Unusual mortalities of the eastern black rhinoceros *(Diceros bicornis michaeli)* due to clostridial enterotoxaemia in Ol Jogi Pyramid Sanctuary, Kenya

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Abstract

Nine eastern black rhinoceroses (*Diceros bicornis michaeli*) developed clinical clostridial enterotoxaemia between May and July 2010 in the Pyramid Black Rhino Sanctuary within the Ol Jogi Conservancy, Laikipia, Kenya. The rhinos presented with a per-acute syndrome characterized by severe abdominal pain manifested by struggling and rolling on the ground and laboured breathing, and they died within three hours after being sighted sick. Necropsy and histopathology revealed severe pathology in the gastro-intestinal tract. Grossly, the small and large intestines were congested and oedematous. All the rhinos had variable amounts of haemorrhagic fluid in the intestines. Microscopically, the most characteristic lesion was severe necrotizing haemorrhagic enteritis. Numerous Gram-positive rod-shaped bacterial colonies that were identified to be *Clostridium* spp. were occasionally seen in the intestinal mucosa. *Clostridium perfringens* type A was isolated from the stomach contents. *C. perfringens* was postulated as the aetiological agent with the infection triggered probably by change of habitat following a prolonged period of drought that was followed by above-normal rainfall.

Additional key words: *Clostridium perfringens*, necrotizing haemorrhagic enteritis

Résumé

Neuf rhinocéros noirs de l'est (*Diceros bicornis michaeli*) ont attrapé une entérotoxémie clostridienne clinique entre mai et juillet 2010 dans le sanctuaire des rhinocéros noirs de la conservation de la Pyramide d'Ol Jogi à Laikipia au Kenya. Les rhinocéros avaient un syndrome suraigu caractérisé par des douleurs abdominales aigues qui se sont manifestées par le débattement et le roulement par terre et une respiration difficile. Ils sont morts trois heures après que l'on avait constaté leur maladie. Une autopsie et une histopathologie ont révélé une pathologie grave dans le tract gastro-intestinal. En peu de mots, les petits et les grands intestins étaient congestionnés et œdémateux. Tous les rhinocéros avaient des quantités variables de liquide hémorragique dans les intestins. Au microscope, la lésion la plus caractéristique était une entérite hémorragique nécrosante aigue. On a parfois vu dans la muqueuse intestinale de nombreuses colonies bactériennes gram-positives en forme de tige identifiées comme *Clostridium* spp. On a isolé le *Clostridium perfringens* de type A à partir des contenus stomacaux. On a postulé que le *Clostridium perfringens* était l'agent étiologique à l'origine de l'infection déclenchée probablement par le changement d'habitat à la suite d'une période de sécheresse prolongée suivie par une pluviométrie supérieure à la normale.

Mots-clés supplémentaires: *Clostridium perfringens*, entérite hémorragique nécrosante

Introduction

The black rhinoceros (*Diceros bicornis*) is listed as critically endangered as its population has declined by an estimated 97.6% since 1960, reaching a low of 2410 in 1995, mainly as a result of poaching and loss of habitat. Since then, numbers have been steadily increasing at a continental level with numbers doubling to 4800 by the end of 2010. However, the current numbers are still more than 90% lower than three generations ago (Emslie, 2011).

There are four recognized subspecies of the black rhinoceros: *D.b. bicornis, D.b. longipes, D.b. michaeli and D.b. minor.* The subspecies *D.b. michaeli*, also known as the eastern black rhinoceros, was distributed from southern Sudan, Ethiopia and Somalia, through Kenya into northern-central Tanzania and Rwanda. Its current stronghold is Kenya, which currently holds approximately 85% of the total wild population. Smaller but growing numbers occur in northern Tanzania and as introduced and subsequently translocated populations in South Africa (IUCN, 2008; Emslie, 2011; Kenya Rhino Program, 2011). The Kenyan population, estimated at about 597 animals, is mainly found in national parks, private sanctuaries and community land tenure systems (Kenya Rhino Program, 2011).

Clostridium perfringens is a Gram-positive, spore-forming, anaerobic rod-shaped bacterium responsible for diseases such as gas gangrene, food poisoning and diarrhoea in humans (Greco et al., 2005) as well as for enterotoxaemia and haemorrhagic gastroenteritis in many domestic and wild animals (Niilo, 1987; Bacciarini et al., 2001; Greco et al., 2005; Bertelsen and Weese, 2006; Das et al., 2008; Diab et al., 2011; Merck & Co., 2011). The bacterium is ubiquitous in the environment and foods and forms part of the normal gut flora in humans and animals (Blood and Radostits, 1989; Merck & Co., 2011).

