

U. Wernery¹H.S.H. Seifert²A.M. Billah¹M. Ali¹

Predisposing factors in enterotoxemias of camels (*Camelus dromedarius*) caused by *Clostridium perfringens* type A

WERNERY (U.), SEIFERT (H.S.H.), BILLAH (A.M.), ALI (M.). Facteurs prédisposants des entérotoxémies à *Clostridium perfringens* type A chez le dromadaire (*Camelus dromedarius*). *Revue Elev. Méd. vét. Pays trop.*, 1991, 44 (2) : 147-152

Clostridium perfringens type A a été isolé à partir de différents organes et d'intestins prélevés sur des dromadaires d'élevage et de course, morts des suites d'entérotoxémies aiguës et subaiguës dans deux foyers distincts. Les modifications pathologiques du tube digestif étaient légères chez les dromadaires d'élevage mais importantes chez les animaux de course. Un sérum anticlostridien polyvalent d'origine bovine, administré par voie intraveineuse, a eu un effet salvateur sur les dromadaires d'élevage mais est resté inefficace pour les autres. Dans les deux foyers, 50 p. 100 des dromadaires d'élevage souffraient d'une affection aiguë à *Trypanosoma evansi* et 25 p. 100 des dromadaires de course étaient atteints de salmonellose. Les auteurs pensent que ces deux affections ont joué un rôle important, comme facteurs prédisposants, dans l'apparition de ces deux foyers d'entérotoxémie à *C. perfringens*. *Mots clés* : Dromadaire - *Camelus dromedarius* - Entérotoxémie - *Clostridium perfringens* type A - Émirats Arabes Unis.

INTRODUCTION

Clostridial organisms are of major importance in animals as primary causes of disease. Their pathogenicity relies on the production of exotoxins. Modern methods for the identification of clostridial bacteria with gas chromatography have shown that the old classical scheme of relating a single disease syndrome to a specific clostridial organism must be considered with caution. Based on research carried out in tropical countries, it becomes more obvious that no narrow etiological connection exists between a specific clostridial organism and a disease syndrome. For example, it was found that clostridia other than *C. chauvoei* can cause blackleg (19). This new development is shown in table I.

C. perfringens is probably more widespread than any other potentially pathogenic bacteria. All types can occur in the soil and the digestive tract of healthy animals, making the cultural demonstration of *C. perfringens* of little value. Outbreaks of disease appear to be caused by predisposing factors which remain poorly understood (2).

TABLE I Arrangement of clostridial species (proposed by Seifert, 1991) according to the disease syndromes produced in livestock.

Enterotoxemia complex	per os enteral	<i>C. perfringens</i> type A-E(F) <i>C. sordellii</i> <i>C. difficile</i>	Improper feeding and management, intensive grazing, crowding
Gasoedema complex	per os parenteral	<i>C. chauvoei</i> <i>C. chicamensis</i> <i>C. haemolyticum</i> <i>C. histolyticum</i> <i>C. novyi</i> type A-C	Alteration of intestinal permeability, lesions of skin and mucose membranes
		<i>C. perfringens</i> type A-E <i>C. septicum</i> <i>C. sordelli</i> Madagascar field strains 217, 335, 735, Mexico field strains 809, etc.	
Toxin complex	per os	<i>C. botulinum</i> type A-F <i>C. perfringens</i> type A-F	Improper management, mineral deficiency (P), plant intoxication
	parenteral	<i>C. tetani</i>	Deep anaerobic injuries

High protein or carbohydrate diets, abrupt change of weather and handling of animals (e.g. transport, weighing) are thought to be predisposing factors of *C. perfringens* outbreaks in cattle. Fattening cattle may die peracutely or they may show signs of enterotoxemia (17).

Literature on *C. perfringens* infection in camels is scarce. IPATENKO (8) has reported enterotoxemia caused by *C. perfringens* types C and D in Mongolia. The disease occurred in acute and subacute forms. Excitement, running, staggering and convulsions preceded death in acute cases. In subacute cases, diarrhoea developed, and most animals died within 20 days.

