Ciliated intestinal protozoa of black (*Diceros bicornis michaeli*) and white rhinoceroses (*Ceratotherium simum simum*) in Kenya

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Abstract

The aim of this study was to survey ciliated intestinal protozoa of the black rhinoceros (*Diceros bicornis michaeli*) and white rhinoceros (*Ceratotherium simum simum*) in Kenyan National Parks. Faecal samples from 28 rhinoceroses that were chemically immobilized for translocation were opportunistically collected. Presence of ciliates was assessed using faecal floatation and sedimentation techniques. The ciliates were identified using cellular morphological features. *Ophryoscoleciidae, Cycloposthiidae* and *Blepharocrythiidae* were the three ciliate families represented. *Ophryoscoleciidae* had nine genera, *Cycloposthiidae* six genera and *Blepharocorythiidae* 1 genus. The dominant ciliate genus in all the rhinoceroses that were sampled was *entodinium*. It was found that the nutrient composition of the diet influences the diversity and numbers of intestinal ciliates, which in turn regulates the nutrient available to the animal. This interplay of nutrient composition of diet, ciliate diversity and nutritional benefits to the host has been used as an index to assess the nutritional state of ruminants. Because of the occurrence of rumenal ciliates in the hindgut fermentative chamber of the rhinoceros, such an index can be used to guide the formulation of feed mixtures for rhinoceros in captivity and remote nutritional assessments of rhinoceros both in free-range and in captivity.

Key words: ciliates, intestinal, Kenya, protozoa, rhinoceros

Résumé

Le but de cette étude était de contrôler les protozoaires ciliés de l'intestin des rhinocéros noirs (*Diceros bicornis michaeli*) et blancs (*Ceratotherium simum simum*) des parcs nationaux kenyans. Nous avons profité de l'immobilisation chimique de 28 rhinocéros qui devaient être déplacés pour récolter des échantillons fécaux. On a évalué la présence des ciliés par les techniques de flottaison et sédimentation, et on les a identifiés par leurs caractéristiques morphologiques cellulaires. Les trois familles de ciliés représentées étaient les *Ophryoscoleciidae,* les *Cycloposthiidae* et les *Blepharocrythiidae*. Les *Ophryoscoleciidae* comprenaient neuf genres, les *Cycloposthiidae* six et les *Blepharocorythiidae* un seul. Le genre de cilié dominant chez tous les rhinos échantillonnés était *Entodinium*. On a découvert que la composition des nutriments dans le régime alimentaire influençait la diversité et l'abondance des ciliés de l'intestin, ce qui, par conséquent, régule les nutriments disponibles pour l'animal. Cette interaction entre composition des nutriments, diversité des ciliés et avantages nutritionnels pour l'hôte a servi d'indice pour évaluer l'état nutritionnel des ruminants. Etant donné la présence de ciliés du rumen dans la dernière chambre de fermentation digestive des rhinocéros, on peut se servir d'un tel indice pour choisir la formule des mélanges de nourriture pour les rhinos en captivité et pour les évaluations nutritionnelles à distance pour les rhinos en liberté et en captivité.

Introduction

Black rhinoceroses (*Diceros bicornis michaeli)* are critically endangered species found in small isolated pockets in the sub-saharan Africa, with majority found in Kenya. They are concentrate selectors that browse on juicy concentrated herbage composed mainly of leaves, flowers and fruits of shrubs and trees. White rhinoceroses are grazers characterized by huge stomachs that are normally filled to capacity with relatively low-quality feed composed mostly of grass (Hofmann, 1973). Both black and white

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rhinoceroses are non-ruminant hindgut fermenters. Their intestinal system contains a characteristic consortium of bacteria, ciliated protozoa, anaerobic fungi and bacteriophages (Kamra, 2005) just like other wild grazers and browsers (Dehority & Odenyo, 2003). These intestinal microbial flora and fauna exist in a symbiotic relationship with the host, and act synergistically to bioconvert ingested food. They survive in the intestinal ecosystem under such constraints as antinutritional factors in feed, which sometimes limit their growth (Kamra, 2005).

Although numerous reports have been published on the ciliated intestinal protozoa of different ruminants, only a limited number of studies have been reported on the ciliated microfauna occurring in the hind stomach of the rhinoceros. Although, Van Hoven, Gilchrist, & Stenson (l998) have published studies on ciliates occurring in the caecum and colon of black and white rhinoceroses in South Africa, similar surveys have not been conducted in the eastern black rhinoceros (*Diceros bicornis michaeli*) and the white rhinoceros (*Diceros simum simum*) found in Kenya. To bridge this knowledge gap, this survey was conducted while translocating rhinoceroses from Lake Nakuru and Nairobi National Parks during dry peak season in the month of January.

