Vol 19 No 2, p 149-152

Clostridium perfringens A HYPERIMMUNE SERUM SAVES DROMEDARY LIVES FROM CLOSTRIDIAL ENTEROTOXAEMIA

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ABSTRACT

Clostridium (C.) perfringens A enterotoxaemia is an important bacterial disease in racing dromedaries in the UAE. The course of the clostridial enterotoxaemia is rapid and therefore an effective treatment with antibiotics is unrewarding. The administration of a specific anti alpha-toxin produced in a homologous system is an efficient short-term prophylactic method to save the life of valuable dromedaries. Ten racing dromedaries suffering from typical *C. perfringens* A enterotoxaemia diagnosed through clinical signs and change in some blood parameters as well as low *C. perfringens* antibody levels, recovered when 100 ml of anti alpha-toxin containing 14g IgG/l were given intravenously.

Key words: *C. perfringens* A, dromedary, homologous hyperimmune serum

Clostridium (C.) perfringens is an anaerobic Gram-positive bacterial rod carrying a wide range of diseases. Clostridia are found ubiquitously in the environment and can be detected in the intestine in small numbers where they cause little or no harm. However, following disturbances in the intestinal flora or sudden dietary changes, clostridial species can multiply in large numbers releasing potent toxins which can be fatal (Seifert and Boehnel, 1995).

Enterotoxaemia occurs in racing dromedaries in the UAE (Wernery and Kaaden, 2002) and it is mainly caused by *C. perfringens* A (Wernery *et al,* 2009a). Investigations have shown that 85% of racing dromedaries have natural high levels of antibodies to *C. perfringens* A and are therefore most probably protected against enterotoxaemia. This natural immunity may explain why only single dromedary succumb to this disease but often these are very valuable and high-priced athletes (Wernery *et al,* 2009b). To avoid fatalities of this kind, the dromedaries with low levels or no antibodies against *C. perfringens* A must be vaccinated or in case of an outbreak must be immediately treated with an alpha-toxin hyperimmune serum. The above mentioned authors proved that 3 subcutaneous vaccinations with a toxoid bacteria *C. perfringens* A vaccine were necessary to obtain high levels of antibodies for at least one year. The researchers also stressed that dromedaries that already possessed natural high levels of antibodies did not show any further significant antibody rise after vaccination. They 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.

The clinical course of clostridial enterotoxaemia is peracute and effective treatment with antibiotics often fails. Therefore, therapy with hyperimmune serum is an efficient short-term prophylactic method. The antitoxin given intravenously will neutralise the toxin circulating in the sick animal.

This paper describes the successful treatment of clostridial enterotoxaemia of dromedaries in the UAE with *C. perfringens* A antitoxin.

Materials and Methods

Production of *C. perfringens* A hyperimmune serum

For the production of a *C. perfringens* A hyperimmune serum, an alpha-toxin from a *C. perfringens* A strain was used. For this purpose the CVRL *C. perfringens* strain 939/06 was chosen. The strain originated from a 2 year-old female racing dromedary which had died from an acute clostridial enterotoxaemia. It was identified by ELISA and PCR as type A strain. The quantitative alpha-toxin estimation was carried out in the lecithovitellin test

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using culture supernatant from Trypticase Glucose Yeast Extract Broth (TGYEB) according to Wernery *et al* (2009a). The *C. perfringens* A strain 939/06 used for manufacturing the alpha-toxin FLB37/07 possessed 512 minimal phospholipase units (MPU) per ml.

Two three year-old male castrated dromedaries kept at CVRL were chosen for the immunisation. Five ml PBS containing 0.4g toxin were mixed with 5 ml of Advax adjuvant (ADVAX™ CXL vaccine adjuvant, Vaccine Pty Ltd, Australia) and subcutaneously given at the base of the neck. In total 4 immunisations were carried out, one each week. Thirty days after the fourth immunisation when both dromedaries showed high levels of antibodies to the alpha-toxin with BIO-X Diagnostics ELISA kit BIOK 221, they were bled. Approximately six litres of blood were withdrawn from the jugular vein in blood bags and after centrifugation, the plasma was frozen at -20°C. When 30 litres of plasma was gained, IgG was extracted following a method described by Wernery *et al* (2009a) and Joseph *et al* (2012).