Enterotoxaemia describes a disease caused by absorption of toxins produced by the growth of *C. perfringens* biotypes (Merck & Co., 2011). The bacteria can produce four major lethal toxins: alpha, beta, epsilon and iota, which are used for toxin typing of the species (Songer, 1996). There are five biotypes of the bacteria based on the differential production of these lethal toxins: A, B, C, D and E (Niilo, 1987; Greco et al., 2005). The bacteria also produce nine minor toxins (or soluble antigens)—delta, theta, kappa, lambda, mu, nu, gamma, eta and neuraminidase—that may play a role in its pathogenicity as well as the enterotoxin that is responsible for food-borne illnesses caused by *C. perfringens* (Niilo, 1987; Greco et al., 2005). In this paper, we report, for the first time to our knowledge, the outbreak of clostridial enterotoxaemia due to *C. perfringens* type A in a wild population of the eastern black rhinoceros in a sanctuary in Kenya.

Materials and methods

Study area

The Pyramid Black Rhino Sanctuary, which covers 50 km^2, is located between 37′00″ E to 37′05″ E and 0′15″ N to 0′20″ N, about 60 km from Nanyuki town in Laikipia District, Kenya (fig. 1). It was established as a black rhino sanctuary in 1979. The sanctuary and the Ol Jogi ranch comprise the Ol Jogi Conservancy.

The sanctuary has so far managed to breed and donate 36 rhinos to other rhino sanctuaries in Kenya. Due to the relatively small size of the sanctuary, male rhinos have in the past been translocated into Ol Jogi Ranch to prevent sometimes fatal territorial fights. Before the mortalities there were 38 rhinos with 17 in Pyramid Sanctuary and 21 in the ranch. The two parts of the conservancy are separated by the Nanyuki–Dol Dol Road and each side is completely enclosed by an electric fence such that animals cannot move from one side to the other. In addition to wildlife conservation, the ranch also keeps livestock. The sanctuary is purely for conservation and ecotourism activities.

Soils in Ol Jogi Conservancy are mainly chromic Cambisols, Fluvisols and eutric Cambisols (Sombroek et al., 1982). The vegetation is a mosaic of grassland, acacia woodland and shrubs (Mizutani, 1999). The mean annual rainfall between 2000 and 2010 was 459 mm.

Case report

Nine eastern black rhinos died in the Pyramid Sanctuary on diverse dates between 17 May and 23 July 2010. The mortalities comprised both young and old animals of both sexes and were distributed in all parts of the 50-km^2 sanctuary. Table 1 summarizes the mortalities by rhino identity, sex, age and date of death.

Eight of the rhinos—Alberto, Il Polei, Jilali, Kwetu, Malaika, Margo, Shatoosh and Tuyie—were observed sick before they died by the game scouts

Figure 1. Ol Jogi Conservancy and Pyramid Sanctuary, Laikipia County.

responsible for rhino monitoring. These animals displayed signs of severe abdominal pain—struggling and rolling on the ground and grunting followed by death within three hours. Some of these animals were also seen trying to roll themselves in ponds of water; one (Margo) had laboured breathing (dyspnoea). The animals displayed no indications of illness before these signs were observed.

After the death of the fifth rhino, it was decided that to avert further losses the remaining breeding females be moved from Pyramid Sanctuary to the ranch, which had been without incident during the entire period. This was aimed at reducing exposure of the animals to the yet-unknown trigger factors leading to the mortalities. Five females, which included one with a calf, were relocated to the ranch to supplement a breeding population established in 2007 while two calves whose mothers had died were vaccinated and covered with antibiotics and moved to a 1-ha enclosed boma within Pyramid Sanctuary. Two bulls were vaccinated, covered with antibiotics and left on site. Table 2 summarizes these interventions, which were undertaken between 22 and 24 June 2010. Two of the rhinos, Margo and Il Polei, died soon after.

All 10 animals were covered with an intramuscular injection of 100 ml of the long-acting antibiotic

Table 1. Summary of the rhino mortalities by rhino identity number, sex, age and date of death

Rhino name	ID no.	Sex	Date of birth	Age (yr.mo)	Date of death
Alberto	3538	M	29 Jan 06	4.4 (subadult)	17 May 2010
Il Polei	3547	F	31 Jan 09	1.5 (calf)	22 July 2010
Jilali	3549	M	24 Feb 09	1.3 (calf)	23 June 2010
Kwetu	3548	M	14 Feb 09	1.4 (calf)	17 June 2010
Malaika	3506	F	06 Oct 84	25.7 (adult)	13 June 2010
Margo	3528	F	22 Sep 01	8.7 (adult)	23 June 2010
Piotr	3539	M	06 Jul 06	4.0 (subadult)	30 May 2010
Shatoosh	3512	F	01 Jan 83	24.0 (adult)	09 June 2010
Tuyie	3511	F	01 Mar 90	20.3 (adult)	20 June 2010