This paper describes two separate outbreaks of disease in camels caused by *C. perfringens* type A and the roles of *Trypanosoma evansi* and *Salmonella* spp. as predisposing factors.

1. Central Veterinary Research Laboratory, POB 597, Dubai, United Arab Emirates.

2. Institute for Applied Biotechnology in the Tropics at the Georg-August University, Goettingen, Germany.

Reçu le 22.1.1991, accepté le 19.4.1991.

MATERIALS AND METHODS

Nine breeding camels became sick from a herd of 90. They were housed in one location, but in different pens. Six animals died and 3 recovered after treatment. The clinical signs of affected camels were : sweating, shivering, hyperexcitement, ataxia and convulsions. Two animals became aggressive. Untreated animals died within 1 hour after the onset of symptoms.

In the second outbreak, 10 racing camels of a group of 55 had developed slight intermittent diarrhoea over several weeks. The cause could not be determined. The entire group was moved to a fenced area with good grazing. After several weeks, about 20 camels became sick and developed watery diarrhoea. The camels had access to water ponds shared by other animals and birds. Three camels died within 4 days after the onset of diarrhoea.

Post-mortem examinations were performed on 5 breeding camels and 3 racing camels. The necropsies were carried out between 2 to 4 hours after death. Abomasum, intestines, liver and kidney were examined for histopathological lesions.

Fluid contents of the abomasum and duodenum were centrifuged at high speed for 10 min at + 4 °C and the supernatant filtered (Sartorius membrane filter, 0.45 µm). The sterile filtrate was then injected into white mice intravenously (0.5-1.0 ml).

Pieces of organs were placed into test tubes containing hot (60 °C) Sahidi-Ferguson-Perfringens (SFP) agar (16) and spread onto Zeissler agar containing antibiotic supplement (Oxoid, SR93). The plates were incubated under anaerobic conditions (Gas generating kit, Oxoid) at 37 °C for 24 h. When black colonies and gas production were observed in the SFP-agar (the following day), the clostridial organisms were spread on Zeissler agar and incubated anaerobically. *C. perfringens* was identified by Gram stain (directly from organs and the media), by motility test and the appearance on Zeissler agar, which showed typical double-zoned haemolysis around the colonies. The strains were then sent for further testing to the Institute for Applied Biotechnology in the Tropics in Goettingen, Germany. (The procedures of type identification will be reported separately).

Organ samples from necropsied camels, faecal samples from all racing camels [55] and water samples from 6 different ponds were enriched in tetrathionate broth followed by culture on brilliant green phenol red lactose agar and pril mannitol agar (13).

Blood was taken from all 90 breeding camels. The sera were checked with the Testryp CAT-test (Smith Kline) for the detection of antibodies against *Trypanosoma gambiense* (sleeping sickness) and wet blood films were tested for live trypanosomes.

Three sick breeding camels and 3 racing camels were each given 100 ml polyvalent gangrene antiserum of bovine origin (Rhône-Mérieux) intravenously.

RESULTS

Gross pathological lesions

Five breeding camels and 3 racing camels were necropsied. Pathological lesions were present in the same organs in all camels, but in racing camels they were more severe. The lesions were as follows : petechial haemorrhages in coastal muscles ; petechiae in cerebellum and brain stem ; petechiae haemorrhages in the mucosa of the pharynx ; subepicardial petechiae and hydropericardium ; ecchymotic haemorrhages in reticulum, omasum and abomasum (photo 1), petechiae and ecchymoses in the intestinal tract (photo 2) ; dark kidneys, adherent kidney capsule could only be removed with subsequent loss of renal parenchyma.

Histopathology

Sections from different parts of the intestine as well as from the omasum and abomasum showed diffuse ulceration and acute haemorrhagic inflammation (photo 3). Kidneys were acutely congested and livers showed central lobular haemorrhages.

Bacteriology

C. perfringens type A was isolated from the reticulum, omasum, abomasum, small intestine, duodenum, colon, liver and kidney but not from muscles and lymphnodes. They were also observed in large numbers in direct smears of the intestinal tract with Gram stain (photo 4).

