Protozoa in the digestive tract of wild herbivores in South Africa. I: Warthogs (*Phacochoerus aethiopicus*)

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Abstract

Seventeen warthogs were harvested during the winter hunting seasons of May to July 2001 and May to June 2002. All animals were taken from their natural habitat. Samples (200 ml each) were collected and weighed from the stomach, cecum and colon of each animal for protozoa counts. *Telamodinium onyx* was the only species present in 7 animals and the predominant species in all others. *Megadinium aethiopicum* was observed in 8 animals and *Teratodinium sphaeredon* was present in at least 2 of the warthogs. Several different species of protozoa were seen in a few animals, two of which are considered normal to the rumen.

Keywords: Warthog, *Phacochoerus aethiopicus*, protozoa, grazer, hind-gut fermentation.

Introduction

The warthog (*Phacochoerus aeththiopicus*) is a grazer and a hindgut fermentor. Only two reports could be found in the literature where the gastrointestinal protozoa of warthogs were mentioned. The first was that of Latteur and Dufey (1967) who established a new sub-family, Telamodiniidae in the family Spirodinidae. In the subfamily they established three new genera with a single species in each genus (*Telamodinium onyx, Tetratodinium sphaeredon* and *Megadinium aethiopicum*). The second report was by Grain (1990), who only mentioned that ciliate protozoa in the subfamily Telamodiniidae occurred in warthogs. To the best of our knowledge, no other in depth studies have been reported on the gastrointestinal protozoa of warthogs, especially over an extended collection period.

Materials and methods

Samples were collected during the winter hunting seasons of 2001 and 2002 near the town of Ellisras in the north-western part of the Limpopo province (Former Northern Transvaal) in South Africa. The farm is about 300 Km north of Pretoria. Seventeen animals were sampled: four adult males (AD, M) (warthogs no. 3, 5, 8, 11), 9 adult females (AD, F) (warthogs no. 1, 2, 6, 9, 12, 13 14 and 17), 2 juvenile females (JU, F) (warthogs no. 4 and 13) and 2 Juvenile males (JU, M) (warthogs no. 7 and 16).

Samples were obtained from the animals within 40 min after death. The whole intestinal tract was removed and the different sections were separated. Only contents of the stomach, cecum, and colon were saved. Weight of the contents of each organ were measured with an electronic scale capable of measuring up to 2 decimal points. A 200ml sample of digesta was taken from

each organ and weighed to establish its density. For transporting back to the laboratory, samples were preserved by the addition of 20-50 ml of 70% alcohol.

In the laboratory, the samples were washed through a set of sieves with an inner and outer chamber . The sample is poured into the inner sieve with pore size of 110 μm and washed with water. Particulate matter and protozoa pass through to the outer sieve which has a pore size of 37 μm. The outer sieve has a draining tap to allow the contents to be drained into a bottle. This washed sample is then allowed to stand for 30min and the volume adjusted back to 200ml by decanting the excess water. Two drops of Brilliant Green stain were added to the sample and allowed to stand for 24h (Dehority, 1984). In some cases a Giemsa (Fig 7 and 8) or Methyl blue (Fig 3 and 12) stain was used to study different structures in the cell. Three aliquots of 0.1 ml from each sample were placed on separate microscope slides, fitted with cover slips and using a standard light microscope, cells were examined and counted. A camera was attached to the microscope to record the different protozoa. In those cases where cells were over-stained so that organs were not visible, a drop of lactophenol was added to the slide to partially decolorize the cell. The protozoa were tentatively identified to the species level and their individual concentrations were determined by multiplying the mean of all three counts by 10, thus giving a count per ml.

Results and discussion

Contents from all warthog stomachs were negative for protozoa and no data is presented for these samples.

Table 1 gives animal weight plus the total weight, volume and density of the cecum and colon contents. Body weights for adult males (4) ranged from 75-85 kg; for adult females (9),

from 34-65 kg; for juvenile males (2), 12 and 17 kg; and juvenile females (2), 12.5 and 15 kg. As might be expected**,** the body weight of adult males was greater than that of adult females, both of which were heavier than the juveniles. The range of values for cecum contents were: total weight, 76-638 g; volume, 197-1393 ml; density, 0.29-1.36 g/ml. For colon contents, values ranged as follows: total weight, 414-6408 g; volume, 758-10728 ml; density, 0.51-1.15 g/ml. The weight and volume of colon contents exceeded those values in the cecum, which probably is a reflection of organ size.

