Protozoa and digestive tract parameters in Blue wildebeest (*Connochaetes taurinus*) and Black wildebeest (*Connochaetes gnou*), with description of *Entodinium taurinus* n.sp.

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Abstract

Rumen contents from four blue wildebeest (*Connochaetes taurinus*) and six black wildebeest (*Connochaetes gnou*) were collected from two locations during two winter culling seasons. A total of 16 species of protozoa were found in blue wildebeest with *Dasytricha ruminantium, Opisthotrichum janus* and *Ostracodinium gracile* occurring in all four animals. In black wildebeest, 23 species of protozoa were observed with only *Diplodinium bubalidis* f. *bubalidis* and *Ostracodinium damaliscus* being present in all animals. In the blue wildebeest, the total number of species in an individual animal varied from 9 – 11 and in the black wildebeest, the number ranged from 4 – 14 species per animal. Concentrations of cells per ml ranged from 1110 to 5880 in the blue wildebeest and 3120 to 6240 in the black wildebeest. This study is the first report on protozoa species in the blue and black wildebeest. A new species of *Entodinium* is described, *Entodinium taurinus* n. sp., observed in the rumen contents of three blue wildebeest. Several physical parameters of the

digestive tract were also measured, including distribution, pH, temperature and density of gastrointestinal contents in different sections of the total tract. In vitro gas production was estimated for rumen, cecum and colon contents.

Keywords: Blue wildebeest, Black wildebeest, *Connochaetes gnou, Connochaetes taurinus*, *Entodinium taurinus* n. sp., protozoa

Introduction

There are two species of wildebeest in Africa, The Blue wildebeest (*Connochaetes taurinus*), sometimes known as the common wildebeest, and the Black wildebeest (*Connochaetes gnou*) or White-tailed gnu. Both species have been described by Hofmann and Stewart (1972) as fresh grass grazers who require a source of water daily. Because of their blunt mussels both species are able to graze short green grass (Estes 1991). However, according to observations by game rangers, the black wildebeest differs slightly from the blue wildebeest in that they tend to prefer grazing on the taller forages. Dehority and Odenyo (2003) previously reported protozoa concentrations and generic distribution in two blue wildebeest from Kenya. However, no previous reports could be found on the species composition of rumen protozoa in either species of wildebeest.

Material and methods

Animals

Two blue wildebeest (*Connochaetes taurinus*) were harvested from the Ruimte Game Reserve 150 Km north of Pretoria (24°23' 20.47"S, 29°01' 43.57"E). Two additional Blue wildebeest and 6 Black wildebeest (*Connochaetes gnou*) were collected from the Rietvlei Game Reserve 50Km south of Pretoria (25°52' 41.17"S, 28°16' 17.83"E). All animals were

adult males, harvested during a controlled winter culling. After killing, the animals were weighed and their digestive tracts removed and separated into different anatomical sections by ligation (rumen, omasum, abomasum, small intestine, cecum, colon, spiral colon and transverse colon and rectum).

Measurements

Weight of organ contents was determined by weighing the organ full and empty (to the nearest 0.1g), using a Mettler PJ4000 electronic balance; pH of the contents was measured with a portable, battery-powered pH meter (Eutech, model EC-PH-10/01N); temperature of the contents was measured by inserting an electronic thermometer into the middle of the organ contents,

Fermentation

Separate 200 ml samples of ingesta were obtained from the rumen, cecum and colon. Each sample was placed in a 250ml glass bottle fitted with a rubber stopper. A 20 gage stainless steel needle, to fit a 10ml glass syringe, was inserted through the stopper. The bottles were incubated in water-bath at 39ºC and gas production was measured every minute for 45 minutes by displacement of the syringe plunger. The weight of each sample was estimated after the fermentation.

Protozoa

Handfuls of the rumen or cecum contents were taken at random and the fluid squeezed out by making a fist with the thumb pointed downward so the fluid could run down into a container. This was repeated until a 200ml sample was obtained from each organ. Due to the fine particle size of colon contents, a 40ml container was used to randomly scoop up samples until the 200ml volume was reached. One hundred ml of 70% ethanol was added to each of the 200 ml samples for preservation.