*Salmonella saint-paul** was isolated from all organs of the necropsied racing camels but not from breeding camels. *Salmonella saint-paul* was also cultivated from 12 faecal samples and *Salmonella cerro* from 2 faecal samples of the 55 racing camels. *Salmonella saint-paul* was also recovered from one of the 6 water ponds.

Injection of mice

The mice were injected with bacteria free-filtrates which were prepared from the intestinal contents of necropsied camels and died after 2 to 4 h with the characteristic symptoms of opisthotonos. This demonstrated the presence of clostridial toxins.

* The authors thank Dr. PIETZSCH, Bundesgesundheitsamt, Berlin, for the *Salmonella* serotyping.



Photo 1 : Haemorrhagic omasitis caused by *C. perfringens* A, mild inflammation, camel suffered from trypanosomiasis.



Photo 2 : Haemorrhagic colitis caused by *C. perfringens* A, severe inflammation, camel suffered from Salmonella infection.



Photo 3 : *C. perfringens* A enterotoxemia, acute haemorrhagic inflammation of the colon.

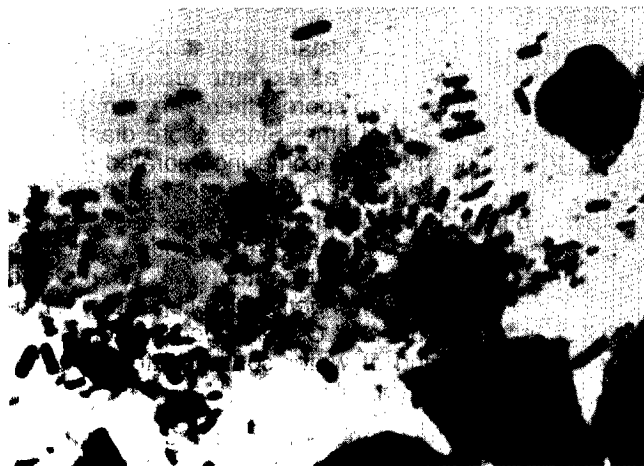


Photo 4 : *C. perfringens* A enterotoxemia, *C. perfringens* organisms found in increased numbers in the colon (direct smear, Gram stain).

Tests for the detection of trypanosomes

Trypanosoma evansi was detected microscopically in the bloods of 45 camels (50 %). Antibodies against trypanosomes were found in 45 camels, too. However, the serologically positive cases did not always correspond with the direct detection of trypanosomes in blood smears. Nineteen camels, in which no blood parasites were detected, had antibodies against trypanosomes which raised the number of positive animals to 64 (71 %). All camels with clostridial infection had trypanosomes in their blood.

Treatment

Three breeding camels, which showed symptoms of peracute *C. perfringens* infection, were successfully treated

with hyperimmunserum. They recovered within 30 min. Racing camels which received the same treatment did not respond and subsequently died.

DISCUSSION

Acute and subacute enterotoxemias as well as haemorrhagic enteritis caused by *C. perfringens* types A, C and D have been described in camels by IPATENKO (8), GAMEEL *et al.*(7) and CHAUHAN *et al.* (5). Enterotoxemias caused by *C. perfringens*, A, C and D are also reported in South American camelids (6). *C. perfringens* type A, has been reported to cause a fatal haemoly-

tic disease in sheep and cattle in Australia (15) and an acute haemorrhagic enteritis in calves (12) and adult cattle (20) in the United Kingdom. An equine intestinal infection with watery diarrhoea and high mortalities caused by *C. perfringens* A was reported by WIERUP (24).

In our study, *C. perfringens* type A was isolated during two different outbreaks from organs, stomach compartments and intestines of breeding and racing camels suffering from peracute and acute enterotoxemias.

C. perfringens A is ubiquitous, and it has been isolated from different feedstuffs as well as air, soil, dust, manure, lakes, streams and rivers (4). Among the 5 different types, type A is most common in the intestine of healthy animals (1). Because of its ubiquitous nature in the environment and because it multiplies in cadavers and invades muscles and organs after death, it is difficult to prove a causal relationship with any condition under investigation.

