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Ruminal Ciliated Protozoa in Bison†

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Ruminal contents from 79 slaughtered bison and 2 ruminally cannulated bison were collected to obtain information on total numbers and species distribution of ciliated protozoa. The bison originated from numerous herds throughout the Great Plains and were grouped into three dietary categories: (i) only forage; (ii) forage with moderate levels of supplementation; and (iii) feedlot concentrate-silage diet. Total ciliate counts were highest in bison receiving grain supplementation (210.1×10^4/g) and lowest in bison consuming only forage (27.1×10^4/g). All protozoan species found in bison have been reported in domestic livestock, although *Ophryoscolex* sp., a relatively common protozoan in cattle, was detected at low concentrations in only eight bison. The uncommon holotrich *Microcetus lappus* was present in five bison in concentrations reaching 8.4% of the total ciliate population. *Charonina ventriculi*, another infrequently observed species, was present in 18 bison, with the highest concentrations in forage-fed animals. Thirty bison possessed a type B protozoan population, characterized by *Epidinium* sp., *Eudiplodinium maggii*, and *Eudiplodinium bovis*. Thirty-eight bison possessed a mixed A-B population, characterized by *Polyplastron* sp. coexisting with low numbers of *Eudiplodinium maggii* or *Epidinium* sp. or both. Thirteen bison possessed populations lacking any remnant type B ciliate species. At least 29 of the bison possessing *Polyplastron* sp. were known to have been in contact with cattle, whereas all bison isolated from cattle had type B populations. The reduction of type B populations in bison becomes increasingly likely as bison production expands into areas inhabited by domestic livestock.

Ruminal microorganisms have been examined in many herbivore species, but there is very little information on ciliated protozoa in bison. Pearson (24) concluded that protozoa in bison appeared similar to those found in domestic livestock; however, preliminary investigations comparing ruminal microbial populations indicated that ciliated protozoa differed both quantitatively and qualitatively between bison and cattle (G. Towne, T. G. Nagaraja, R. C. Cochran, C. E. Owensby, and D. L. Harmon, J. Anim. Sci. **65**:503, 1987). Thus, the objective of this study was a comprehensive survey of total numbers and species distribution of ciliate protozoa in bison.

MATERIALS AND METHODS

Between June 1987 and March 1988 at numerous locations in Kansas, ruminal fluid was collected from 79 slaughtered bison and 2 ruminally cannulated bison either grazing pasture or fed various amounts of forage and grain. Samples represented not only native Kansas animals, but also bison originating from herds in Colorado, Missouri, Nebraska, North Dakota, Oklahoma, South Dakota, and Texas. The bison did not fast before being sampled. Immediately after evisceration, ruminal contents from several locations within the ruminoreticulum were withdrawn and composited.

Approximately 20 ml of mixed ruminal samples were pipetted with a wide-orifice pipette into tared flasks containing 10% (vol/vol) Formalin. Flasks were reweighed, and additional Formalin was added to obtain a 1:1 (wt/wt) dilution of ruminal contents. A portion of the preserved sample was diluted with staining solution containing methyl green in phosphate buffer with 30% (vol/vol) glycerol. Total numbers and species distribution of ciliate protozoa were counted from 20 microscope fields in a Sedgwick-Rafter counting chamber. Ciliates were classified by the method of Hungate (17), with additional species identifications based on descriptions from Kofoid and MacLennan (19), Ogimoto and Imai (22), and Orpin and Mathiesen (23). Individuals of the genus *Entodinium* were not identified to the species level, nor was *Isotricha prostoma* differentiated from *Isotricha intestinalis*. The length and width of 20 randomly selected cells from each ciliate species, except entodinia, were measured with a calibrated ocular micrometer. Average cell dimensions of *Entodinium* species were determined from 60 randomly selected cells. Relative cell volumes were calculated by a rotational ellipsoid formula, assuming that thickness was in constant proportion to width (16).

For statistical analysis, bison were grouped into three dietary categories: (i) grazing pasture or fed only hay; (ii) fed forage with moderate levels of grain supplementation; and (iii) fed a concentrate-silage feedlot diet for at least 90 days. Protozoan species counts were converted to percentage of the total population for each bison. If a species was present in only two dietary categories, variances were tested for equality by an F test, and means subsequently were separated with the appropriate t test. When a species was present in all three dietary categories, medians were compared by the Kruskal-Wallis nonparametric test (28). Total ciliate numbers and cell volumes likewise had heterogeneous variances and were tested nonparametrically.