Table 2. Summary of interventions done to the surviving rhinos following the mortalities

Rhino name	ID no.	Sex	Age (yr.mo)	Interventions done
Cecilia	3535	F	5.5	Relocated, vaccinated, antibiotics given
Il Polei	3547	F	1.2	Vaccinated, antibiotics given, moved to boma
Kili	3514	F	17.8	Relocated, vaccinated, antibiotics given
Manuela	3536	F	5.2	Relocated, vaccinated, antibiotics given
Margo	3528	F	8.7	Relocated, vaccinated, antibiotics given
Moyo	2027	M	17.6	Vaccinated, antibiotics given, released on site
Nyiro	3544	M	1.9	Relocated, vaccinated, antibiotics given
Pyramid	3542	M	2.7	Vaccinated, antibiotics given, moved to boma
Twala	3518	F	14.9	Relocated, vaccinated, antibiotics given
Uhuru	2028	M	15.4	Vaccinated, antibiotics given, released on site

Duplocillin LA® (MSD Animal Health, Wellington, New Zealand) consisting of 150,000 IU of procaine penicillin and 150,000 IU of benzathine penicillin per millilitre. Each rhino was also covered with 5 ml of the multivalent bacterin-toxoid ULTRABAC® 7 (Pfizer Animal Health, New York, USA) that contains killed standardized cultures of various *Clostridium* species for protection against different clostridial infections. This vaccine was divided into halves and injected subcutaneously at two different sites.

Investigation and findings

Post-mortem revealed good body conditions (body scores ranged between 3.5 and 4 on a scale of 1 to 5) with no obvious lesions externally. The main gross lesions in all the rhinos were in the gastro-intestinal tract (GIT) where there was severe pathology. The small and large intestines were diffusely congested, oedematous and filled with haemorrhagic fluid (fig. 2 and 3). The caecum in particular had large oedematous swellings (fig. 4). The mesenteric lymph nodes were enlarged and congested.

Samples of the heart, lung, liver, spleen, kidney, small and large intestines, mesenteric lymph nodes and brain were collected in 10% buffered formalin and submitted to different laboratories for histo-pathological analysis. Intestinal contents from different parts of the GIT and tissue samples from the brain, liver and kidneys were also collected in cool boxes with ice packs for bacteriological culture and toxicological analysis. These laboratories included the University of Nairobi Veterinary School and the Government Chemist in Kenya, Onderstepoort

Figure 2 (left) and 3. Gross pathological changes in a black rhino that died suddenly showing congestion and haemorrhages in the intestines.

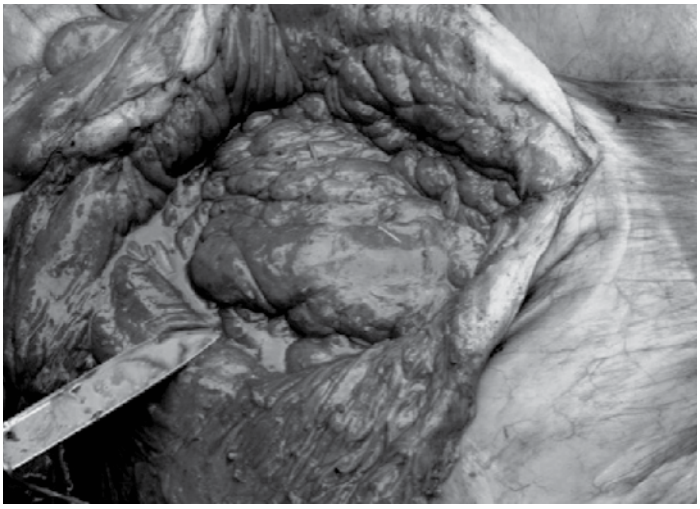

Figure 4. Gross pathological changes showing oedematous swellings on the mucosa in the caecum of a black rhino that died suddenly.

Veterinary Institute and IDEXX Laboratories in South Africa, and the Central Veterinary Research Laboratory (CVRL) in the United Arab Emirates.

Based on the clinical and post-mortem observations, differential diagnosis of clostridial enterotoxaemia, salmonellosis or toxicity was made. However, salmonellosis was considered highly unlikely because it is rarely per-acute and is usually associated with food poisoning, which was not likely in free-ranging rhinos that browse on fresh vegetation. There was no evidence of the animals being supplemented.

