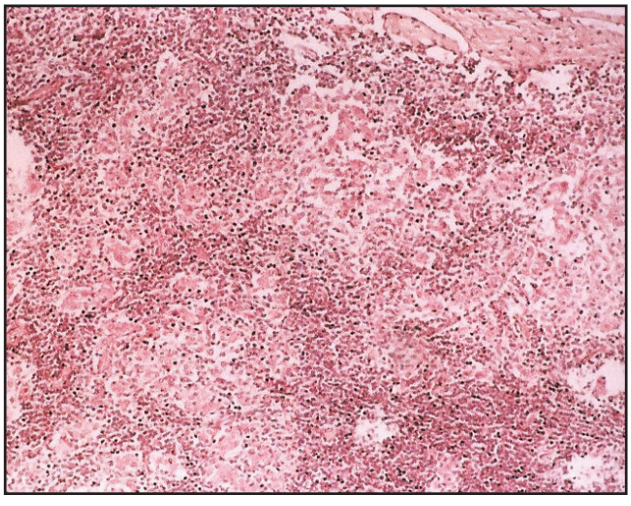

Fig 6. Mesenteric lymph node granuloma containing large number of macrophages. H&E x250.

cleared the infection within 5 - 9 weeks. This variation in duration of response of the diseased camels to treatment with rifampin and streptomycin could be attributed to the complex nature of the immunity against paratuberculosis infection (Stabel, 2006). The interplay between the mycobacterium and the host immune system determines the outcome of MAP infection. After the uptake of mycobacteria by macrophages, they might be destroyed immediately or they might be established as persistent infection. The host immune system activates T-cells that interact with infected macrophages through cytokines and receptors (Coussens, 2001). Despite these efforts to get rid of infection, the mycobacteria might persist resulting in severe intestinal damage. The variation in the duration to treatment in the different camels could therefore be attributed to the level of immunity of the host against invading mycobacteria as well as the virulence of the pathogen.

Literature search did not reveal any previous report describing treatment of paratuberculosis in camels. However, paratuberculosis was successfully treated in ruminants. Dosing with rifampin, isoniazid and streptomycin was reported to treat a goat with clinical paratuberculosis (Slocombe, 1982). Paratuberculosis in goats was also found to be sensitive to injections with combination of streptomycin sulphate, rifampin and levamisole or streptomycin, rifampin and dapsone (Das *et al*, 1992).

However, treatment of paratuberculosis in animals is usually not recommended because of difficulties in the diagnosis of sub-clinical cases (Al-Hajr *et al,* 2007), the high cost of medication, the long duration of treatment and the for hospitalisation and special care for the inpatient animal. The bacterium can be found dormant inside macrophages and thus resist antimicrobial and antibodies attack (Sigurdardottir *et al,* 1999). St-Jean and Jernigan (1991) reviewed the antimicrobial drugs that can be used to treat paratuberculosis in ruminants. They recommended that treatment should not be a method for controlling the disease because the anti-tuberculosis drugs are not recommended for animal treatment and the food products of treated animals are not fit for human consumption.

However, treatment of certain expensive breeds of camels infected with paratuberculosis can be highly justifiable since the price of some of the racing breeds might reach millions of dollars. The owners of the expensive camel might bear cost of the drugs and the hospitalisation.

Pathological investigation results showed that Silag is an extremely severe disease becuase its lesions reached the hepatic and mediastinal lymph nodes. Yet, the disease caused granulomatous reactions similar to those described for other ruminants (Mahmoud *et al,* 2002).

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