Ciliate measurements are given as means ± standard error.

RESULTS

Total protozoan counts and cell volumes were both lower ($P < 0.0001$) in bison consuming only forage compared with bison fed other diets (Table 1). Numbers and cell volumes of ruminal protozoa were similar in supplemented and feedlot-fed bison.

The common holotrich genera *Dasytricha* and *Isotricha* were present in the majority of bison, the former existing at

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TABLE 1. Total numbers and volume of ciliate protozoa in ruminal contents of bison fed different diets

Diet	No. of protozoa (10^4/g)	Volume of protozoa (10^8 μm^3/g)
Forage	27.1[a]	297[a]
Forage + grain	210.1[b]	1,058[b]
Concentrate	169.2[b]	917[b]

[a,b] Means in the same column with different superscripts are different ($P < 0.05$).

the highest concentrations in forage-fed animals (Table 2). *Isotricha* concentrations were not significantly different among diets.

Microcetus lappus, a recently discovered holotrich, was detected in five bison at concentrations from 0.9 to 8.4% of the total ciliate population. *Microcetus lappus* was absent in feedlot bison, but was present in similar concentrations in animals fed the other two diets. *Charonina ventriculi*, another infrequently observed holotrich, was present in 18 bison, with the highest concentrations occurring in forage-fed animals.

Entodinium was the only protozoan genus present in every bison, exceeding 60% of the total ciliate population in all but 10 animals. Bison consuming only forage had significantly lower percentages of *Entodinium* species and correspondingly higher proportions of most other ciliates compared with bison fed other diets.

Diplodinium, *Eudiplodinium*, *Metadinium*, and *Ostracodinium* concentrations were all highest in bison consuming only forage. In feedlot-fed bison, most species of Diplodiniinae were virtually absent (Table 3). The relatively uncommon ciliate *Elytroplastron bubali* was present in eight bison, all of which had been fed a supplemented diet.

Epidinium ecaudatum thrived with all diets and was the only ciliate in significantly higher numbers in bison on a feedlot diet. *Epidinium* concentrations ranged up to 24.5% on roughage diets, but proportions in one feedlot bison reached 63.5% of the total ciliate protozoa.

DISCUSSION

Dietary supplementation increased total protozoan numbers over those of forage-fed bison, primarily because *Entodinium* concentrations significantly increased with starch intake. With high-grain diets, species diversity declined, probably because sensitivity to lower ruminal pH and faster rate of passage eliminate most protozoa (1, 3). Considerable variation in total ciliate numbers occurred among individual bison within each dietary group, ranging from 17×10^4 to 822×10^4 cells per g on forage diets, 126×10^4 to $4,076 \times 10^4$ cells per g on concentrate diets, and 420×10^4 to $4,187 \times 10^4$ cells per g on grain-supplemented diets. Wide fluctuations in total numbers and sporadic occurrence of many species illustrates the inadequacy of quantifying protozoan populations by sampling only a few animals.

All ciliates found in bison have been reported in domestic livestock, and apparently no species are specific to only bison or cattle. However, *Ophryoscolex purkynei*, a relatively common protozoan in cattle, was detected in only eight bison at very low concentrations. Although Pearson (24) reportedly found *Ophryoscolex caudatus* in Utah bison, the organism more likely was *Epidinium ecaudatum* var. *caudatum*, which would be more compatible with the protozoan species he observed.

The presence of *Microcetus lappus* in bison represents its first reported occurrence in North American ruminants; it was previously detected only in Norwegian cattle (23). However, Orpin and Mathiesen (23) have suggested that *Oligoisotricha bubali*, a similar holotrich found on one occasion in North American cattle (11), is also *Microcetus lappus*. Positive identification of *Microcetus lappus* was based on the presence of two distinct cytopharyngeal rods.

Quantitative differences in some protozoan species among bison are due to different population types rather than dietary influences. Eadie (14, 15) designated two separate ruminal protozoa populations: type A, characterized by the presence of *Polyplastron multivesiculatum*, *Ophryoscolex* species, and *Metadinium affine*; and type B, denoted by *Epidinium ecaudatum*, *Eudiplodinium maggii*, and *Eudiplodinium bovis* (*Eremoplastron bursa*). Most other ciliate

TABLE 2. Percent generic distribution and occurrence of ciliate protozoa in ruminal contents of bison fed three different diets

