The effect of acid drinking water on rumen protozoa in the blesbok (*Damaliscus dorcas phillipsi*)

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Abstract

Rumen contents were collected from ten adult female blesbok, five from a mine area with only acid drinking water available and five from a control group consuming normal, non-polluted drinking water. The mean concentration of total protozoa in the normal water group was almost double that in the acid drinking water group, 24.9 x 10^3 versus 14.7 x 10^3 . Percent of *Entodinium* was higher and *Diplodinium* lower in those animals drinking the acid water. The number of different protozoa species present in animals from both locations was fairly similar. *Diplodinium bubalidis, Ostracodinium gracile* and *Diplodinium consors* were present in the highest percentage in the normal water group, 18.8, 18.4 and 17.7 %, respectively. The same three species, plus *Entodinium dubardi,* were also highest in the acid water group, *O. gracile*, 21.3 %; *D. consors,* 12.6 %; *E. dubardi,* 11.4 % and *D. bubalidis,* 10.3 %. Seventeen species of protozoa found in this study were a new host record for the blesbok, bringing the total number of species reported from the blesbok to 29.

Keywords: Acid drinking water, Blesbok, *Damaliscus dorcas phillipsi*, Rumen, Protozoa.

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Introduction

Because of reported blesbok deaths in the area of an abandoned gold and uranium mine, Nothling et al. (2014), conducted a study in which they measured lead isotope ratios in bone ash of animals consuming acid water draining from the mine. In addition, these same measurements were taken on animals from a different area consuming non-contaminated drinking water. They found that the blesbok drinking the acidic water had accumulated higher concentrations of uranium in their tibia bones, presumably as a result of this element leaching from uraniferous rocks. The present study was undertaken to determine whether the acid mine water may have also contributed to changes in the rumen protozoa and affected animal health.

Materials and methods

Collection sites

Five female blesbok were harvested from the Krugersdorp Game Reserve (KGR) (26º 05 ' 11" S and 27º '42' 45" E) in the Gauteng Province of South Africa. These animals only had access to acidic drinking water. The control group of five female blesbok, drinking from a normal water supply, were harvested from the Rhino and Lion Nature Reserve (RLNR) which is located approximately 15 km north-east of KGR.

Drinking water

A sample of the water being consumed by the blesbok was collected at each site and returned to the lab for measurement of pH. Water from the mine area had a pH of 3 while the normal drinking water had a pH of 7.

Sample collection

Handfuls of rumen digesta were squeezed in the fist with the thumb pointing downwards so that liquid could run down into a plastic container. This was repeated until a 40ml sample was obtained. This volume was then transferred to a 100ml bottle and 40ml of 40% formalin was added to preserve the sample.

Counting protozoa

Because of the high concentration of protozoa, a 1ml sub sample of the fixed rumen fluid sample was diluted to 5.0ml by adding 4.0ml of water. Two drops of Brilliant Green Stain were added and the sample was allowed to stand overnight. Using a finnpipet,

0.025ml of the stained sample was placed on a glass microscope slide, covered with a glass cover slip and the protozoa were counted. Each sample was counted in triplicate. Results of the 0.025ml count were multiplied by 400 to give the concentration of protozoa per ml of rumen fluid.

Results and Discussion

Concentration and generic distribution of rumen protozoa in the blesbok drinking normal or acid water are shown in Table 1. The overall mean value for protozoa concentration/ml was almost double for the animals drinking normal water, i.e., 24.9 x 10^3 compared to 14.7 x 10^3. The range of values in the normal drinking water group, 8.2 – 39.4 x 10^3/ml, was much greater than that in the acid drinking water group, 12.4-19.2 x 10^3/ml. This is reflected in the higher standard deviation in the control group, ±12.6 versus that in the acid water group, ±2.7. A number of studies have shown that lowering of rumen pH by feeding high concentrate diets markedly lowers protozoa concentrations (Latham et al.,1971; Abe et al., 1973; Mackie et al.,1978; Towne et al., 1988; Franzolin and Dehority, 1996). Dehority (2005), working in vitro with monocultures of *Entodinium caudatum, Entodinium exiguum, Epidinium caudatum and Ophryoscolex purkynjei,* observed that numbers of all four species decreased markedly below pH 5.8 and the protozoa were no longer viable at a pH of 5.3. Unfortunately, in the present study pH of the rumen contents was not measured. However, drinking water with a pH of 3.0 should have markedly reduced rumen pH.