Materials and methods

Area of study

The areas of study were Lake Nakuru National Park (LNNP) and Nairobi National Park (NNP) in Kenya. LNNP is located 4 km from Nakuru town-centre between 0°50′S–1°00′S and 36°20′E–36°25′E. The park is completely fenced covering an area of 188 km^2, of which 44 km^2 lie in the shallow alkaline soda lake. The area around the lake is flat lowland of 1200-m altitude and the surrounding hills and cliffs rise to 1750-m altitude. The park receives mean annual rainfall of 850 mm and its vegetation consists of open grassland, *Acacia* forest, invasive *Tarchonanthus* bush land, deciduous and *Euphorbia* forest (Kutilek, 1974).

Nairobi National park is 10 km from the city of Nairobi and lies between 2°18′S–2°20′S and 36°23′E–36°28′E covering an area of 117 km^2. The park is partially fenced with an opening of approximately 20 km on its southern boundary for animal migration. The gentle undulating elevations in the park reach altitude of 1790 m around woodland areas in the northwest to the mosaic grasslands of lowland plains of 1508 m in the southeast. The vegetation cover includes riverine thorn forests, *Acacia* and deciduous forests, shrubs and grasslands (Smith & Verdicourt, 1962; Muya & Oguge, 2000). The park is a sanctuary for black rhinoceroses, which coexist with a wide variety of other animal species.

Collection and processing of faecal samples

Faecal samples were collected opportunistically from 28 rhinoceroses that had been chemically immobilized for translocation. These included ten black rhinoceroses from NNP, and nine black and nine white rhinoceroses from LNNP. The samples were collected from the rectum by hand covered in polythene sleeve and were immediately placed in airtight plastic jars. The jars were packed in a cool box and transported to the laboratory where they were stored in the refrigerator at 4°C until processed.

Two qualitative techniques were used to assess the presence of ciliates in the faecal material, namely simple test tube floatation and sedimentation. In the simple test tube floatation technique, 3 g of faecal sample was mixed with 50 ml of saturated solution of sodium chloride in a container. The resulting faecal suspension was filtered through a tea strainer into a second container. The filtered faecal suspension was then poured into a test tube in a rack and filled up to a convex meniscus. A clean cover slip was gently placed on the meniscus, let to remain for 20 min and then lifted gently and placed on a microscope slide together with the drop of fluid adhering to it. A drop of Lugol's Iodine was applied at the edge of the cover slip and then observed under a light microscope at a magnification of ×200 and ×400. This process was repeated for the same sample stained with acidified methylene blue.

In the sedimentation technique, 5 g of faeces was mixed with 200 ml of water in a beaker. The filtrate was poured through a tea strainer and the debris discarded. It was then left to remain for 10 min and 70% of the supernatant was poured off and the beaker refilled with the same volume of water. This process was repeated three-to-five times, until the supernatant was clear. The final supernatant was carefully decanted and the sediment in the beaker was poured into a Petri dish. Using a Pasteur pipette, a drop of the sediment was placed on a slide and a drop of either Lugol's iodine or acidified methylene blue stain was added. A coverslip was placed and the sediment was observed under a light microscope at a magnification of ×200 and ×400. Species of ciliates were identified using their cellular morphological features. The nuclei, and the cytoplasmic granules and processes were stained with acidified methylene blue while the skeletal plates were stained with Lugol's iodine (Dehority, 1993).

Results

Black rhinoceroses in LNNP had more ciliate species followed by those in NNP. The least number of ciliate species was found in white rhinoceroses in LNNP. *Ophryoscoleciidae*, *Cycloposthiidae* and *Blepharocorythiidae* were the three ciliate families represented. The first two were found in black rhinoceroses while *Blepharocorythiidae* was found in the white rhinoceroses. *Ophryoscoleciidae* had nine genera, *Cycloposthiidae* six genera and *Blepharocorythiidae* one genus. *Entodinium* was the dominating genus in all the rhinoceroses. The occurrence of ciliates species in rhinoceros in the two parks are presented in Table 1. The number of ciliates in different rhinoceros groups is presented in Fig. 1. The number of ciliates in each genus in the different rhinoceros groups is presented in Fig. 2.