Genus	% Distribution			Occurrence (%)
	Forage (n = 21)	Forage + grain (n = 45)	Concentrate (n = 15)	
Dasytricha	5.7[a]	0.7[b]	2.6[a,b]	66.7
Isotricha	1.2	0.9	1.2	64.2
Microcetus	0.2	0.4	0	6.2
Charonina	3.5[a]	0.2[b]	0	22.2
Entodinium	55.6[a]	90.2[b]	77.2[c]	100.0
Diplodinium	10.0[a]	1.1[b]	0	40.7
Eudiplodinium	12.4[a]	2.0[b]	4.0[b]	71.6
Metadinium	2.3	1.7	0	59.3
Ostracodinium	5.3[a]	0.2[b]	<0.01[b]	51.9
Elytroplastron	0	0.6	0	9.9
Polyplastron	0.8	0.4	0	38.3
Epidinium	3.6[a]	1.8[a]	14.9[b]	39.5
Ophryoscolex	<0.1	0.1	0	9.9

[a,b,c] Means in the same row with different superscripts are different ($P < 0.05$).

TABLE 3. Percent species distribution of Diplodiniinae in ruminal contents of bison fed different diets

Species	% Distribution		
	Forage (n = 21)	Forage + grain (n = 45)	Concentrate (n = 15)
Diplodinium			
anisacanthum	5.4[a]	0.6[b]	0
dentatum	0.3	0.4	0
lobatum	2.5[a]	<0.1[b]	0
polygonale	1.7	<0.1	0
posterovesiculatum	0.1	<0.1	0
Eudiplodinium			
bovis	2.9[a]	0.2[b]	3.3[a]
dilobum	3.0[a]	<0.1[b]	0.2[a,b]
maggii	4.1[a]	1.2[b]	0.6[b]
rostratum	2.4[a]	0.6[b]	0
Metadinium			
affine	0.2[a]	1.3[b]	0
medium	1.0[a]	0.1[b]	0
ypsilon	1.2	0.3	0
Ostracodinium			
clipeolum	0.5[a]	<0.1[b]	0
dentatum	2.1[a]	<0.1[b]	<0.1[b]
gracile	0.9[a]	0.2[b]	0
obtusum	1.9[a]	<0.1[b]	0

[a,b] Means in the same row with different superscripts are different ($P < 0.05$).

species apparently coexist satisfactorily in either type A or type B populations. However, the two populations are antagonistic, and if they are intermixed, *Polyplastron multivesiculatum* will selectively devour *Epidinium ecaudatum*, *Eudiplodinium maggii*, and *Eudiplodinium bovis*, irrevocably transforming a type B into a type A population (6, 15).

Coleman (5) subsequently designated an additional population category, type O, characterized by the presence of only *Entodinium* species or holotrichs or both. Type O populations prevail in feedlot animals, in which increased ruminal acidity frequently eliminates less tolerant species (12, 20); in young ruminants, in which entodinia are usually the first species to become established (13, 21); and in many cervine species, which have a high ruminoreticular turnover rate (7, 25).

In this study, 30 bison possessed a type B population, 38 bison had a mixed A-B population, and 12 bison possessed a type A population. One feedlot bison possessed a type O population, which was probably due to a low ruminal pH eliminating other species. At least 58% of the bison with type A or mixed A-B populations ($n = 50$) were known to have been in contact with cattle, whereas all individuals from isolated wild bison herds had type B populations. The two ruminally cannulated bison both possessed a mixed A-B population, but 15 months earlier had harbored a type B population before accidental contact with nearby cattle. Apparently, bison harbor a type B population unless exposed to ruminants possessing *Polyplastron multivesiculatum*. Brazilian water buffalo (*Bubalus bubalis*) (9), muskoxen (*Ovibos moschatus*) (7, 10), African antelopes (*Redunca* spp.) (27), and New Zealand cattle (4) likewise are characterized by type B populations, whereas North American cattle commonly possess either type A or mixed A-B populations. Contagious cross-inoculation and the subsequent reduction in relic type B populations becomes increasingly likely as bison production expands into areas inhabited by domestic livestock.

Type A populations had significantly higher concentrations of protozoa than either type B or mixed A-B populations; however, ciliate cell volume was not different among populations. In type B populations, higher concentrations of more massive entodiniomorphs, particularly *Eudiplodinium maggii* and *Epidinium ecaudatum*, dominated total cell volume. But in other population types, higher concentrations of smaller entodinia were responsible for most of the protozoan cell volume.