Toxicology samples tested negative for commonly used pesticides such as organophosphates, organochlorines, carbamzates and arsenic. The University of Nairobi, CVRL, Onderstepoort and IDEXX Laboratories reported severe necrotizing-haemorrhagic enteritis characterized by haemorrhages, oedema, and infiltration by large numbers of neutrophils and mononuclear leukocytes as well as loss of mucosal epithelium. Numerous rod-shaped bacteria infiltrate were also found in the intestinal walls. These bacteria colonies appeared clostridial. The lesions appeared more severe in the distal portions of the GIT than the anterior sections. CVRL isolated *C. perfringens* type A from the stomach contents after enrichment because viable rods were dead due to exposure to oxygen and only spores were left. In one rhino (Jilali), some *Salmonella* spp. were also observed in the intestinal walls. Nevertheless, it was unlikely that even this rhino was killed by salmonella. No such colonies were observed in the other rhinos and neither was salmonella isolated from the stomach contents. Two of the rhinos (Shatoosh and Jilali) had pulmonary oedema, which could represent a terminal event as a result of heart failure or circulation of toxins. No other notable lesions were observed in the remaining tissues and organs.

Discussion

Based on the per-acute and the painful clinical presentation, the post-mortem lesions as well as the laboratory findings, a diagnosis of clostridial enterotoxaemia was made. The clinical manifestation and the severe pathology in the GIT and lack of other remarkable findings were considered compatible with this diagnosis and have been described elsewhere (Blood and Radostits, 1989; Niilo, 1993; McMillan, 2006; Diab et al., 2011; Merck, 2011). The isolation of *C. perfringens* type A by culturing was also a significant finding in this diagnosis.

All types of *C. perfringens* cause profound enterotoxaemia with sudden death as the principal manifestation (Niilo, 1980,1993; Merck, 2011). In types A, B, C and E, there is severe enteritis with diarrhoea and dysentery (Niilo, 1980,1987,1993; Merck & Co., 2011). These types produce a highly necrotizing and lethal ß-toxin that causes severe intestinal damage (Niilo, 1987,1993; Diab et al., 2011). The post-mortem lesions in type D enterotoxaemia, also called 'pulpy kidney disease', which is characterized by rapid post-mortem autolysis of the kidneys, are minimal, especially if the course is short (Blood and Radostits, 1989; Merck & Co., 2011). Various types of clostridial enterotoxaemia have also been described in different species of wildlife (Sato and Matsuura, 1998; Bacciarini et al., 2001; Greco et al., 2005; Bertelsen and Weese, 2006; Das et al., 2008).

Confirmatory diagnosis for clostridial enterotoxaemia is made by detection of toxins from intestinal filtrates and subsequent identification by neutralization with specific antiserum (Niilo, 1987; Greco et al., 2005). It was not possible to characterize the biotype responsible for these mortalities because of various limitations. However, it is highly likely that

the responsible biotype was A, following the isolation of this bacterium in the stomach contents.

Clostridium spp. are normal GIT flora and the factors that trigger the development of the disease are not well understood but it is presumed that some alteration in the normal GIT environment permits excessive multiplication of the bacteria that produce the toxins capable of causing intestinal damage and systemic effects such as shock (Blood and Radostits, 1989; Merck & Co., 2011). The sanctuary experienced a devastating drought in 2009, which almost wiped out the populations of grazer species in particular. It was estimated that over 600 impalas and 400 buffaloes representing over 95% of each species died but there were no losses of rhinos as a result of the drought. The sanctuary later received higher than normal rainfall during the long rains of April 2010, leading to rapid overgrowth of foliage. In the absence of grazers, particularly the buffaloes, this resulted in markedly noticeable changes in the diversity of thriving flora in the area. It is presumed that these changes resulted in unusual amounts of green plants in the digestive system of the rhinos. These highly digestible plants with high amounts of proteins and carbohydrates and little fibre, possibly with other predisposing factors that were not identified, played a role in changing the normal gut environment in the rhinos triggering the proliferation of *C. perfringens*.

The mortalities tended to be found along ephemeral river systems, which are more or less like the riverine habitats that most animals prefer. These systems provide suitable habitats that stay green longer and are sources of water as well as shade. Thus, animals spend less time in search of browse and water than they do in open areas. In relation to this outbreak, it was presumed that animals in ephemeral river systems were more exposed to the change in foliage that triggered the disease. The management measures undertaken (i.e. treatment, vaccination and movement of the rhinos) were appropriate and arrested further mortalities. It is highly likely that the two rhinos that died soon after these interventions were made were already infected and their condition was exacerbated by the stress of capture and translocation.

Conclusion

Emergency preparedness and rapid response measures should be further strengthened in Ol Jogi Conservancy and all rhino areas to ensure close monitoring of the situation with a view to making a rapid diagnosis to institute appropriate control measures. The area should not be condemned but should be given time for observations and recovery. To restore the ecology of the area, it would be appropriate to introduce buffaloes to control excessive herbage after the rains.

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