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Short Communication

NATURAL ACQUIRED *Clostridium perfringens* α-TOXIN ANTIBODIES PROTECT DROMEDARIES FROM CLOSTRIDIAL ENTEROTOXAEMIA

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ABSTRACT

Thirty one dromedaries of different age and gender were necropsied and their sera tested for antibody levels to *C. perfringens* α-toxin. It was found that 15/31 dromedaries had low or no antibodies to *C. perfringens* A and therefore succumbed to clostridial enterotoxaemia. Sixteen dromedaries (16/31) had high *C. perfringens* A antibody titres. They died from different diseases.

It is proposed to test all racing camels for *C. perfringens* A antibodies and vaccinate those animals which have low (<50%) or no titres to *C. perfringens* A.

Key words: Dromedary, *C. perfringens* antibody, ELISA

Clostridium (C.) perfringens is an anaerobic Gram-positive bacterial pathogen causing a wide range of diseases. It is widely spread in the soil and gastrointestinal tract of animals, and it is characterised by its ability to produce potent exotoxins. These toxins can cause specific enterotoxaemias in man and various animal species. In the United Arab Emirates (UAE), *C. perfringens* infections have become the most important bacterial disease in dromedaries, gazelles and hunting falcons (Wernery *et al,* 1998; Wernery *et al,* 2009). Predisposing factors to clostridial enterotoxaemias are believed to be concurrent diseases, dehydration, over training, excess energy feeding and management problems. With this study we investigated the question if dromedaries with high antibody levels to *C. perfringens* α-toxin succumb to clostridial enterotoxaemia.

Materials and Methods

Thirty one dromedaries of different age and sex which were submitted to CVRL in Dubai in 2008 were necropsied using routine laboratory methods to define the cause of death.

For the isolation and identification of *C. perfringens* bacteria, the following procedures were performed: Swabs from the mucosa of the small intestines and other organs were taken and spread onto Zeissler agar containing antibiotic supplements (Oxoid, SR 93). Smears were also prepared from affected organs and stained after Gram. The Zeissler agar plates were incubated under anaerobic conditions (Gas generating kit, Oxoid) AN 0025A at 37°C for 24hrs. *C. perfringens* colonies grown on Zeissler agar were subjected to identification and classification by the typical growth, green pigment production and typical appearance in Gram stain of pure colonies from Zeissler agar and direct smears from the intestinal mucosa.

For the classification of the isolated anaerobes an ELISA (Enterotoxaemia ELISA, Cypress Diagnostics, Belgium) was used. The ELISA works with culture supernatants derived after sub-cultivation of *C. perfringens* -suspicious colonies from Zeissler agar and further incubation under anaerobic conditions for 4 hours in Trypticase Glucose Yeast Extract broth (Merck Cat No. 1.05459, Wernery *et al,* 2003). The Cypress ELISA detects alpha (a), beta (b) and epsilon (e) toxins of *C. perfringens* as well as identifies the bacteria itself (id).

C. perfringens α-toxin antibody levels were tested using the BIO-X Diagnostic ELISA BIO K221. The test is designed to monitor the animal's serological response after natural contact with *C. perfringens* bacteria or vaccination. It is a competitive ELISA and can therefore be used for all animal species.

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The results are expressed in % inhibition which is calculated from optical densities of positive and negative sera using special formulas.

Calculated value	Degree of positivity
% inhibition <20	0
% inhibition 20-40	+
% inhibition 40-60	++
% inhibition 60-80	+++
% inhibition 80>	++++

The antibody levels of all 31 necropsied camels were tested from sera taken during necropsy.

Results

The cause of death of 31 dromedaries is summarised in Table 1, and the diseases are compared with the *C. perfringens* findings. Eight camels from which *C. perfringens* organisms were cultured and which showed antibodies above 50% did not die from clostridial enterotoxaemia. Also 8 camels which possessed only antibodies above 50% without positive *C. perfringens* culture did not succumb to clostridial enterotoxaemia. However, 15 camels which had low or no antibodies to *C. perfringens* α-toxin died from clostridial enterotoxaemia.

All isolated strains of *C. perfringens* were confirmed as α-toxin producer.

Discussion

The aim of this study was to investigate the question if dromedaries which had acquired a natural immunity to *C. perfringens* α-toxins are protected against clostridial enterotoxaemia. Therefore, we examined 31 dromedaries of different age and sex for *C. perfringens* α-toxin antibodies and compared these results with the cause of their deaths. Fifteen dromedaries which had no (11) or low (4) antibodies to *C. perfringens* α-toxin died from clostridial enterotoxaemia. From these animals *C. perfringens* organisms were isolated from the small intestines and other organs. The Gram stain of the mucosa swabs was also positive and the isolated *C. perfringens* bacteria were α-toxin producers. These animals died acutely or subacutely and showed the typical enterotoxaemia lesions which are described elsewhere (Wernery and Kaaden, 2002).

In contrary, 16 dromedaries which showed high *C. perfringens* antibodies died from different diseases

Table 1. Cause of death of 31 necropsied dromedaries with and without *C. perfringens* α-toxin antibodies.

Positive C. *perfringens* culture	Positive Gram stain	Positive α-toxin producer	High (>50%) *C. perfringens* antibodies in %	Disease
8	8	8	8	Colisepticaemia 1 Salmonellosis 2 White Muscle Disease 2 Fracture 1 Peritonitis 1 Butazolidon Intoxication 1

Negative C. *perfringens* culture	Negative Gram stain	Not tested*	High (>50%) *C. perfringens* antibodies in %	Disease
8	8	8	8	Septicaemia 2 Salmonellosis 1 Pleuritis 1 Acidosis 1 White Muscle Disease 1 Tumor 1 Coccidiosis 1

	Positive C. *perfringens* culture	Positive Gram stain	Positive α-toxin producer	High (>50%) *C. perfringens* antibodies in %	Disease
No antibodies	11	11	11	11	Clostridial enterotoxaemia
Low antibodies <50%	4	4	4	4	

* Test not performed because no *C. perfringens* was cultured

although from 8 of these animals *C. perfringens* α-toxin producing organisms were cultivated.

Our investigations clearly showed that natural acquired *C. perfringens* α-toxin antibodies protect dromedaries from clostridial enterotoxaemia. Similar results can be expected when dromedaries are vaccinated against *C. perfringens* A (Wernery *et al*, 2009). The high number of dromedaries with high antibody levels (15/31) may also explain our observations in the UAE that only few racing dromedaries succumb to the disease. It is therefore proposed to test all racing dromedaries with the *C. perfringens* antibody ELISA, and to vaccinate only animals which have no or low levels of *C. perfringens* A antibodies.

References

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