The anomaly of *Polyplastron multivesiculatum* coexisting with low numbers of *Epidinium ecaudatum* and *Eudiplodinium maggii* may be due to developmental polymorphism in the prey protozoa. In bison possessing mixed A-B populations, surviving *Epidinium ecaudatum* were almost exclusively the five-spined variety, *Epidinium ecaudatum* var. *cattanei*; but in type B populations, other *Epidinium* varieties prevailed, and *Epidinium ecaudatum* var. *cattanei* was undetected. The implication that selective divergence of *Epidinium* biotypes is an inducible defense to *Polyplastron* predation is supported by investigations of other ruminant species, in which *Epidinium ecaudatum* var. *cattanei* was not detected whenever *Polyplastron multivesiculatum* was also absent (4, 9, 10, 18). *Epidinium* transformation, however, apparently is limited, because *Epidinium ecaudatum* var. *cattanei* was present in only four bison in concentrations ranging from 0.2 to 2% of the total ciliate protozoa. In dairy cows, Abou Akkada et al. (2) observed no apparent antagonism between *Polyplastron multivesiculatum* and *Epidinium ecaudatum* var. *tricaudatum*, but that variety was nonexistent in bison with mixed A-B populations.

A more successful defensive response to avoid ingestion from *Polyplastron multivesiculatum* was used by *Eudiplodinium maggii*. In mixed A-B populations, *Eudiplodinium maggii* was significantly larger (length, 187.6 ± 4.5 μm; width, 142.0 ± 3.6 μm) than in type B populations (length, 133.8 ± 3.3 μm; width, 87.2 ± 1.6 μm). Enlarged *Eudiplodinium maggii* cells were not detected in type B populations, suggesting that polymorphic plasticity is an inducible defense to deter *Polyplastron* attack. Alternatively, enlarged *Eudiplodinium maggii* may have been present at undetected levels in type B populations and subsequently increased in numbers after smaller cells were engulfed by *Polyplastron multivesiculatum*. In type A populations, *Eudiplodinium maggii* apparently was unable to rapidly develop morphotypic changes in response to *Polyplastron* intrusion.

In the presence of prey protozoa, *Polyplastron multivesiculatum* has been observed to increase in size (6). Morphological plasticity presumably would enable *Polyplastron multivesiculatum* to continually engulf larger prey. However, dimensions of *Polyplastron multivesiculatum* from type A populations were not significantly different from those of cells from mixed A-B populations. Large and smaller *Polyplastron* organisms occurred in both population types (ranging in size from 128 to 236 μm in length to 84 to 208 μm in width), suggesting that remnant prey protozoa remained at undetectable levels in type A populations.

With one exception, *Eudiplodinium bovis* was absent in bison possessing type A or mixed A-B populations, indicating that it cannot coexist with *Polyplastron multivesiculatum*. The aberrant sample may have been from a bison recently exposed to *Polyplastron multivesiculatum*, since *Epidinium ecaudatum* was also present. Eadie (15) observed that upon intermixing, *Polyplastron multivesiculatum* preferentially engulf smaller prey protozoa first, followed by *Epidinium ecaudatum* and then *Eudiplodinium maggii*.

Diplodinium anisacanthum was not found in bison possessing type A or mixed A-B populations, suggesting that it is incompatible with *Polyplastron multivesiculatum*. Antagonisms may also exist with *Dasytricha ruminantium* and *Ostracodinium dentatum*, both of which were significantly fewer in populations inhabited with *Polyplastron multivesiculatum* than in type B populations. *Ostracodinium* species were initially designated as type B organisms that are eliminated when exposed to *Polyplastron multivesiculatum* (15). However, except for *Ostracodinium dentatum*, concentrations of *Ostracodinium* species did not differ between type B and mixed A-B populations. Other investigators have also observed *Ostracodinium* species coexisting with *Polyplastron multivesiculatum* in cattle (26) and sheep (8).

In addition to the three ciliate species characterizing type A populations (*Metadinium affine*, *Ophryoscolex* species, and *Polyplastron multivesiculatum*), six species were absent from type B populations (*Charonina ventriculi*, *Diplodinium dentatum*, *Diplodinium lobatum*, *Diplodinium polygonale*, *Metadinium ypsilon*, and *Microcetus lappus*). These species were associated with type A and mixed A-B populations, and their presence in bison apparently is indicative of type B populations contaminated with inoculum from other ruminants.

The percent species distribution of ruminal protozoa may vary among geographic areas, but ruminal populations likely include the species reported here. Although a few relatively minor ciliate species occasionally found in North American cattle were undetected in this survey, we have no reason to

suspect that they could not occur in bison depending on diet and exposure to other ruminants.

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