FIELD NOTE

Composition of intestinal ciliate fauna of free-ranging African elephants in Tsavo West National Park, Kenya

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Abstract

This study aimed at surveying intestinal protozoan ciliates of the African elephant (*Loxodonta africana*) in Tsavo West National Park, Kenya. Faecal samples of seven elephants chemically immobilized for translocation were collected opportunistically and flotation-sedimentation techniques used to assess the presence of ciliates. Identification of ciliates was based on morphological features such as shape, presence and morphology of external spines and lobes, micro- and macronuclei, and internal skeletal plates. Seven ciliate families and 27 genera were found in the faecal samples of the elephants. The families included Blepharocorythidae, Buetschliidae, Cycloposthiidae, Isotrichidae, Ophryoscoleciidae, Paraisotrichidae and Troglodytellidae. The dominant families were Buetschliidae with nine ciliate genera and Cycloposthiidae with eight. These ciliates in the caecum and colon of elephants are similar to those in the rumen and reticulum of ruminants. They digest plant fibres (principally cellulose and hemicellulose) that otherwise could not be used since elephants have no fibre-digesting enzymes of their own. This preliminary information will guide comparative studies on the rumen ciliate diversity and population of various hosts in different regions.

Résumé

Cette étude voulait analyser les protozoaires ciliés présents dans l'intestin des éléphants africains (Loxodonta africana) du Parc national de Tsavo-ouest, au Kenya. On a profité de l'immobilisation chimique de sept éléphants qui devaient être déplacés pour récolter des échantillons de crottes et l'on a utilisé des techniques de flottaison-sédimentation pour évaluer la présence de ciliés. L'identification des ciliés s'est faite sur la base de caractéristiques morphologiques telles que la forme, la présence et la morphologie de crêtes ou de lobes externes, de micro- et macronucléus, et de plaques squelettiques internes. Sept familles de ciliés et 27 genres furent découverts dans les échantillons fécaux des éléphants. Les familles incluent les Blepharocorythidae, les Buetschliidae, les Cycloposthiidae, les Isotrichidae, les Ophryoscoleciidae, les Paraisotrichidae et les Troglodytellidae. Les familles dominantes étaient les Buetschliidae, avec neuf genres de ciliés et les Cycloposthiidae, avec huit. Ces ciliés présents dans le caecum et le colon des éléphants sont semblables à ceux que l'on trouve dans le rumen et l'ergastoplasme des ruminants. Ils digèrent les fibres végétales (principalement la cellulose et l'hémicellulose) qui, sans cela, ne pourraient pas être utilisées puisque les éléphants ne produisent pas d'enzymes leur permettant de digérer les fibres par eux-mêmes. Cette information préliminaire pourra servir de guide pour des études comparatives de la diversité et les populations des ciliés du rumen de divers hôtes dans différentes régions.

Introduction

Ciliates are the most abundant protozoa in the stomach contents of both wild and domestic ruminants and camelids (Gocmen et al. 2001). However, they are also found in non-ruminant herbivores like the elephant, where they are involved in host metabolism and digestion of plant material (Regensbogenova et al. 2004). Ciliate protozoa are classified on the basis of the micro- and macronucleus and the presence and morphology of exterior spines and lobes or internal skeletal plates as well as the shape and size of cells (Williams and Coleman 1992). The ciliate composition is determined by phylogenetic factors, geographical distribution areas, type and amount of feed consumed (Dehority 1978). Feed-related stress, such as starvation and physiological condition of the host, also influence the fauna (Ogimoto and Imai 1981; Williams and Coleman 1992). Although some investigations have been conducted in various geographical areas on the ciliate population occurring in ruminants, knowledge about the overall distribution of protozoa in different animal host in different countries around the world is limited (Gocmen et al. 2001). Due to the nutritional importance of ciliates in elephants, this study sought to investigate the composition of intestinal microfauna of elephants in Tsavo West National Park during a translocation exercise. Since the composition of ciliates is determined by geographical area, this work will provide preliminary data that can be used in pretranslocation assessment and nutritional optimization in captive elephants. The protozoa ciliates were identified to the genus level.

Materials and methods

Study area

Tsavo West National Park in Kenya (2°42´S, 38°10´E) covers 7065 km^2 stretching from Mtito Andei along the Nairobi–Mombasa highway to the Tanzanian border. The park's habitats include open plains alternating with savanna bush and semi-desert scrub, acacia woodlands, belts of riverine vegetation and palm thickets.