In the laboratory, a 10 ml subsample was taken from each of the protozoa samples with a 1ml pipette, 1 ml at a time until 10 ml was acquired. Two drops of Brilliant Green Stain were added and the sample was allowed to stand overnight. Using a finnpipet, a 0.25ml aliquot of the 10ml stained sample was dropped onto a microscope glass slide, and fitted with a cover slip. Counts and species identifications were made using a standard light microscope. Results of the counts were multiplied by 4 to give the counts per ml of the preserved sample and then by 1.5 to give counts per ml of original fluid.

Results

Animals

Body weights for the four blue wildebeest varied from 143 to 360 Kg, averaging 262±90 Kg. The range of body weights in the six black wildebeest was quite narrow, 241– 261 Kg, with an average weight of 252±10 Kg.

Physical and Physiological Parameters

The average weight of gastrointestinal contents in four blue wildebeest and four black wildebeest is given in Table 1, along with the percentage distribution in each of the eight sections of the tract, i.e., from the rumen to the rectum. Most of the contents were found in the rumen, 76–84% in the blue and black wildebeest, respectively. The next highest percentage was in the small intestine; however, values were less than 10%. In general, pH varied from about 6.0 in the rumen and omasum, falling to approximately 4.0 in the abomasum and increasing back to around neutrality (7.0) down the rest of the tract (Table 1). Temperature of the contents was also measured, and was found to show an overall gradual decrease from the rumen to the rectum.

Density of the contents was measured in all organs except the omasum and abomasum, and ranged from 0.57-0.96, with one exception, 1.14 in the small intestinal contents from the

Table 1. Physical and physiological parameters of the gastrointestinal tract of the blue and black wildebeest

	Total weight (g)	Rumen	Omasum	Abomasum	Small intestine	Cecum	Colon	Spiral colon	Transverse colon and rectum
		Percent							
Gastrointestinal contents									
Blue wildebeest	36464±15537	75.8±7.6	3.4±1.7	2.9±1.0	8.6±3.3	3.4±1.3	1.5±1.0	0.8±0.2	3.6±2.3
Black wildebeese[a]	37109±18004	84.2±5.5	1.4±0.9	1.7±0.7	6.8±4.0	1.5±0.4	1.5±0.9	0.8±0.5	2.2±0.9
		pH							
pH									
Blue wildebeest		6.10±0.24	6.00±0.42	3.52±0.42	7.16±0.30	7.12±0.12	7.29±0.44	7.04±0.31	7.01±0.12
Black wildebeest[a]		6.02±0.67	6.35±0.57	4.10±0.84	7.05±0.74	6.74±0.39	6.78±0.32	7.12±0.28	6.86±0.52
		Degrees C							
Contents temperature									
Blue wildebeest		37.0±1.4	32.1±3.3	31.4±2.8	25.7±1.3	28.7±3.2	29.0±3.0	25.9±2.1	22.4±2.2
Black wildebeest[a]		35.4±6.1	32.7±4.1	27.6±1.6	24.5±4.7	25.2±3.5	24.6±2.6	22.9±2.8	23.8±1.5
		g/ml							
Density									
Blue wildebeest		0.70±0.17	-	-	0.83±0.37	0.96±0.41	0.67±0.24	0.59±0.18	0.77±0.16
Black wildebeest[b]		0.63±0.17	-	-	1.14±0.22	0.85±0.20	0.72±0.14	0.57±0.09	0.92±0.36
		ml gas/gm of wet ingesta							
Gas production									
Blue wildebeest		0.15±0.02	-	-	-	0.11±0.03	0.13±0.02	-	-
Black wildebeest[b]		0.18±0.05	-	-	-	0.09±0.04	0.11±0.05	-	-

[a]Based on only 4 animals (SWB1, SWB4, SWB 5 and SWB 6), data for omasal contents was not recorded.

[b]Based on data from all 6 animals.

black wildebeest (Table 1). Gas production, measured with ingesta from the rumen, cecum and colon, was slightly greater with the rumen contents, but relatively low in all three sites.

No statistical differences were found between the species for any of the above parameters.

Protozoa

Total protozoa concentrations in rumen fluid ranged from 1110 to 5880 per ml in blue wildebeest and from 3120 to 6240 per ml in black wildebeest (Table 2). No ciliate protozoa were found in either cecum or colon fluid.