In brief

Hyperimmune sera were subjected to a series of processes, which included solvent-detergent extraction that effectively inactivates possible lipid-enveloped viruses. Serum proteins of the extract were then precipitated by caprylic acid (octanoic acid) without loss of yield and purity. Subsequent filtration and chromatographic separations resulted in highly purified IgGs.

For the *C. perfringens* alpha-toxin, the purified IgGs were then further concentrated to 14g/l and filled in 50 – 100 ml sterile transfer bags (Compoflex, Fresenius Kabi AG, Germany).

Treatment of dromedaries against clostridial enterotoxaemia

Over a period of 6 months, ten racing dromedaries stabled in different locations in the Emirate of Dubai were selected by the field veterinarian for the treatment of *C. perfringens* A enterotoxaemia. Hundred ml of anti-alpha-toxin was intravenously given immediately at the onset of clinical signs of clostridial enterotoxaemia. Before the anti-toxin was administered, blood samples were taken for haematology, biochemistry and *C. perfringens* A antibody levels. The dromedaries were re-tested 2 to 5 days later to record any change of blood values. Clostridial antibodies were tested according to Wernery *et al* (2009a).

Results

Ten dromedary racing camels which were chosen by the treating veterinarian for this experiment suffered from *C. perfringens* A enterotoxaemia. The clinical signs were as followed: fever above 40°C, depression, off feed, severe alteration in many blood parameters as documented in Table 1. The most prominent changes were observed in WBC, RBC, Hb, platelets and iron. The dromedaries possessed low or no antibodies to *C. perfringens* A in the ELISA.

The values returned to normal when the bloods were retested 2 to 5 days after the treatment with hyperimmune serum. Also after treatment the *C. perfringens* A antibodies increased above 80% inhibition.

Discussion

*C. perfringens*is is widely spread in the soil and gastrointestinal tract of animals and is characterised by its ability to produce potent exotoxins which are responsible for a variety of diseases in human and animal species.

In the United Arab Emirates (UAE), *C. perfringens* infections have become the most important bacterial disease in dromedaries causing often live threatening enterotoxaemia with typical clinical signs and pathological lesions described by Kinne and Wernery (2012). In the majority of cases *C. perfringens* A is isolated from pathological samples and from the faeces and identified by a multiplex PCR and Cypress ELISA. The multiplex PCR identifies 6 genes which are α, β, β2, ε, ι and enterotoxin (Gkiourtzidis *et al,* 2001 and Tansuphasti *et al,* 2002) whereas the enterotoxaemia ELISA detects α, β, ε toxins of *C. perfringens* as well as the bacteria itself.

The clinical course of clostridial enterotoxaemia is rapid and therefore an effective treatment with antibiotics is unrewarding. However, the administration of a specific hyperimmune serum is an efficient short term prophylactic method to save valuable racing dromedaries. The antitoxin given IV as soon as the disease starts will neutralise the toxin circulating in the sick animal. It was unknown which amount of IgG must be administered to a 350 Kg dromedary to neutralise the circulating toxin but our experiment clearly showed that 100 ml of hyperimmune serum containing 14g IgG/l saved the lives of 10 racing dromedaries. It was of great importance to start the treatment as soon as possible because delayed treatment after 24 hours or even after longer periods would reduce the effect of the hyperimmune serum due to organ damages.

Table 1. Haematology, biochemistry and *C. perfringens* A blood parameters before and after treatment of clostridial enterotoxaemia in dromedaries with hyper immune serum.