Sample processing

Faecal samples of seven elephants from five different families, chemically immobilized for translocation, were collected opportunistically. Faecal samples were collected from the rectum and placed in airtight plastic jars. Simple test tube flotation and sedimentation techniques were used to assess the presence of the ciliates. In simple test tube flotation, 3 g of faecal sample was mixed with 50 ml of flotation fluid (saturated solution of sodium chloride) in container 1. The resulting faecal suspension was filtered through a tea strainer into container 2. The faecal suspension from container 2 was then poured into a test tube. The test tube was placed in a rack and gently topped with faecal suspension until it left a convex meniscus at the top of the tube. A clean cover slip was gently placed on top of the test tube and the tube let stand for 20 minutes. The cover slip was then lifted gently together with the drop of fluid adhering to it, and immediately placed on a microscope slide. A drop of Lugol's iodine was applied at the edge of the cover slip and then observed under a light microscope at x200 and x400 magnification. This process was repeated for the same sample, but stained with acidified methylene blue.

In the sedimentation technique 5 g of faeces was mixed in a beaker with 200 ml of water. The mixture was poured through a tea strainer and the debris in the strainer discarded. It was left to stand for 10 minutes and then 70% of the filtrate poured off and the beaker refilled with the same volume of water. This process was repeated 3–5 times, until the supernatant was clear. Most of the final supernatant was carefully decanted off and the sediment in the beaker poured into a Petri dish. Using a Pasteur pipette, a drop of the sediment was placed on a microslide and a drop of stain added. A coverslip was placed on top and observation made under light microscope at x200 and x400 magnification.

Ciliate species were identified through cellular morphological features using acidified methylene blue staining nucleus, cytoplasmic granules and cytoplasmic processes, with Lugol's iodine used to stain skeletal plates (Dehority 1993).

Results

All the elephants sampled were positive for intestinal microfauna. Seven ciliate families and 27 genera were identified in the faecal samples of the elephants. The families were Blepharocorythidae, Buetschliidae, Cycloposthiidae, Isotrichidae, Ophryoscoleciidae, Paraisotrichidae and Troglodytellidae. The dominant families were Buetschliidae with nine ciliate genera and Cycloposthiidae with eight. The distribution of ciliate families and genera are shown in table 1.

Discussion

This is the first documentation of ciliate microfauna of African elephants in the Tsavo West ecosystem. The microbial ecosystem is well studied for the rumen of domesticated animals like cattle, sheep and goats, but it is poorly studied in wild ruminants, and scarcely in hind-gut fermenters like the elephant. According to Mohr et al. (1982) African elephants appear to have a more complex ciliate assemblage than other cellulose-eating mammals so far studied and this concurs with the finding of seven families and 27 genera of ciliates in Tsavo West elephants. Although a large number of protozoal species have been found to exist in different animals and under different conditions, the number of species in a specific animal is generally limited to 35 or fewer (Dehority and Orpin 1997). The high amount of ciliate diversity in Tsavo West elephants may be attributed to the elephnats' free-ranging state since gut microfauna have been reported to be less varied in captive elephants than in wild elephants (Smith et al. 1982).

Table 1. Distribution of ciliate families and genera found in Tsavo West elephants

Family	Genus
Blepharocorythidae	*Blepharocorys*
Buetschliidae	*Alloiozona* *Ampullacula* *Blepharoconus* *Blepharoprosthium* *Buetschlia* *Cucurbella* *Didesmis* *Polymorphella* *Prodonopsis*
Cycloposthiidae	*Cycloposthium* *Ditoxum* *Prototapirella* *Rhabdothorax* *Tetratoxum* *Triadinium* *Tripalmaria* *Triplumaria*
Isotrichidae	*Dastricha* *Isotricha*
Ophryoscoleciidae	*Entodinium* *Epidinium* *Eudiplodinium* *Ophryoscolex* *Ostracodinium*
Paraisotrichidae	*Paraisotricha*
Troglodytellidae	*Troglodytella*