Sixteen different species of protozoa were identified in the rumen of blue wildebeest, with *Dasytricha ruminantium, Ostracodinium gracile* and *Opisthotrichum janus* occurring in all 4 animals. Twenty species were found in black wildebeest, with only *Diplodinium bubalidis* f. *bubalidis* and *Ostracodinium damaliscus* common to all 6 animals (Table 2). *Entodinium nanellum*, *E. parvum*, *E. simplex*, *Diplodinium bubalidis* f. *aspinosum, Eudiplodinium kenyensis, Ostracodinium tenue, O. trivesiculatum,* and *Enoploplastron triloricatum* and were found only in black wildebeest while *Entodinium longinucleatum*, *E. taurinus* n. sp., *Metadinium affine* and *Epiplastron africanum* were found only in blue wildebeest. The number of species occurring in an individual animal varied from 9-11in blue wildebeest and 4-14 in black wildebeest.

A new species of *Entodinium* was observed in the rumen fluid of three blue wildebeest, and a description of this new species is given below. All dimensions are given in μm (± standard deviation) and are based on measurements from 20 specimens.

***Entodinium taurinus* n. sp. (**Figs. 1 and 2)

Description: Body slightly truncated in side view and teardrop shaped when viewed from the dorsal side; a long posterior spine on the ventral side and a short blunt to pointed lobe on the posterior dorsal side; body length 39 ± 5 (35-44); body width 21 ± 2 (19-24); posterior spine 21 ± 4 (18-25); L/W 1.85 ± 0.29 (1.32-2.25); macronucleus 15 ± 2 (13-17); macronucleus

Table 2. Concentration and percent distribution of protozoa species in the rumen of the blue and black wildebeest.

	Blue Wildebeest				Black wildebeest					
	Ruimte		Rietvlei		Rietvlei					
Subfamily	July				July			August		Sept.
Genus	2006		2007		2006					
Species	BWB1	BWB2	BWBR1	BWBR2	SWB1	SWB2	SWB3	SWB4	SWB5	SWB6
Total protozoa per ml x 10^3	1.11	4.02	4.80	5.88	3.66	6.24	3.12	4.35	3.77	3.81
	----------%----------									
Dasytricha ruminantium	16.2	29.8	13.8	12.2	4.9	-	-	-	-	-
Entodiniinae	10.8	17.9	18.8	22.4	21.2	18.3	0	9.0	13.6	7.8
Entodinium										
E. caudatum	5.4	-	-	-	1.6	7.7	-	-	-	3.1
E. dubardi	5.4	10.4	-	1.0	9.8	2.9	-	9.0	8.0	4.7
E. exiguum	-	1.5	-	-	3.3	-	-	-	-	-
E. longinucleatum	-	-	-	6.1	-	-	-	-	-	-
E. nanellum	-	-	-	-	-	1.0	-	-	-	-
E. parvum	-	-	-	-	3.3	6.7	-	-	-	-
E. simplex	-	-	-	-	1.6	-	-	-	-	-
E. taurinus n. sp.	-	6.0	18.8	15.3	-	-	-	-	-	-
E species (unknown)[a]	-	-	-	-	1.6	-	-	-	-	5.6
Diplodiniinae	37.8	29.9	63.7	58.1	73.8	81.7	98.0	83.6	78.5	58.3
Diplodinium										
D. bubalidis f. *aspinosum*	-	-	-	-	6.6	3.4	-	-	-	3.1
D. bubalidis f. *bubalidis*	-	17.9	13.8	19.4	9.8	28.8	59.6	37.2	42.2	27.6
Eudiplodinium										
E. gigantium	-	-	1.2	-	3.3	-	-	2.8	-	1.6
E. kenyensis	-	-	-	-	3.3	-	-	-	-	-