Infections caused by *C. perfringens* are soil-borne. The spores which are formed in the carcass or parts of the carcass and faeces are resistant to destruction by environmental influences such as extreme drought or frost. The soil contaminated with spores, therefore, remains so, for an unlimited period of time. Since spore diameter is less than lum, the spores become incorporated into the soil structure (18), particularly where livestock has been kept for long periods and where the soil has become heavily contaminated. Camels which are housed in paddocks in the desert are in constant contact with spores and vegetative forms of *C. perfringens*. Despite regular removal of feces from paddocks, *C. perfringens* is continually ingested through food or water contaminated with soil from faeces of carrier animals.

Under normal circumstances ingested *C. perfringens* organisms are kept to low numbers by inhibitory factors present in the duodenum and jejunum, although some survive in the duodenum where multiplication occurs and toxin is produced (21). Toxemia, however, does not occur because the movement of ingesta keeps the bacterial population and toxin concentration down to low levels. In certain circumstances, however, sudden outbreaks of clostridial enterotoxemias occur.

There is evidence that alterations must occur in the normal environment of the digestive tract before rapid multiplication of pathogenic organisms can occur, but very little is known about the intestinal conditions which predispose to this condition (3). Clostridial enterotoxemias are known to be initiated by a sudden alteration in food supply, usually from a poor diet to rich one. According to SEIFERT (18), fodder rich in carbohydrates can provide optimal conditions for the proliferation of *C. perfringens*. The bacteria proliferate on carbohydrates, but when sugar supplies are depleted, spore production ends and toxins are released which are absorbed from the gut. Other factors such as heavy milk feeding may have the same effect (2).

JANSEN (9) has shown that phenothiazine given to sheep in therapeutic doses can induce the development of enterotoxemia if the sheep harbour *C. perfringens* type D in their intestines. WENSVOORT (pers. comm. 1990) reported enterotoxemias in lambs suffering from coccidiosis which did not occur when monensin (a coccidiostat) was administered. THOMAS and DOWNEY (23) observed a high incidence of enterotoxemia in sheep in association with heavy tapeworm infestation.

Factors other than change in diet or parasite infestation may predispose to outbreaks of clostridial enterotoxemia. *Trypanosoma evansi* causes the most widespread and important disease in camels (14). Trypanosomes are mechanically transmitted by haematophagous biting flies. Their bite may cause localised swellings. From the skin they enter lymph nodes and then blood, where they divide rapidly (22). They also invade tissues resulting in damage to organs. The animal shows progressive anaemia, remittent fever and emaciation. Erythrophagocytosis may play a role in anaemia but there is evidence that the anaemia is immunologically mediated. The immune mechanisms are profound and produce mononuclear cellular infiltrates and reticuloendothelial proliferation in the spleen and liver (10).

Immunosuppression occurs to various degrees in trypanosomiasis of livestock and is severe in acute infections with trypanosomes (11). Experimental infections in cattle are often associated with acute septicemia and enteric forms of salmonellosis. Another indication of immunosuppression in cattle with trypanosomes is the lowered serological response to bacterial and viral vaccines. In laboratory animals it was found that the mechanisms leading to immunosuppression were associated with the suppression of lymphocytic responses, reduction of complement and involvement of suppressor cells (11).

Fifty percent of the breeding camels in this study suffered from acute trypanosomiasis. All necropsied camels, as well as successfully treated ones, had trypanosomes in their blood. This acute trypanosome infection predisposed to outbreaks of peracute *C. perfringens* A enterotoxemia in breeding camels. There were no environmental stress factors involved. The camels were watered and fed daily with good quality hay.