The blesbok primarily consumes grasses, which would account for the low percentage of *Entodinium* species, 5.0 to 17.1% in the control animals. In a previous study on protozoa in wild African ruminants, those animal species which consumed only grasses and roughages, the hartebeest and wildebeest, had percentages of *Entodinium* ranging from 6 to 16% (Dehority and Odenyo, 2003). As in the present data, the majority of protozoa were *Diplodinium* and *Ostracodinium* species. In contrast, the concentrate and intermediate feeders, which presumably had a lower rumen pH, had *Entodinium* percentages ranging from 41 to 100%. This is closer to the range in the acid water group, 10 to 61%.

Table 1. Concentration and generic distribution of rumen protozoa in blesbok drinking normal or acid water.

	Normal drinking water						Acid mine drinking water					
	1	2	3	4	5	Mean±SD	1	2	3	4	5	Mean±SD
Total protozoa (x10^3/ml)	8.2	18.0	23.6	39.4	35.2	24.9±12.7	12.9	14.4	19.2	12.4	14.4	14.7±2.7
Genus (%)												
Entodinium	17.1	14.9	16.9	5.1	14.8	13.8±5.0	19.4	30.6	10.4	61.3	27.8	29.9±19.2
Diplodinium	68.3	36.2	27.1	40.6	21.6	38.8 ±18.1	24.8	22.2	29.2	22.6	41.7	28.1±8.1
Eudiplodinium	0	0	15.3	12.2	19.9	9.5±9.1	0	2.8	4.2	0	2.8	2.0±19
Ostracodinium	4.9	46.8	37.3	37.6	38.6	33.0±16.2	31.0	33.3	56.3	16.1	27.8	32.9±14.7
Epidinium	0	0	0	0	0	0	0	2.8	0	0	0	0.6±1.1
Opisthotrichum	9.8	2.1	3.4	4.1	4.5	4.8±3.0	24.8	8.3	0	0	0	6.6±10.8
Dasytricha	0	0	0	0.5	0	0.1±0.2	0	0	0	0	0	0

In general, the percentage *Entodinium* was increased and *Diplodinium* and *Eudiplodinium* decreased in the acid drinking water group. The percentage of *Ostracodinium* was similar between the two groups. The increase in *Entodinium* at lower rumen pH values would agree with earlier in vivo observations (Vance et al., 1972; Lyle et al., 1981; Towne et al., 1988; Franzolin and Dehority, 1996). However, the in vitro studies by Dehority (2005) did not suggest any lower pH tolerance for *Entodinium* species.

Table 2 presents the species distribution of protozoa in the two groups. For those animals drinking normal water, a total of 19 species were observed among the five animals. A total of 13 species occurred in one animal followed by 12,10, 9 and 5 in the remaining blesbok. Over 50% of the total population was comprised of three species, *Diplodinium bubalidis* (18.8%), *Ostracodinium gracile* (18.4%) and *Diplodinium consors* (17.7%).

In the group drinking acid water, 22 species were present. One animal contained 14 species, one animal had 10 species, two had 9 and the fifth animal contained 8 species. The same three species as in the normal group made up 44% of the fauna: Ostracodinium gracile (21.3%), *Diplodinium consors* (12.6%) and *Diplodinium bubalidis* (10.3%). *Epidinium dubardi* comprised 11.4% compared to only 6.2% in the normal drinking water group. Only 14 species were common to both groups.

The protozoa faunas of 26 young blesbok were studied by van Hoven (1978). The animals were harvested from a farm in the Western Transvaal province of South Africa. The animals ranged in age from 3 to 21 months with a mean protozoa concentration of 29 x 10^4/ml. This concentration is almost 10-fold higher than found in the normal group in the present study. However, no information is available on the diet of the animals in either study. A total of 12 species were identified in the 26 animals, two of which were not found in this study, *Ostracodinium garstangi* and *Entodinium fyferi.* Across all animals, the average percentages of *Ostracodinium gracile, Diplodinium consors* and *Diplodinium bubalidis* were 4.9, 12.8 and 15.5%, respectively, for a total of 33% of the total species. This is somewhat lower than the total for these three species found in the present study; however, these were young animals compared to adults. Only two species of *Entodinium* were found, *Entodinium fyferi* and *Entodinium parvum.*

Table 2. Percent species distribution of rumen protozoa in blesbok drinking from a normal or acid drinking water supply.

