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CLOSTRIDIUM PERFRINGENS TYPE B ENTEROTOXAEMIA IN A KENYAN CAMEL

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ABSTRACT

Clostridium perfringens toxin type B was isolated from the small intestine of a 2 year old weaned camel calf (*Camelus dromedarius*) in North Kenya that had died from severe haemorrhagic enteritis. The case occurred at the end of the dry season after early the onset of the long rains in a pasture area, heavily stocked with sheep. *Clostridium perfringens* toxin type B was not found in faecal samples from 7 cohort animals in the same herd and from 30 age mates on the same pasture or in the same district. Sheep cannot be ruled out as a possible source of this infection.

Key words: Camel, *Clostridium perfringens* toxin type B, haemorrhagic enteritis, lamb dysentery

Clostridium perfringens occurs worldwide and causes disease in sheep, cattle, goats, pigs, equine foals, Old and New World Camelids and humans (Buxton, 1983; Odendaal, 1994; Smith and Sherman, 1994; Younan and Drescher, 1996; Mueller *et al,* 1998; Fowler 1998, Wernery and Kaaden, 2002; OIE 2005). *Clostridium perfringens* is also a common cause of food poisoning (OIE, 2005). According to production of major lethal toxins (alpha, beta, epsilon) *Cl. perfringens* toxin types A, B, C and D can be differentiated and are related to specific disease syndromes (Table 1).

Clostridium perfringens toxin type B is an obligate gut parasite and the cause of lamb dysentery, a highly fatal haemorrhagic enteritis in young lambs and kids under 2 weeks of age (Buxton, 1983; Smith and Sherman, 1994; Kriek *et al,* 1994). *Clostridium perfringens* type B enterotoxaemia is also seen in 7 to 10 day old bovine calves and, exceptionally, in equine foals (Kriek *et al,* 1994). Different toxigenic varieties of *Clostridium perfringens* type B occur, including one that is responsible for haemorrhagic enteritis in adult sheep and goats in the Middle East (Nilo, 1993).

Acute and subacute enterotoxaemia and haemorrhagic enteritis due to *Cl. perfringens* types A, C and D have been described in old and in new world camelids, (Fowler, 1998; Wernery and Kaaden, 2002). McGrane and Higgins (1986) stated that clostridial diseases appear to be rare in camels other than tetanus.

Clostridium perfringens toxin type B was isolated from the small intestine of a 2 year old weaned camel calf (*Camelus dromedarius*) in North Kenya that had died from severe haemorrhagic enteritis.

Material and Methods

A loop of small intestine was received from a dead 2 year old weaned camel calf belonging to a herd, kept on communal pastures in the Laikipia district of North Kenya. Faecal samples were collected from 7 live camel weaners, aged 2 to 4 years, in the same herd. In addition faecal samples were also collected from 30 weaners, aged 2 to 3 years, in 4 camel herds in the Laikipia district [(herd B (n=2), herd C (n=3), herd D (n=9), herd E (n=16)]. Herds B and C were using exactly the same communal grazing area at the time of the outbreak, while herds D and E were grazing inside adjacent ranches.

Post mortem specimen smears were prepared from contents of the small intestine and examined as direct wet smears and after staining according to Gram and Giemsa. The gut contents were cultured on blood agar (BA; Oxoid No. CM 271) under aerobic and anaerobic (GasPak® System, Becton-Dickinson) conditions at 37ºC for 24 hours. Growth patterns of both cultures were compared. Gram stain and reverse CAMP-test (Eisgruber and Reuter, 1987) were used for presumptive identification of *Cl. perfringens* isolates. For faecal samples from clinically healthy animals a 24 hour anaerobic cooked meat medium (Oxoid CM0081B) culture was subsequently sub-cultured onto BA, incubated under anaerobic conditions for 24 hours and examined as described above. Species identification for all *Cl. perfringens*

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Table 1. Diseases associated with *Clostridium perfringens* toxin types (According to: Sterne and Batty, 1957; Buxton, 1983; Odendaal, 1994; Wernery and Kaaden, 2002).

***Clostridium perfringens toxin* type (associated disease)**	**Major Lethal Toxins produced**		
	alpha	**beta**	**epsilon**
A (Enterotoxaemia in racing camels)	+	-	-
B (Lamb Dysentery)	+	+	+
C (Struck)	+	+	-
D (Pulpy Kidney)	+	-	+

isolates was confirmed by API 20 A (BioMérieux #20300). Confirmed *Cl. perfringens* isolates were typed by indirect Enzyme Immuno Assay (EIA, as described by Younan *et al,* 1994) at the Institute of Animal and Environmental Hygiene, Freie Universitaet Berlin, Germany.

Results

The fatal case occurred at the end of the dry season, about 2 weeks after early onset of the long rains in Laikipia district, North Kenya. At the time of the outbreak the pasture area was heavily stocked with sheep. The affected weaned camel calf was 2 years old and had died within less than 48 hours after falling sick. Symptoms communicated to the investigators were those of a brief but severe bloody-watery diarrhoea, recumbency, ophisthotonus and death. Herdsmen carried out a dissection of the dead weaner. They described red guts as the main feature. A severed loop of haemorrhagic, small intestine was received for laboratory examination. The intestine was red and contained watery bloody liquid. Gram stained smears of gut contents showed abundance of Clostridia-like large square gram-positive rods. Coccidia or helminth stages were not seen at direct microscopic examination of wet smears. While direct aerobic culture showed mixed growth, the direct anaerobic culture of faecal contents on blood agar showed in subpure growth of *Cl. perfringens*. The *Cl. perfringens* isolate was typed by EIA and was identified as toxin type B.