During the second *C. perfringens* A outbreak *Salmonella saint-paul* was isolated from 14 (25 %) faecal samples of racing camels. The organs of all necropsied camels contained salmonellae. The camels developed infection through contaminated water from a pond (which contained the same salmonella type). Salmonellosis is a disease of all animal species which clinically manifests in a peracute septicemia, an acute enteritis or a chronic enteritis. Intestinal lesions are associated with haemorrhage, oedema, necrosis and villous atrophy (10). The infection with *S. saint-paul* altered the mucous membranes of the digestive tract enabling clostridial organisms to proliferate and release toxins. The toxins are absorbed through the

damaged gut. SINKOVICS (21) reported similar findings in piglets with pathogenic *E. coli* infections. He found a marked mobilisation and activation of *C. perfringens* in the small intestine of piglets with *E. coli* enterotoxemia and stated that the toxins of the pathogenic *E. coli* strains altered the mucosa of the gut which favoured clostridial growth.

The antiserum given to 3 sick breeding camels suffering from severe peracute *C. perfringens* A infection had a life-saving effect. The rapid recovery of treated breeding camels was not only due to neutralisation of the clostridial toxins, but also possibly because severe pathological lesions did not exist (see pictures). Severe tissue damage

and acute haemorrhagic enteritis with blood stained intestinal contents were seen in the second *C. perfringens* A outbreak in racing camels. Due to the severity of the lesions the animals did not respond to the treatment.

ACKNOWLEDGEMENTS

These studies were carried out with the support of H.H. Sheik Mohammed bin Rashid Al Maktoum, Dubai, United Arab Emirates.

WERNERY (U.), SEIFERT (H.S.H.), BILLAH (A.M.), ALI (M.). Predisposing factors in enterotoxemias of camels (*Camelus dromedarius*) caused by *Clostridium perfringens* type A. *Revue Élev. Méd. vét. Pays trop.*, 1991, **44** (2) : 147-152

C. perfringens type A was isolated from different organs and intestines from breeding and racing camels which died from peracute and acute enterotoxemias in two separate outbreaks. Pathological changes in the digestive tract were mild in breeding camels, and severe in racing camels. A polyvalent clostridial antiserum of bovine origin given intravenously had a life-saving effect on breeding camels, but not on racing camels. In the two outbreaks, fifty percent of the breeding camels were suffering from an acute *Trypanosoma evansi* infection, and 25 % of the racing camels had developed a salmonellosis. It is suggested that both infections played an important role as predisposing factors for the outbreak of *C. perfringens* enterotoxemias. *Key words* : *Camelus dromedarius* - Enterotoxemia - *Clostridium perfringens* - United Arab Emirates.

WERNERY (U.), SEIFERT (H.S.H.), BILLAH (A.M.), ALI (M.). Factores predisponentes a la enterotoxemia por *Clostridium perfringens* tipo A en el dromedario (*Camelus dromedarius*). *Revue Élev. Méd. vét. Pays trop.*, 1991, **44** (2) : 147-152

Se aisló un *Clostridium perfringens* tipo A de intestinos y diferentes órganos provenientes de dromedarios de cría y de carreras, muertos por enterotoxemia aguda o subaguda, en dos focos diferentes. Las modificaciones patológicas del tubo digestivo fueron leves en los dromedarios de cría, pero más importantes en los animales de carreras. Un suero anti-clostridium polivalente de origen bovino, administrado por vía intravenosa, tuvo un efecto positivo sobre los dromedarios de cría, pero se mostró ineficaz en los otros animales. En los dos focos, 50 p. 100 de los dromedarios de cría sufrían de una infección aguda por *Trypanosoma evansi* y 25 p. 100 de los dromedarios de carreras de salmonellosis. Los autores piensan que estas dos afecciones desempeñan un papel importante, como factores predisponentes en la aparición de estos dos focos de enterotoxemias por *C. perfringens*. *Palabras claves* : *Camelus dromedarius* - Enterotoxemia - *Clostridium perfringens* - Emiratos Arabes Unidos.