Genus Species	Normal drinking water						Acid drinking water					
	1	2	3	4	5	Mean	1	2	3	4	5	Mean
	Percent species distribution											
Dasytricha												
ruminantium Shuberg, 1888	-	-	-	0.5	-	0.1	-	-	-	-	-	0
Entodinium												
acutonucleatum K & M[a], 1930	-	-	1.7	-	-	0.3	-	-	-	6.5	-	1.3
bimastus Dogiel, 1927	-	-	-	-	-	0	3.1	-	4.2	19.4	-	5.3
dubardi Buisson, 1923	-	8.5	10.2	3.0	9.1	6.2	12.4	13.9	2.1	6.5	22.2	11.4
exiguum Dogiel, 1925	-	-	-	2.0	3.4	1.1	0.8	8.3	-	-	-	1.8
furca Cunha, 1914	-	-	-	-	-	0	-	2.8	-	-	-	0.6
nanellum Dogiel, 1922	-	-	-	-	-	0	-	-	-	-	2.8	0.6
***parvum* Buisson, 1923[b]**	-	2.1	-	-	-	0.4	-	5.6	-	-	-	1.1
simplex Dogiel, 1925	-	2.1	-	-	-	0.4	-	-	-	-	-	0
taurinus B & D[c], 2012	4.9	-	3.4	-	-	1.7	-	-	-	29.0	2.8	6.4
Species[d]	12.2	2.1	1.7	-	2.3	3.7	3.1	-	4.2	-	-	1.5

Diplodinium												
aspinosum Ito et al.[e], 1997	-	-	-	-	-	0	3.1	-	-	-	-	0.6
bubalidis Dogiel, 1925	43.9	12.8	16.9	19.3	1.0	18.8	12.4	2.8	20.8	9.7	5.6	10.3
***consors* Dogiel, 1925**	24.4	23.4	8.5	12.2	20.0	17.7	3.1	8.3	8.3	12.9	30.6	12.6
costatum Dogiel, 1925	-	-	-	-	-	0	6.2	-	-	-	-	1.2
dogieli Dogiel, 1925	-	-	1.7	9.1	-	2.2	-	2.8	-	-	2.8	1.1
minor Dogiel, 1925	-	-	-	-	-	0	-	2.8	-	-	-	0.6
rangiferi Dogiel, 1925	-	-	-	-	-	0	-	5.6	-	-	2.8	1.7
Eudiplodinium												
***giganteum* Dogiel, 1925**	-	-	5.1	3.0	-	1.6	-	2.8	4.2	-	2.8	2.0
***impalae* Dogiel, 1925**	-	-	10.2	8.1	9.1	5.5	-	-	-	-	-	0
insigne Dogiel, 1925	-	-	-	1.0	1.0	0.4	-	-	-	-	-	0
neglectum Dogiel, 1925	-	-	-	-	10.2	2.0	-	-	-	-	-	0
Ostracodinium												
***gladiator* Dogiel, 1925**	-	8.5	16.9	3.3	13.6	8.5	-	8.3	2.1	-	8.3	3.7
***gracile* Dogiel, 1925**	-	29.8	11.9	25.4	25.0	18.4	31.0	25.0	31.3	-	19.4	21.3
nanum Dogiel, 1925	-	-	8.5	9.1	-	3.5	-	-	12.5	3.2	-	3.1
***tenue* Dogiel, 1925**	4.9	8.5	-	-	-	2.7	-	-	10.4	12.9	-	4.7

Epidinium												
***lunatus* Van Hoven,1975**	-	-	-	-	-	0	-	2.8	-	-	-	0.6
Opisthotrichum												
***janus* Buisson, 1923**	9.8	2.1	3.4	4.1	4.5	4.8	24.8	8.3	-	-	-	6.6
Total %	100.1	99.9	100.1	100.1	99.2	100	100	100.1	100.1	100.1	100.1	100.1
Number of species	5	9	12	13	10	19	9	14	9	8	10	22

[a]Kofoid and MacLennan.

[b]Species in bold print were previously identified in blesbok by van Hoven (1978). Two additional species reported by van Hoven were not found in the present material: *Entodinium fyferi* and *Ostracodinium garstangi.*

[c]Booyse and Dehority.

[d]Cells were distorted and could not be identified below the genus level.

[e]Ito, Arai,Tsutsumi and Imai.

Although *Entodinium parvum* was only present in two animals in this study, *Entodinium dubardi* was found in 9 of the 10 blesbok. This is of interest based the similarity of these two species (Dehority, 1994).

A total of 27 species were observed in the present study.10 of which were previously reported from the blesbok by van Hoven (1978). These are shown in bold in Table 2. Thus the remaining 17 species constitute a new host record for blesbok. Adding the two additional species reported by van Hoven (1978) brings the total species for this host to 29.

In summary, drinking the acid mine water reduced the concentration of rumen protozoa by about half. Although there was a shift in the generic percentages between groups, the same major species were present in both.

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