Ostracodinium										
O.damaliscus	-	3.0	-	4.1	23.0	16.8	19.2	11.0	25.1	10.2
O. gladiator	10.8	-	7.5	1.0	3.3	1.9	-	5.5	1.6	7.9
O. gracile	10.8	6.0	13.8	19.4	21.3	26.9	19.2	27.6	6.4	-
O. nanum	5.4	-	1.2	1.0	4.9	-	-	-	-	1.6
O. tenue	-	-	-	-	-	-	-	-	-	3.1
O. trivesiculatum	-	-	-	-	-	1.0	-	-	-	-
Metadinium affine	5.4	-	12.5	-	-	-	-	-	-	-
Enoploplastron	-									
E. garstangi	-	3.0	11.2	2.0	1.6	-	-	-	3.2	1.6
E. triloricatum	-	-	-	-	-	-	-	-	-	1.6
Diplodiniinae species (unknown)[a]	5.4	-	2.5	11.2	-	2.9	-	-	-	-
Ophryoscolecinae	35.1	22.4	3.8	7.1	0	0	1.9	6.9	8.0	33.8
Epiplastron africanum	10.8	-	-	-	-	-	-	-	-	-
Opisthotrichum janus	24.3	22.4	3.8	7.1	-	-	1.9	6.9	8.0	33.8
Total number of species[b]	9	9	10	11	14	10	4	7	7	12

[a]Distorted cells could not be identified below the Family or genus level.
[b]Unknown *Entodinium* or Diplodiniinae species not included in total number of species.

located dorsally in the middle to upper portion of cell; micronucleus lies near the middle of the macronucleus on the ventral side; contractile vacuole lies at the anterior end and slightly displaced toward the right side of the cell; esophagus bends toward the macronucleus; cytoproct terminates between the posterior lobe and spine.

Type host: *Connochaetes taurinus,* Blue wildebeest, Republic of South Africa.

Habitat. Rumen

Occurrence: *E. taurinus* n. sp. constituted 6.0, 15.3 and 18.8 % of the total ciliates in three of the four blue wildebeest sampled.

Etymology: *Entodinium taurinus* is named after the host in which it was found.

Remarks: *E. taurinus* is distinguished from previously described species by its long and thin ventral posterior spine. Fig. 1 is a line drawing of *E. taurinus* as viewed from the right side. Fig. 2 presents several photographs focused to show the distinguishing features of this species

Discussion

In general, values for the different parameters listed in Table 1 are fairly similar to those previously reported for the impala in South Africa (Booyse and Dehority, 2011). The relatively low gas production, particularly from rumen contents, is probably a reflection of the procedure. Depending on the time of sampling, the extent of fermentation is undoubtedly controlled by the amount of available substrate. In any further studies, it would be best to add substrate as described by el-Shazly and Hungate (1965), to provide an estimate of microbial concentration.

Rumen protozoa concentrations in this study were considerably lower than reported by Dehority and Odenyo (2003) from two blue wildebeest in Kenya. This difference could be related to season, in that the animals from Kenya were sampled in early spring when forage was readily available as compared to the present animals sampled during the winter.