Discussion

The genera of ciliates that were found in all the rhinoceroses that were sampled belonged to the Order *Entodiniomorphida*, which are endosymbiotic ciliates inhabiting the fermentative digestive organs of most mammalian herbivores (Williams & Coleman, 1991). The *entodiniomorphida* include three suborders, *Archistomatia, Blepharocorythina and Entodiniomorphina* (Lynn & Small, 1997). The *entodiniomorphina* are the most diverse group of ciliates characterised by reduced somatic ciliation forming tufts or bands, a semi rigid pellicle covering extensive nonciliated areas and an adoral band of cilia around the cytostome. This highly diverse group of ciliates was present in large proportion with *entodinium* being the dominant genus in both black and white rhinoceroses. This finding agrees with the findings reported by Dehority & Odenyo (2003) in their study on the influence of diet on the numbers and types of protozoal fauna in indigenous wild ruminants in Kenya. According to these writers *entodinium* was in considerably higher proportion in concentrate selectors than in roughage eaters in relation to the general concentrations of other ciliates (Dehority & Odenyo, 2003). The present

Table 1 Species of intestinal ciliates found in rhinoceroses in Kenya

	BRLnnp	BRnnp	WRLnnp
Family: Ophryoscoleciidae			
Genus: *Entodinium*			
E. bursa	+	+	–
E. dubardi	+	+	+
E. minimum	+	+	+
E. caudatum	+	+	+
E. bicornutum	+	+	+
E. furca	+	–	+
E. lobospinosum	+	+	+
Genus: *Epidinium*			
E. caudatum	+	+	–
E. bulbiferum	+	+	+
E. gigas	+	+	+
Genus: *Diplodinium*			
D. dentatum	–	+	–
D. anacanthum	+	–	–
F. anacanthum			
D. anacanthum F. monocanthum	+	–	–
Genus: *Eudiplodinium*			
E. maggi	+	+	+
Genus: *Diploplastron*			
D. affine	+	+	–
Genus: *Metadinium*			
M. medium	+	+	+
Genus: *Polyplastron*			
P. multivesiculatum	+	–	–
Genus: *Elyplastron*			
E. bubali	+	+	–
Genus: *Ostracodinium*			
O. mammosum	+	+	–
O. gracile	+	–	–
O. trivesiculatum	+	–	–
Family: Blepharocorythiidae			
Genus: *Blepharocorys*			
B. jubata	–	–	+
Family: Cycloposthiidae			
Genus: *Cycloposthium*			
C. bipalmatum	+	+	–
C. scutigerum	+	+	–
C. edentatum	+	–	–
C. dentiferum	+	+	–
C. corrugutum	–	+	–
Genus: *Spirodinium*			
S. equi	+	–	–
Genus: *Tridinium*			
T. galea	+	–	–
Genus: *Tetratoxum*			
T. parvum	+	–	–
T. unifasculatum	+	–	–

Table 1 (Continued)

	BRLnnp	BRnnp	WRLnnp
Genus: *Tripalmaria*			
T. dogieli	+	–	–
Genus: *Ditoxum*			
D. funinucleum	+	–	–
Total number of species	30	18	11
Total number of genera	15	8	5

BRLnnp, black rhinoceros at Lake Nakuru national Park; BRnnp, black Rhinoceros at Nairobi National Park; WRLnnp, white Rhinoceros at Lake Nakuru National Park; +, presence of ciliate species; –, absence of ciliate species.

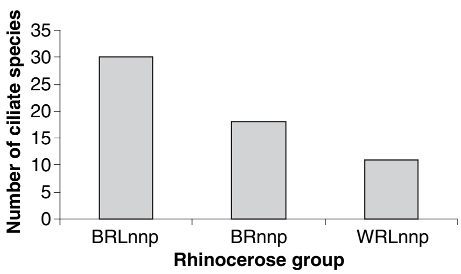

Fig 1 The number of ciliate species in different groups of rhinoceroses in Kenya. Black rhinoceros at Lake Nakuru National Park (LNNP) had more ciliate species, followed by black rhinoceros at Nairobi National Park (NNP). White rhinoceros at LNNP had the lowest diversity of ciliate species diversity

study agrees with these findings and implies that the higher ciliate diversity in black rhinoceros compared with that in white rhinoceros can be attributed to their different modes of feeding.