The faecal samples collected from 37 healthy weaners in herds A-E a total of 19 *Cl. perfringens* were isolated via CM enrichment culture. The isolates were typed by EIA at the Institute of Animal and Environmental Hygiene, Freie Universitaet Berlin, Germany, and were all found to belong to *Cl. perfringens* toxin type A.

Discussion

No *Cl. perfringens* type B were isolated from 7 cohort animals (herd A) and from 30 age mates grazing on the same pasture (herd B and C) or in the same district (herd D and E). Although demonstration of major lethal toxins in autopsy material was not possible, the demonstration of large numbers of *Cl. perfringens* type B in the received guts is diagnostically relevant. Failure to demonstrate toxin does not exclude enterotoxaemia as toxins, in particular beta-toxin, are destroyed by enzymes during autolysis (Sterne and Batty, 1975). Healthy animals harbouring *Cl. perfringens* type B in the intestinal tract are able to form the focus for fatal infection as and when conditions alter to allow rapid multiplication of clostridia (Buxton, 1983). In the reported outbreak there was no indication that camels of the same age group and kept in the same area were harbouring *Cl. perfringens* type B. Hence small ruminants, in particular sheep, cannot be ruled out as a possible source of the infection. The rapid transition from dry season grazing conditions to fresh pasture, triggered by early rains, and the overstocking, particularly with sheep, may have been contributing factors.

Clostridium perfringens type B produces the major lethal toxins alpha, beta, and epsilon. The beta-toxin is produced by *Cl. perfringens* in an inactive precursor form during the exponential growth phase, while alpha and epsilon toxins are produced as active toxins. Beta-toxin is transformed into active toxin by trypsin present in the guts of suckling lambs (Sterne and Batty, 1975). In lamb dysentery *Cl. perfringens* beta-toxin, a potent necrotising agent, is primarily responsible for inducing pathological changes (Kriek *et al,* 1994). In the observed case, the animal had been weaned about half a year earlier, making it unlikely that sufficient trypsin for activation of the beta-toxin was present in its guts. It can be assumed that alpha and epsilon toxins were mainly responsible for the pathology seen in this weaner.

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News

WORKSHOP ON REPRODUCTION IN DROMEDARY AND BACTRIAN CAMELS

Scientific Workshop on Reproduction in Camel shall take place at Faculty of Veterinary Medicine, University of Tehran on 25th of July 2007 under the theme Natural Heritage and Our Mission. Eminent camel scientists will present the papers and conduct various sessions. Dr. Amir Niasari-Naslaji will present in Session 1 on Disease and Dr Alex Tinson on common diseases of the dromedary camel. In session 2, Dr Darab Nikjou will present on follicular dynamics and Infertility and Dr Asghar Moghiseh on Controlling Follicular Dynamics in Camel and Dr Alex Tinson on infertility in the female dromedary camel. In session 4, Dr Mojtaba Kafi will present on IVF in dromedary camel, Dr Alex Tinson will present on practical aspects of embryo transfer in dromedary camel and Dr. Amir Niasari-Naslaji on semen collection, processing and cryopreservation in bactrian camel. There will be general discussion before closing session.

DELEGATES OF INTERNATIONAL CAMEL CONFERENCE, BIKANER WERE CLOSE TO CAMELS

There was an inbibed feeling to be close to the camels in the International Camel Conference which held at Bikaner from 16-17 February 2007. Delegates were given option to travel in the camel carts in form of a caravan from faculty house (breakfast venue) to the auditorium (conference venue). Majority of delegates preferred this mode of transport. The caravan was led by a camel being rode by Dr. T.K. Gahlot, Organising Secretary and Sh. Bagdi Ram Raika, representative of the camel pastoralist community. Inaugural function had a great attraction of traditional welcome extended by the college students to all the delegates and every delegate was given a camel cap. A film-"Bikaner-a hub of camel research" was shown in the inaugural function depicting the importance of camels at Bikaner in socioeconomic zone, camel festival, border security force and at two research institutions i.e. Veterinary College and National Research Centre on Camels. All delegates were offered camel milk brought by Dr. U. Wernery and its team from UAE, camel cheese brought by Ms. Nancy Abeiderrahmane from Mauritania and camel milk ice cream brought by Ms. Ilse K Rollefson, Germany from her NGO working at Jaisalmer (Rajasthan). All delegates were taken to a round to Camel Clinic of Veterinary College and research laboratories of National Research Centre on Camel.

CAMEL SERUM TO TREAT SNAKE BITE

Rajasthan has become the first state in India to take fresh steps to address the problem of snake bites. An exclusive snake bite task force comprising Dr.Ian Simpson of the World Health Organisation snake bite treatment group and Dr.PD Tanwar of the SP Medical College snake bite research cell, in Bikaner was formed. The research will focus on developing a new anti snake venom from camel serum which would deal with snake bite cases across the world. Scientists are of the opinion that the reseach being conducted on anti snake venom from camel serum could be more effective as it is more stable in a hot environment, causes less allergies compared to other ASVs and controls snake bite damage more effectively.