The order Entodiniomorphida consists of endo-symbiotic ciliates inhabiting the fermentative diges-tive organs of most mammalian herbivores (Williams and Coleman 1992). The order has three suborders, Archistomatia, Blepharocorythina and Entodini-omorphina (Lynne and Small 1997). The blepharo-corythines are monofamilial (Blepharocory-thidae) and possess a complicated oral apparatus consisting of a conical vestibulum, a dorsal overture, an external adoral ciliary band, and a triangular vesti-bulary cili-ary band (Wolska 1971). The Archistomatia include only a single family, the Buetschliidae, which are characterized by a simple vestibulum, a holo-trichous covering of longitudinal somatic kineties and fully developed concretement vacuoles (Wolska 1964). The Entodiniomorphina are the most diverse group with nine families. Some of the entodinio-morphids found in Tsavo West elephants are Ble-pharo-corythidae, Cycloposthiidae, Ophryoscoleciidae and Troglodytellidae. They are characterized by reduced somatic ciliation, forming tufts or bands, a semi-rigid pellicle covering extensive non-ciliated areas, and an adoral band of cilia around the cytostome (Cameron et al. 2003). The families Buetschliidae, Isotrichidae and Paraisotri-chidae are holotrichs that have typical uniform and simple body ciliation.

The presence of both holotrichs and entodi-nio-morphs in Tsavo West elephant gut may be due to the elephant diet, which most probably requires a wide enzymatic profile. The enzymes responsible for cel-lulose and hemicellulose degradation have been re-ported in the holotrich protozoa but the levels are very low compared with those present in entodiniomorphid protozoa (Williams and Coleman 1985).

Intestinal ciliates vary with the host species and from different geographic areas because trans-fau-nation of ciliates has been assumed to occur only by direct contact between hosts (Ito et al. 1994). Hence, some of these ciliates have been reported in different animals and different geographic areas. The genus *Paraisotricha* had been reported in Kenyan elephants (Mohr et al. 1982), while *Prototapirella* and *Troglodytella* species have been reported in free-ranging lowland gorillas (*Gorilla gorilla gorilla*), in Central African Republic (Freeman et al. 2004). The genus *Troglodytella* has always been associated with gorilla and chimpanzee (*Pan troglodytes*) (Swezey 1934). *Cycloposthium, Prototapirella, Tripalmaria* and *Triplumaria* were the genera found in Tsavo West ele- phants. The genus *Triplumaria* had been reported to include 11 new ciliate species found in the Asian elephant (*Elephas maximus*) and African elephant (Timoshenko and Imai 1995).

The dominant families observed in these elephants were Buetschliidae and Cycloposthiidae, which are generally considered to be endosymbionts of equids (Williams and Coleman 1992). However, the family Isotrichidae, consisting of *Dastricha* and *Isotricha,* is found to occur more widely in domesticated than in wild ruminants (Clark 1977).

Various interacting factors have been found to determine the generic composition and overall size of the gut ciliate population. The more important include the geographical location, type of host, the diet consumed and protozoal interspecies antagonisms (Williams 1986). The variety of ciliates is larger in grazing than in browsing animals in Europe (Giesecke 1970), but there is a greater variety of ciliate species in browsing animals in Africa (Van Hoven 1983). This implies that a mixed feeder host such as an elephant, and especially the African elephant, will have innumerable assemblage as seen in Tsavo West elephants. The geographical location seems advantageous for African elephants due to great feed diversity, and it influences the holotrich ciliates. A study conducted in Kenya to determine if protozoal fauna of indigenous African wild ruminants' diet influenced the numbers and types of protozoa established that the percentage of the genus *Entodinium* was higher in concentrate selectors than in roughage eaters (Dehority and Odenyo 2003). This concurs with the low presence of *Entodinium* in Tsavo West elephants since they are bulk roughage eaters.

The finding from this study is baseline information that can be a useful index to the nutritional state of the elephants. Presence or absence of ciliate protozoa can be used to remotely confirm or negate a nutritional disturbance (Hungate 1978). In addition, comparative studies of the rumen ciliate population of various hosts in different regions will provide information on phylogenetic relations between the ciliates and the hosts (Imai 1988).

Acknowledgement

We would like to thank the Director, Kenya Wildlife Service, the capture team, and the KWS Elephant Programme for supporting this work.

References

Cameron SL, Wright A-D, O'Donoghue JP. 2003. An expanded phylogeny of the Entodiniomorphida (Ciliophora: Litostomatea). *Acta Protozoologica* 42:1–6.

Clark RTJ. 1977. Protozoa in the rumen ecosystem. In: Clark RTJ, Bauchop T, eds., *Microbial biology of the gut*. Academic Press, New York. p. 251–275.

Dehority BA. 1978. Specificity of rumen ciliate protozoa in cattle and sheep. *Journal of Protozoology* 25:509–513.

Dehority BA. 1993. *Laboratory manual for classification and morphology of rumen ciliate protozoa*. CRC Press. Boca Raton, Florida. 120 p.