The concentration and distribution of protozoa in the cecum and colon are presented in Table 2. In general, tentative identification was based on size, body shape, skeletal plates and shape of the macronucleus. These attributes are visible in the cells shown in Figures 1-12. The species *Telamodinium onyx* (Fig. 1-4) was present in all 17 animals. *Megadinium aethiopicum* (Fig. 5 & 6) occurred in 8 animals and *Teratodinium sphaeredon* (Fig. 9) in only two of the warthogs. Warthog no.6 also contained low numbers of two other species of protozoa. One was tentatively identified as *Blepharaconus krugerensis* (Fig. 10&11), a species first described in the intestinal contents of the elephant and more recently in the rumen contents of Brazilian cattle (Dehority, 1986). The second species appeared to belong to the Family Cycloposthiidae (Latteur & Dufey, 1967). Warthog 17 contained two species normally occurring in the rumen, *Diplodinium dentatum* (Fig. 7 and 8) *and Ophryoscolex purkynjei* (Fig. 12). Although unusual, protozoa considered specific to the rumen have previously been observed in the hindgut of the capybara. Once in the feces of two capybara housed in a zoo in the USA and more recently from capybara in a zoo in Japan (Dehority, 1987; Imai et al., 1997).

In general, sizes of the different species observed in this study all fall within the previously reported ranges, except for the *Ophryoscolex* species, which was approximately half

as large as cells found in the rumen habitat (Dehority, 1986; Dogiel, 1927; Latteur & Dufey, 1967).

Although limited, the presence of protozoa in the Families Cycloposthidae, Buetschliidae and Ophryoscolecidae was somewhat unexpected and raises some interesting questions. Since they have not been reported from any other animal, is the Family Telemodiniidae specific to the warthog hindgut? Would species in Telemodiniinae survive in the rumen? Is the warthog hingut a suitable environment for protozoa from these other three Families? Can the apparent specificity be explained solely on the basis of isolation or separation of animals, i.e., not in close enough physical contact to allow cross inoculation of protozoa? These are questions which could be experimentally studied in the future.

Protozoa concentrations were fairly similar in the cecum and colon and extremely low, only 40 – 240 total cells per ml. As compared to foregut fermenting herbivores, rotozoa concentrations in the rumen of cattle and sheep have been reported to range from 12 to 181 x 10^4 per ml (Dehority, 2003). Slightly lower concentrations have been observed in the camel forestomach, 3 to 109 x 10^4 per ml (Kubesy & Dehority, 2002). Considerably lower concentrations were found in forestomach contents of the kangaroo, 4 to 20 x 10^3 protozoa per ml (unpublished, Dehority).

There are two major types of hindgut fermenting herbivores, cecum fermentors and colon fermentors [Dehority, 2003]. In general, the smaller animals, like the warthog are cecum fermentors while the horse, elephant, etc. are colon fermentors, where the contents of the cecum and colon mix . No reports were found for the concentration of protozoa in the warthog or other cecum fermentors; however, several studies have reported protozoal concentrations in the hindgut (cecum and colon) of the horse. Values ranged from 3.8 to162 x 10^3 (Kern et al., 1973;

Moore & Dehority, 1993). Since concentrations in the hindgut of the warthog were markedly lower, anywhere from 95 to 4000 fold less, it seems highly unlikely that the protozoa play a very important role in fiber digestion. Presumably hindgut digestion in these animals is primarily the result of bacterial action.

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Figure Legends

Fig. 1. *Telamodinium onyx.* Length =90Φ; Width = 37Φ; L/W = 2.4. Note ciliary bands.

Fig. 2. *Telamodinium onyx.* Arrow shows skeletal plate which extentds up to anterior end of cell.

Fig. 3. *Telemodinium onyx.* Arrow shows macronucleus (38Φ long)

Fig. 4. *Telamodinium onyx.* Shows circular opening at anterior end.

Fig. 5. *Megadinium aethiopicum.* Length = 225Φ; Width = 128Φ; L/W = 1.76. Note the club-shaped macronucleus (Length = 120Φ) shown by the arrow.