Parameters	SI Units	Reference Values*	Before treatment										After treatment									
			1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Red Blood Cells (RBC)	1012/L	7.0 – 10.5	6.9	5.2	8.7	4.9	6.1	7.0	7.1	6.5	5.8	4.9	7.2	7.3	7.9	8.1	8.2	7.0	7.1	7.3	7.4	7.6
Haemoglobin (Hb)	g/dl	10.5 – 14.5	9.5	8.2	9.4	7.9	8.3	8.5	8.0	8.8	8.1	7.8	11.2	10.7	10.9	10.8	10.4	10.0	9.9	10.3	10.4	10.7
Platelets (Pl)	10^9/L	270 – 600	146	130	181	211	230	191	192	204	205	186	226	280	311	401	312	391	412	381	401	420
White blood cells (WBC)	10^9/L	8 – 15	1.8	3.2	3.6	2.1	2.6	2.4	3.0	2.8	3.1	2.1	11.2	12.0	13.1	9.8	13.0	13.4	12.0	14.0	10.8	12.1
Neutrophils (NEU)	%	40 – 60	50	53	56	58	54	52	53	44	50	51	72	54	58	60	57	55	53	47	50	50
Lymphocytes (LY)	%	25 – 45	43	35	38	35	36	39	41	42	41	40	21	37	36	35	36	34	40	40	40	39
Monocytes (MON)	%	2 – 8	5	5	4	4	7	5	3	7	6	6	3	4	4	3	4	6	5	6	4	5
Eosinophils (EO)	%	0 – 6	1	6	2	3	3	3	2	6	3	3	2	4	2	2	2	5	2	5	6	5
Basophils (BA)	%	0 –1	1	1	0	0	0	1	1	1	0	0	2	1	0	0	1	0	0	2	0	1
Iron	µmol/l	15 – 27	4	9	12	8	4	6	8	7	5	6	17	18	20	23	25	18	21	23	24	26
C. perfringens antibody	Degree of positivity in %	-	20 – 40	<20	20 – 40	20 – 40	<20	<20	<20	20 – 40	<20	20 – 40	>80	>80	>80	>80	60 – 80	>80	>80	>80	>80	60 – 80

*Wernery *et al* (1998)

A haematology and biochemistry check up at the early stages of the disease is an important diagnostic tool and shows alteration in several blood parameters which are typical for clostridial enterotoxaemia. The WBC count is severely decreased most probably due to the effect of the clostridial toxin on the bone marrow. This refers also to the platelets but there are no significant changes in the WBC differential. RBC and Hb are also reduced which can be explained by the diathesis which occurs in the intestinal tract. Severe bleeding is always observed in the intestinal tract especially in the colon ascendens due to changes in the permeability of the intestinal capillaries by the clostridial toxin. Low iron values which are always diagnosed in clostridial enterotoxaemia are a sign of malabsorption in the intestinal tract due to the above mentioned diathesis.

All diseased dromedaries were tested with the *C. perfringens* antibody ELISA and as can be seen from Table 1, all 10 camels showed no or low antibody titers to *C. perfringens*. Hundred ml given IV to diseased dromedaries is in most cases sufficient to save their lives from acute clostridial enterotoxaemia. Animals recover in a short period of time within a couple of hours. Only in few cases a second intravenous application of hyperimmune serum is necessary, most probably because of a very severe enterotoxaemia or when the treatment was delayed. No side effects like allergic reactions or anaphylactic shock have been observed which is unlikely to occur because a homologous system was used for the hyperimmune serum production.

When the treated dromedaries were re-tested 2 to 5 days later, all blood parameters were in the normal range. The dromedaries had no fever and were eating normally. Also the low antibodies to *C. perfringens* observed before the administration of the alpha – antitoxin had reached high levels. It is most likely that the antibody increase was caused by the application of *C. perfringens* IgG. It is known from sheep that anti – toxins will circulate for more than 20 days in the animal's body. This would prevent a recurrence of the toxaemia within this time frame.

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