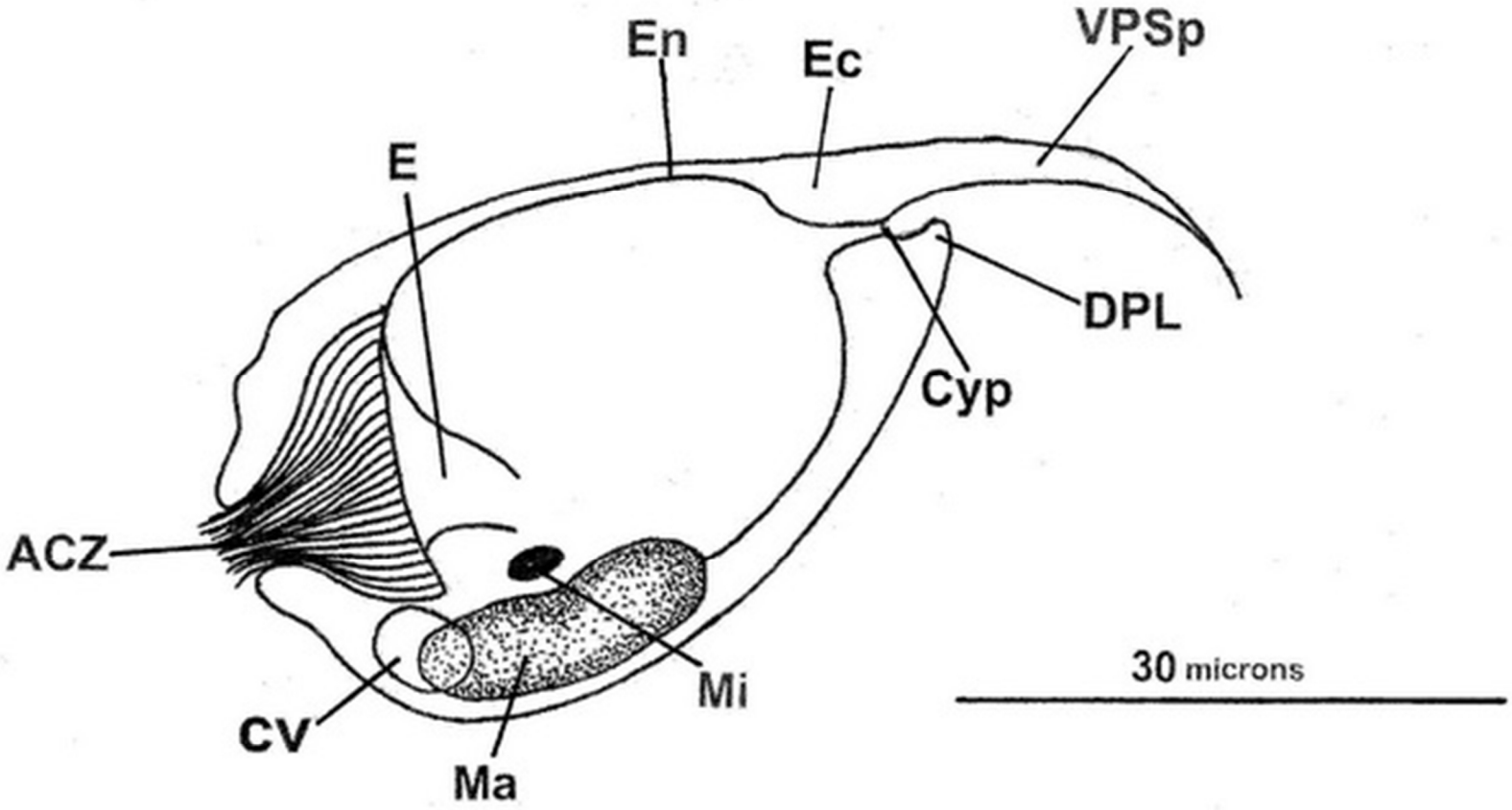

Fig. 1, Line drawing of Entodinium taurinus n. sp., view from the right side. Adoral ciliary zone (ACZ); Contractile vacuole (CV); Macronucleus (Ma); Micronucleus (Mi);Cytoproct (Cyp); Dorsal posterior lobe (DPL) Ventral posterior spine (VPSp); Ectoplasm (Ec); Endoplasmic sac (En); Esophagus (E).

Fig. 2.Photographs of Entodinium taurinus n. sp. A.View from the left side showing macro- and micronucleus.
B. Dorsal view, showing both posterior projections and tear-shape of cell.
C. dorsal view, focused on posterior dorsal lobe.
D. View from the right side showing location of the contractile vacuole.

The percentage *Entodinium* in this study, for all 10 animals, ranged from 0 to 22.4, with an average of 14.0±7.1. This compares quite closely with the values 15.3 and 15.7 found by Dehority and Odenyo (2003) in the two blue wildebeest from Kenya. This low percentage of *Entodinium* differs markedly from most domestic ruminants fed on pasture (Dehority, 2003; Dehority and Orpin, 1997). The major portion of the ciliate population was in the family Diplodiniinae, particularly in the genus *Ostracodinium.* A similar pattern was found by Imai (1988) in zebu cattle living in Kenya. He postulated that this distribution was unique to grazing animals ingesting only roughage, since the Diplodiniinae species possess cellulolytic enzymes. Generic distribution in browsing or mixed feeding wild ruminants is generally very high in *Entodinium*, with values up to 100% in some animals (Dehority, 1995; Dehority and Odenyo, 2003; Dehority, Demaris and Osborne, 1999; Wilkinson and Van Hoven, 1976; Imai and Rung, 1990). Presumably the *Entodinium* species, with a shorter generation time, multiply rapidly utilizing starch and other readily available substrates (Dehority, 1998; 2004).