Fig. 6. *Megadinium aethiopicum.* Length = 225Φ; Width = 133Φ; L/W = 1.69; Macronucleus = 129Φ.Macronucleus shown by arrow. Cilia bands are also visible.

Fig. 7. *Diplodinium dentatum.* Length = 59Φ; Width = 50Φ; L/W = 1.18. Readily distinguished by its wide flange along the dorsal body edge, shown by arrow.

Fig. 8. *Diplodinium dentatum.* Arrows show the short incurved caudal spines typical for this species.

Fig. 9. *Teratodinium sphaeredon.* Length = 130Φ; Width = 109Φ; L/W = 1.19. Note the round body shape Arrow indicates location of macronucleus (Length = 87Φ).

Fig. 10 and 11. Tentatively identified as *Blepharoconus krugerensis.* Length = 70Φ; W = 47Φ; L/W = 1.49. Arrows in Fi. 11 indicate location of cilia. Posterior ciliary tuft barely visible.

Fig. 12. *Ophryoscoles purknjei.* Length = 72Φ; Width = 42Φ; L/W = 1.71. Short main caudal spine. Three circlets of secondary spines are shown by arrows.Note two rows of contractile vacuoles.

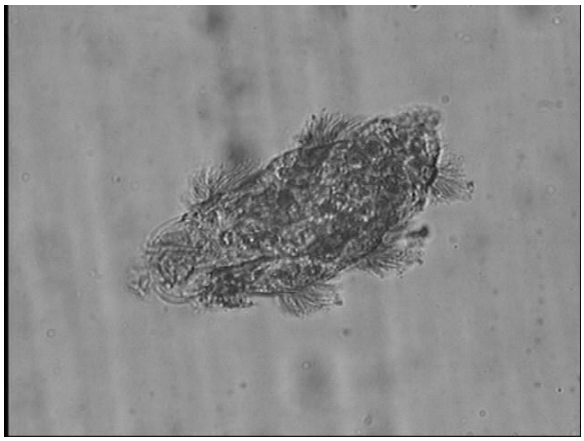

Fig 1
Telamodinium onyx

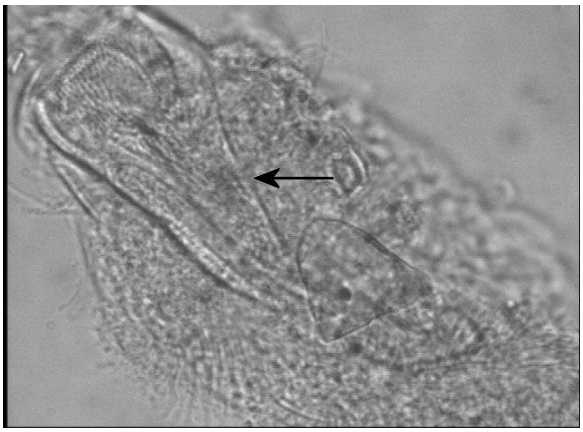

Fig 2
Telamodinium onyx

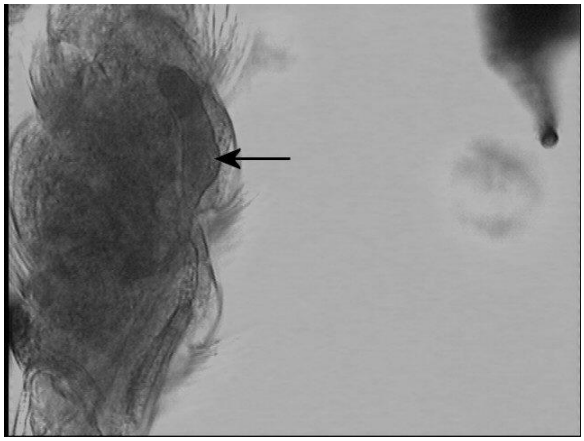

Fig 3
Telamodinium onyx

Fig 4
Telamodinium onyx

Fig 5
Megadinium aethiopicum

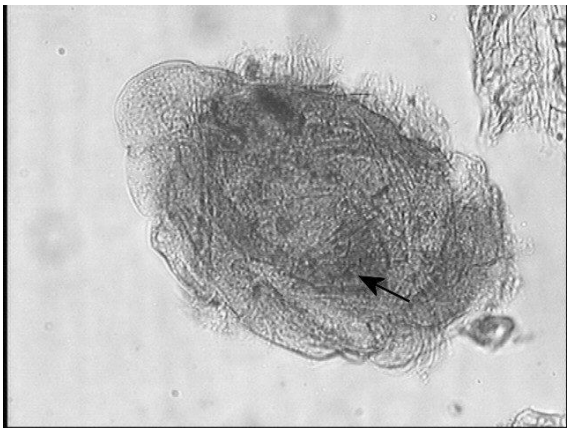

Fig 6
Megadinium aethiopicum

Fig 7
Diplodinium dentatum

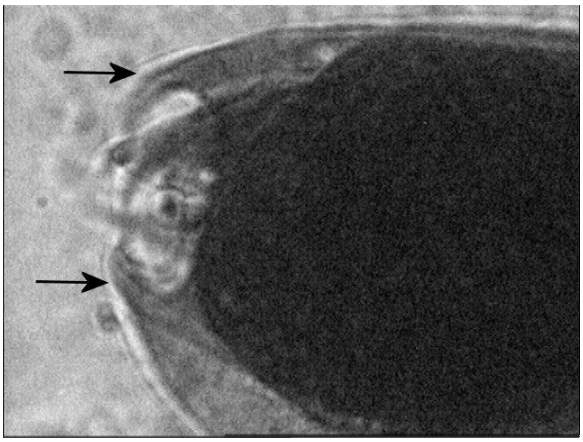

Fig 8
Diplodinium dentatum

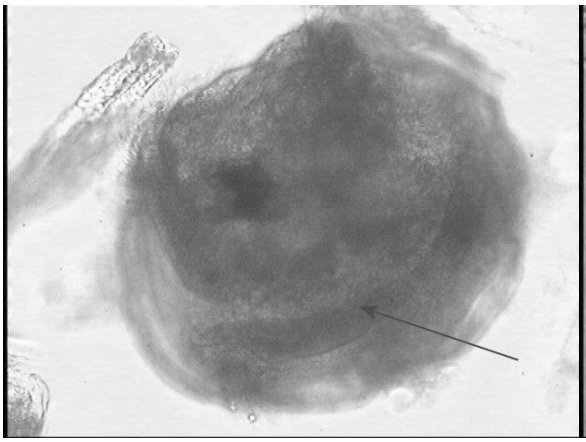

Fig 9
Teratodinium sphaeredon

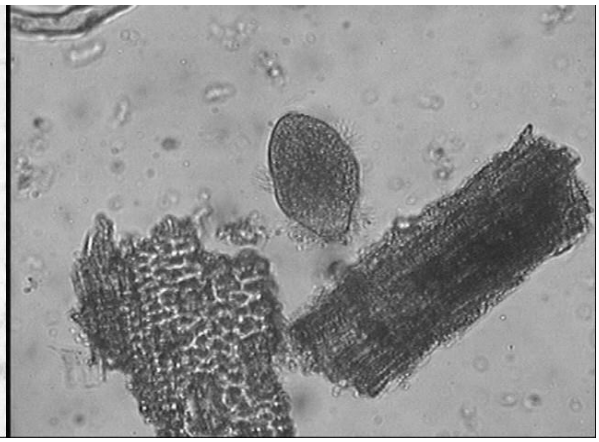

Fig 10
Blepharoconus krugerensis

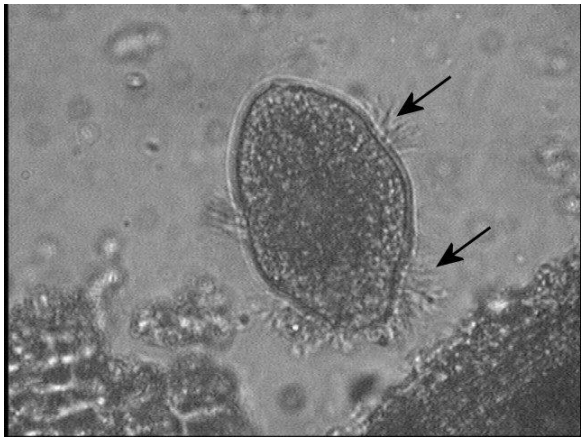

Fig 11
Blepharoconus krugerensis

Fig 12
Ophryoscolex purkenjei

Table 1. Description of individual warthogs and weight.volume and density of their cecum and colon contents.