Dehority BA, Odenyo AA. 2003. Influence of diet on the rumen protozoal fauna of indigenous African wild ruminants. *Journal of Eukaryotic Microbiology* 50(3): 220–223.

Dehority BA, Orpin CG. 1997. Development of, and natural fluctuations in, rumen microbial populations. In: Hobson PH, Stewart CS, eds., *The rumen microbial ecosystem*, 2nd ed. Chapman and Hall, London. p. 196–245.

Freeman AS, Kinsella JM, Cipolleta C, Deem SL, Karesh W. 2004. Endoparasites of western lowland gorillas (*Gorilla gorilla gorilla*) at Bai Hakou, Central African Republic. *Journal of Wildlife Diseases* 40(4):775–781.

Giesecke D. 1970. Comparative microbiology of the alimentary tract. In: Philipson AT, Annison EF, Armstrong DG, Balch CC, Comline RS, Hardy RN, Hobson PN, Keynes RD, eds., *Physiology of digestion and metabolism in the ruminant*. Oriel Press. Newcastle-upon-Tyne, England. p. 306–318.

Gocmen B, Dehority BA, Talu GH, Rastgeldy S. 2001. The rumen ciliate fauna of domestic sheep (*Ovis ammon aires*) from the Turkish Republic of Northern Cyprus. *Journal of Eukaryotic Microbiology* 48(4):455–459.

Hungate RE. 1978. The tureen protozoa. In: Krier JP, ed., *Parasitic protozoa vol 2*. Academic Press, New York. p. 655–695.

Imai S. 1988. Ciliate protozoa in the rumen of Kenyan zebu cattle (*Bos taurus indicus*) with the description of four new species. *Journal of Protozoology* 35:130–136.

Ito A, Imai S, Ogimoto K. 1994. Rumen ciliate composition and diversity of Japanese beef black cattle in comparison with those of Holstein-Friesian cattle. *Journal of Veterinary Medical Science* 56:707–714.

Lynne DH, Small E. 1997. A revised classification of the phylum Ciliophora (Dolfein, 1901). *Revista de la Sociedad Mexicana Historia Natural* 47:65–78.

Ogimoto K, Imai S. 1981. *Atlas of rumen microbiology.* Japan Scientific Societies Press, Tokyo. 231 p.

Mohr JE, Jollie KG, Smith TP. 1982. Ciliates of elephant caecums. *Journal of Protozoology* 29:482.

Regensbogenova M, Kisidayova S, Michalowski T, Javorsky P, Moon-van der Staay SY, Moon-van der Staay GWM, Hackstein JHP, McEwan NR, Jouany J-P, Newbold JC, Pristas P. 2004. Rapid identification of rumen protozoa by restriction analysis of amplified 18SrRNA gene. *Acta Protozoologica* 43:219–224.

Smith TP, Jollie KG, Mohr JL. 1982. Gut protozoans of zoo elephants. *Journal of Protozoology* 29:482.

Swezey WW. 1934. Cytology of *Troglodytella abrassarti*, an intestinal ciliate of the chimpanzee. *Journal of Morphology* 56:621–630.

Timoshenko O, Imai S. 1995. Eleven new ciliate species of the genus *Triplumaria* (Ciliophora, Entodiniomorphida) from Asian elephant (*Elephas maximus*) and African elephant (*Loxodonta africana*). *Journal of Protozoology Research* 5(4):157–175.

Van Hoven W. 1983. Rumen ciliates with description of two new ciliates from three African reedbuck species. *Journal of Protozoology* 30:688–691.

Williams AG. 1986. Rumen holotrich ciliate protozoa. *Microbiological Reviews* 50(1):25–49.

Williams AG, Coleman GS. 1985. Hemicellulose degrading enzymes in rumen ciliate protozoa. *Current Microbiology* 12:85–90.

Williams AG, Coleman GS. 1992. *The rumen protozoa*. Brock/Springer Series in Contemporary Bioscience. Springer-Verlag, New York.

Wolska M. 1964. Infraciliature of *Didesmis ovalis* Fior. and *Blepharozoum trizonum* (Hsiung)—Fam. Buetschliidae (Ciliata, Rhabdophorina). *Acta Protozoologica* 6:153–158.

Wolska M. 1971. Studies on the family Blepharocorythidae, Hsiung VI. Phylogenesis of the family and description of the new genus *Circodinium* gen.n with the species *C. minimum* (Gassovsky, 1918). *Acta Protozoologica* 15:171–194.