Although the study by Dehority and Odenyo (2003) only identified protozoa to the generic level, all genera they reported were found in this study. In addition, species in two additional genera were observed, *Metadinium affine* and *Epiplastron africanum.*

Of particular interest was the much higher percentage of Diplodiniinae species in the black wildebeest and two blue wildebeest harvested at Rietvleil, 74.5±13.9%, compared to 33.8±5.6% in the two blue wildebeest harvested at Ruimte ($P < 0.01$). In the animals at Rietvlei, most of the Diplodininiinae were species of *Ostracodinium.* Percentages of *Ostracodinium* species were 9 and 27 in the two wildebeest at Ruimte and ranged from 22.5 to 52.5 in those animals from Rietviel. Mean values were 18.0±12.7% and 35.7±11.5% in the wildebeest from Ruimte and Rietvlei respectively. The major difference in the blue wildebeest from .the two sites was in the Ophryscolecinae, 35.1 and 22.4 % from Ruimte and 3.8 and 7.1% from Rietvlei.

Other than the occurrence of *Dasytricha* and *Entodinium taurinus* n. sp. There appeared to be little difference between the two species of wildebeest. Differences were more closely associated with location,

References:

1. Booyse, D. G., Dehority, B. A., 2011. Protozoa and digestive tract parameters of the impala. Onderstepoort Journal of Veterinary Research 78(1), Art. #327, 5 pages. doi:10.4102/ojvr.v78i1.3278, 1
2. Dehority, B. A., 1995. Rumen ciliates of the pronghorn antelope (*Antilocapra americana*), mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*) and elk (*Cervus canadensis*) in the northwestern United States. Archiv Fur Protistenkunde 146, 29-36.
3. Dehority, B. A., 1998. Generation times of *Epidinium caudatum* and *Entodinium caudatum*, determined *in vitro* by transferring at various time intervals. J. Anim. Sci. 76, 1189-1196.
4. Dehority, B. A., 2004. In vitro determination of generation times for *Entodinium exiguum*, *Ophryoscolex purkynjei* and *Eudiplodinium maggii*. J. Eukaryot. Microbiol. 51, 333-338. .
5. Dehority, B. A., 2003. Rumen Microbiology. Nottingham University Press. Nottingham, UK.
6. Dehority, B. A., Odenyo, A. A., 2003. Influence of diet on the rumen protozoal fauna of indigenous African wild ruminants. J. Eukaryot. Microbiol. 50, 220-223.
7. Dehority, B. A., Demarais, S., Osborn, D. A., 1999. Rumen ciliate protozoa of white-tailed deer (*Odocoileus virginianus*), axis deer (*Axis axis*), sika deer (*Cervus nippon*) and fallow deer (*Dama dama)* from Texas. J. Eukayrot. Microbiol. 46,125-131.

8. Dehority, B. A., Orpin, C. G., 1997. Development of, and natural fluctuations in rumen microbial populations. In: Hobson, P. N. and Stewart, C. S.(Eds.), The Rumen Microbial Ecosystems, Second edition, Blackie Academic and Professional, London, pp. 6-245.

9. el-Shazly, K., Hungate, R. E., 1965. Fermentation capacity as a measure of net growth of rumen microorganisms. Appl. Microbiol. 13, 62-69.

10. Estes, R., 1991. The Behaviour Guide to African Mammals, Including Hoofed Mammals, Carnivores, Primates. The University of California Press. Los Angeles, pp. 150-156

11. Hofmann, R. R., Stewart, D. R. M., 1972. Grazer or Browser: A classification based on the stomach- structure and feeding habits of East African ruminants. Mammalia, Paris, 36: 226-240.

12. Imai, S., 1988. Ciliate protozoa in the rumen of Kenyan zebu cattle, *Bos Taurus indicus,* with the description of four new species. J. Protozool. 35, 130-136.

13. Imai, S., Rung,G., 1990. Rumen ciliates from the Mongolian gazelle, *Procapra gutturosa.* Jpn. J. Vet. Sci. 52, 1063-1067.

14. Wilkinson, R. C.,Van Hoven, W., 1976.Geographical distribution and population structure of springbok *Antidorcas marsupialis* rumen protozoa in southern Africa. Koedoe 19, 17-26.