Animal number, age,sex And weight [a]	Total weight of contents (g)	Density of contents (g/ml)	Volume of Sample (ml)	Animal number, age, sex and weight	Total weight of contents (g)	Density of contents (g/ml)	Volume of Sample (ml)
1 AD, F 65Kg				10 AD, F 34Kg			
Cecum	296.5	1.36	217.7	Cecum	114	0.57	198.3
Colon	1831	1.15	1587.7	Colon	5554.3	0.59	9414.1
2 AD, F 48Kg				11 AD, M 83Kg			
Cecum	277.9	0.78	356.3	Cecum	638.1	0.458	1393.2
Colon	2368	0.83	2853.0	Colon	6407.6	0.60	10728.5
3 AD, M 85Kg				12 AD, F 42Kg			
Cecum	366.9	0.93	394.6	Cecum	296	0.56	524.1
Colon	2544.4	0.92	2755.9	Colon	2639.2	0.59	4499.9
4 JU, F 12.5 Kg				13 AD, F 43Kg			
Cecum	170	0.33	517.5	Cecum	291.4	0.44	660.0
Colon	414.4	0.55	757.6	Colon	2986.5	0.65	4570.0
5 AD, M 81 Kg				14 AD, F 40Kg			
Cecum	341.4	0.58	586.1	Cecum	263.8	0.44	597.5
Colon	2163.2	0.72	3012.8	Colon	2733.6	0.65	4183
6 AD, F 47 Kg				15 JU, F 15Kg			
Cecum	237.8	0.43	550.5	Cecum	75.7	0.3845	196.9
Colon	2692.1	0.78	3467.0	Colon	1364.5	0.503	2712.7
7 JU, M 17 Kg				16 JU, M 12Kg			
Cecum	174.1	0.29	597.8	Cecum	153.2	0.6035	253.9
Colon	1138.1	0.63	1798.7	Colon	1376.3	0.564	2440.2
8 AD, M 75 Kg				17 AD, F 50Kg			
Cecum	359.6	0.55	656.5	Cecum	228.6	0.38	604.8
Colon	5853.2	0.64	9192.3	Colon	3306.1	0.58	5656.3
9 AD, F 50Kg							
Cecum	210	0.41	517.6				
Colon	2647.6	0.88	2995.9				

[a]AD, M=adult male, AD, F=adult female, JU, M=juvenile male, JU, F = juvenile female.

Table 2. Concentration and distribution of protozoa in the cecum and colon contents of 17 warthogs

Animal	Age and sex[a]	Date collected	Protozoa /ml			
			Telamodinium onyx	*Megadinium aethiopicum*	*Teratodinium sphaeredon*	Other
Warthog 1	AD, F	May 2001				
Cecum			30			
Colon			100			
Warthog 2	AD, M	May 2001				
Cecum				40		
Colon			10			
Warthog 3	AD, M	June 2001				
Cecum			30			
Colon			50			
Warthog 4	JU, F	June 2001				
Cecum			30			
Colon			40			
Warthog 5	AD, M	July 2001				
Cecum			70			
Colon			40			
Warthog 6	AD, F	July 2001				
Cecum			10	10		*Blepharoconus krugerensis* & Family Cycloposthiidae
Colon			10	10		
Warthog 7	JU, F	July 2001				
Cecum			10		10	
Colon			20		40	
Warthog 8	AD, M	July 2001				
Cecum			30		10	
Colon			30		40	
Warthog 9	AD, F	July 2001				
Cecum			30			
Colon			100			
Warthog 10	AD, M	May 2002				

Cecum			150	20	
Colon			70		
Warthog 11	AD, F	June 2002			
Cecum					
Colon			30	30	
Warthog 12	AD, F	June 2002			
Cecum			50		
Colon			20		
Warthog 13	AD, F	June 2002			
Cecum			40		
Colon			20	30	
Warthog 14	AD, F	June 2002			
Cecum			30		
Colon			30		
Warthog 15	JU, F	June 2002			
Cecum			20	10	
Colon			30	10	
Warthog 16	JU, M	June 2002			
Cecum				70	
Colon			20	50	
Warthog 17	AD, F	June 2002			
Cecum			50	20	*Diplodinium dentatum*
Colon			30		*Ophryoscoles purkkynjei*

[a]AD, M=adult male; AD, F=adult female; JU, M=juvenile male; JU, F=juvenile female.