Black rhinoceroses highly select herbs and shrubs preferring *Solanum incanum, Dichrotachys cinera, Acacia* spp. (Mukinya, 1977), *Grewia similes* and *Commelina africana* with selectivity depending on the availability of these plant species (Muya & Oguge, 2000). On the other hand, white rhinoceroses feed largely on grasses such as *Pennisetum, Panicum*, *Urochlioa* and *Digiteria* (Owen-Smith, 1975). The different types of feed selectively influences the numbers and types of ciliates in an individual host. *Entodiniomorphs* and *holotrichs* are efficient converters of soluble sugar, starch and cellulose and therefore, feeds that are high in these nutrients enrich the population of these ciliates. Diets rich in starch and deficient in sugars inhibit *dastricha* and some small species of *entodinium,* while large *entodiniomorphids* such as *Ophryoscolex* and *Polyplastron* flourish (Abe & Iriki, 1978).

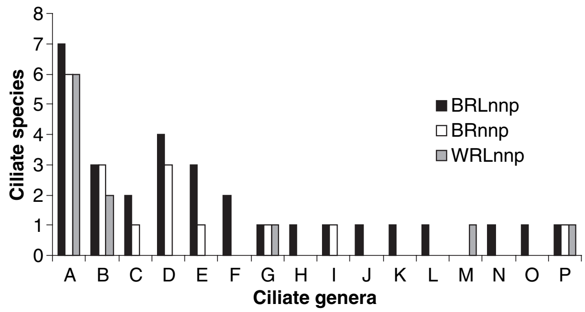

Fig 2 Diversity in ciliate genera among rhinoceros groups sampled in Kenya. Genus *Entodinium* was the predominant genus in all the Rhinoceros groups. *Epidinium* was the second most predominant genus in all the rhinoceros groups. Black rhinoceros at Lake Nakuru had 15 genera, while the Black Rhinoceros at Nairobi National Park had eight genera. White rhinoceros at Lake Nakuru National Park had five genera. A, Entodinium; B, Epidinium; C, Diplodinium; D, Cycloposthium; E, Ostracodinium; F, Tetratoxum; G, Eudiplodinium; H, Diploplastron; I, Elyplastron; J, Tridinium; K, Tripalmaria; L, Ditoxum; M, Blepharocorys; N, Spirodinium; O, Polyplastron; P, Metadinium

Rumenal ciliates have been found to vary in hosts in different geographical regions or ecosystems (Bayram *et al.*, 2005). This finding agrees with the present study in which ciliate composition between black rhinoceros in LNNP and NNP varied and therefore, could be attributed to differences in geographical location and ecosystems in these two parks. The two parks have different types of feed whose nutrient composition and availability vary, which could influence the diversity of ciliates in the hosts. The prevailing dry season could also affect the availability and quality of feed in these two parks to different degrees. This finding agrees with the study reported by Demeyer (1981), who found that the total number of rumen protozoa is variable and depends on the availability and condition of feed, and the season of the year. The effect of season on feed quality has been reported by Bax & Sheldrick (1963), who concluded that during dry season in Kenya, grasses contain 5–7% crude protein whereas legumes and herbs contain 10–12% crude protein. The proportion of crude protein may not affect the numbers but it is likely to influence the ciliate diversity as most *entodiniomophs* and *holotrichs* thrive in soluble sugar and starch diets.

The presence in the hind-stomach of rhinoceroses of *Ophryoscolecids*, which are normally associated with ruminant animals, is in agreement with the findings of Williams & Coleman (1991). According to these authors, the number of ciliate species in an individual host is highly variable and various hosts share many ciliate species.

In conclusion, black rhinoceroses had more diverse ciliated protozoa than white rhinoceroses. This was attributed to the difference in their mode of feeding and diet composition. The variation in composition of ciliates in black rhinoceroses between the two parks in this study is most likely related to ecological differences closely tied to seasonal impacts that influence availability and quality of feed. It was also observed that both black and white rhinoceroses had *entodiniomophids*, endosymbionts that are commonly found in ruminants, suggesting a similarity in the protozoal ecosystems of the rumen and hindgut fermentative chambers. It is likely that these ciliates play a symbiotic role in both black and white rhinoceroses similar to the one they play in ruminants. The interplay of all these factors influences the type and numbers of ciliates in an individual host, which in turn regulates the nutrient availability to the animal. This relationship between nutrient composition of diet, ciliate diversity and nutritional benefits to the host has been used as an index to assess the nutritional state of ruminants (Hungate, 1978). Because of the occurrence of rumenal ciliates in the hindgut fermentative chamber of the rhinoceros, such an index can be used to guide the formulation of feed mixtures for rhinoceros in captivity and remote nutritional assessments of rhinoceros both in free-range and in captivity.

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