REFERENCES

1. BISPING (W.), AMTSBERG (G.). Colour atlas for the diagnosis of bacterial pathogens in animals. Berlin and Hamburg, Verlag Paul Parey, 1988.
2. BLOOD (D.C.) RADOSTITS (D.M.). Veterinary medicine. 7th ed. London, Baillière Tindall, 1989.
3. BULLEN (J.J.). Enterotoxemia of sheep. *Clostridium welchii* type B in the alimentary tract of normal animals. *J. Path. Bact.*, 1952, **64** : 201-206.
4. CARTER (G.R.). Essentials of veterinary bacteriology and mycology. Philadelphia, Lea and Febiger, 1986.
5. CHAUHAN (R.S.), KULSHRESHTHA (R.C.), KAUSHIK (R.K.). A report of enterotoxaemia in camels in India. *Indian vet. J.*, 1985, **62** (10) : 825-827.
6. FOWLER (M.E.). Medicine and surgery of South American camelids. Ames, Iowa State University Press, 1989.
7. GAMEEL (A.A.), EL-SANOUSI (S.M.), MUSA (B.), EL-OWNI (E.E.). Association of some pathogenic bacteria with haemorrhagic enteritis in camels. Camel Research Paper, Camel research Unit, University of Khartoum, Sudan. 1986. P. 50-55 (N° SRC 12).
8. IPATENKO (N.G.). Infectious enterotoxemia of camels. *Vet. Bull.*, 1974, **44** (4) : 1481-1484.
9. JANSEN (B.C.). The occurrence of pulpy kidney in sheep dosed with phenothiazine. *J. S. Afr. vet. med. Ass.*, 1960, **31** (2) : 209-210.

10. JONES (T.C.), HUNT (R.D.). Veterinary pathology. 5th ed. Philadelphia, Lea and Febiger, 1983.
11. LOSOS (G.J.). Infectious tropical diseases of domestic animals. Avon, The Bath Press, 1986.
12. MAC RAE (D.R.), MURRAY (E.G.), GRANT (I.G.). Enterotoxaemia in young suckled calves. *Vet. Rec.*, 1943, **55** (19) : 203-204.
13. PIETZSCH (O.). Ein Nachrboden zur Schwaermhemmung und gleichzeitigen Unterscheidung der Proteuskeime von Salmonellen. *Fleischwirtschaft*, 1967, **47** (1) : 31-32.
14. ROETTCHE (D.), SCHILLINGER (D.), SWEYGARTH (E.). Trypanosomiasis in the camel (*Camelus dromedarius*). *Revue sci. tech. Off. int. Épizoot.*, 1987, **6** (2) : 463-470.
15. ROSE (A.L.), EDGAR (G.). Enterotoxaemic jaundice of sheep and cattle. A preliminary report on the aetiology of the disease. *Aust. vet. J.*, 1936, **12** : 212.
16. SAHIDI (S.A.), FERGUSON (A.R.). New quantitative, qualitative, and confirmatory media of rapid analysis of food for *Clostridium perfringens*. *Appl. Microbiol.*, 1973, **21** : 500-506.
17. SEIFERT (H.S.N.). Untersuchungen zur Aetiologie von Anaerobierinfektionen bei Rindern und Schafen in Lateinamerika. *Zentbl. VetMed. B*, 1975, **22** (3) : 60-86 und 177-195.
18. SEIFERT (H.S.H.). Hygiene management on extensive and intensive cattle units in the tropics. *Anim. Res. Dev.*, 1978, **7** (4) : 49-102.
19. SEIFERT (H.S.H.). Tropentierhygiene. Jena, Verlag Gustav Fischer, 1992.
20. SHIRLEY (G.N.). Clostridial enteritis in cattle. *Vet. Rec.*, 1958, **70** (23) : 478-480.
21. SINKOVICS (G.). Quantitative changes of Clostridia in the intestine of early-weaned pigs diseased in *Coli-enterotoxemia*. *Acta vet. hung.*, 1972, **22** (2) : 133-139.
22. The Merk veterinary manual. 6th ed. Rahway, N.J., Merck and Co., 1986.
23. THOMAS (P.L.), DOWNEY (N.E.). Mortality in lambs due to enterotoxemia associated with heavy infestations of *Moniezia expansa*. *N.Z. vet. J.*, 1956, **4** : 161-163.
24. WIERUP (M.). Equine intestinal clostridiosis. *Acta vet. scand.*, Suppl. 1977, **62** : 1-4.