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Omasal Ciliated Protozoa in Cattle, Bison, and Sheep†

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Omasal contents were collected from slaughtered cattle (n = 54), bison (n = 15), and sheep (n = 40) to determine numbers and generic distribution of ciliated protozoa. Total protozoan numbers were significantly lower in omasal contents than in ruminal contents of all three species, but the percent composition of all protozoan genera was similar between omasal and ruminal populations. The highest numbers of omasal protozoa found were 7.61×10^5/g in cattle, 7.01×10^5/g in bison, and 1.29×10^6/g in sheep. Omasal dry matter was significantly higher than ruminal dry matter in all species and ranged up to 51.5% in cattle fed high-concentrate diets. The omasal pH was similar to the ruminal pH in all species. The number of omasal laminae averaged 149, 145, and 74 for cattle, bison, and sheep, respectively. Although protozoan concentrations in omasal contents were approximately 80% lower than those in ruminal contents, the omasum harbored relatively high numbers of ciliated protozoa. The resident omasal protozoa are extremely difficult to remove, particularly in cattle, and apparently are responsible for reinoculating transiently defaunated rumens.

Although the omasum is generally ignored as a site of microbial activity, Smith (26) characterized it as an environmental niche that is suitable for ruminal microbial growth. Ciliated protozoa have been detected in the omasal contents of cattle (18) and in effluent entering (29) and exiting (15, 17) the omasal canal of sheep. However, effluent samples reflect emigrating organisms associated with liquid passage so may not be representative of protozoans actually residing within the omasum. Because the omasa of sheep and cattle are dissimilar in size and morphology (16), their indigenous protozoan populations may not be analogous. Therefore, we were interested in quantifying ciliated protozoa sequestrating within the omasum and comparing the omasal structure of different ruminant species.

MATERIALS AND METHODS

Animals and sampling. Over a 10-month period, omasal and ruminal samples were collected from cattle (n = 54), bison (n = 15), and sheep (n = 40) at numerous slaughterhouses. Sampled cattle breeds included Hereford, Angus, Holstein, Ayrshire, Red Shorthorn, and various crosses, whereas the sheep were Suffolk and Suffolk crosses. The diets of the animals varied considerably, ranging from all-forage to various amounts and types of grain supplementation. Some animals fasted before slaughter, but most animals had access to food and water until just before death.

Immediately after evisceration, the omasum was excised and opened with a longitudinal incision, and samples of digesta were collected from among the laminae with a spoon. To minimize reticular or abomasal contamination, we did not collect samples of digesta in the omasal canal. The rumen also was slit, and ruminal samples were collected from several ruminal-reticular locations. After the pH was measured, approximately 20 g of a mixed sample was preserved in a preweighed flask containing 10% (vol/vol) Formalin. The flasks later were reweighed, and additional Formalin was added to obtain either a 1:1 or a 1:2 (wt/wt) dilution of digesta. Duplicate samples of both omasal and ruminal contents were collected for dry matter measurements. Total omasal contents of some animals were determined by measuring the difference in omasal weight following thorough washing. The omasal laminae of at least seven animals for each ruminant species were counted.

Protozoan enumeration. A portion of each preserved sample was diluted with staining solution containing methyl green in phosphate buffer with 30% (vol/vol) glycerol. Total numbers and generic distribution of ciliated protozoa were determined from 20 microscopic fields in a Sedgwick-Rafter counting chamber. The minimal dilution for counting protozoa was 1:20, but higher dilutions were frequently required to provide 30 to 50 cells per microscopic field. Classification of protozoan genera was done as described by Hungate (10), with supplemental identification based on descriptions from other sources (19, 22). Relative protozoan cell volumes were calculated from a rotational ellipsoid formula, assuming that thickness was proportional to width (8).

Statistical analyses. Protozoan genus counts were converted to a percentage of the total population for each animal. Differences in protozoan concentrations between ruminal and omasal contents within each ruminant species were analyzed statistically with the paired t test. Because of the disparity in dietary quality and animal management, omasal and ruminal protozoan numbers were not compared among ruminant species. Dry matter and pH were tested with analysis of variance, and means were separated by least significant differences ($P < 0.05$).

RESULTS

Total protozoan numbers and protozoan cell volumes were significantly lower in omasal contents than in ruminal contents of all three ruminants (Tables 1 to 3). However, the percent composition of all protozoan genera was similar between ruminal and omasal populations. Four cattle and two sheep that were fed high-concentrate finishing diets were both ruminally and omasally defaunated (or possessed protozoan concentrations below the minimal detectable level of 566 cells per g); otherwise, however, omasal protozoa were present in all animals. The highest numbers of omasal protozoa found were 7.61×10^5/g in cattle, 7.01×10^5/g in bison, and 1.29×10^6/g in sheep.

Omasal dry matter was significantly higher than ruminal

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TABLE 1. Ciliated protozoa in ruminal and omasal contents of 54 cattle

Parameter or genus	Mean % (SD) in:	
	Rumen	Omasum
Total protozoa (10^3/g)	635.1[a] (895.0)	69.2 (125.7)
Protozoan volume (10^8 μm^3/g)	275.8[a] (310.1)	37.6 (63.5)
Isotricha	1.8 (5.9)	3.3 (14.3)
Dasytricha	1.8 (3.9)	1.1 (3.8)
Charonina	2.2 (4.7)	0.9 (2.8)
Microcetus	0.8 (4.4)	0.5 (2.6)
Entodinium	71.0 (34.0)	69.5 (36.4)
Diplodinium	1.9 (3.5)	2.1 (5.6)
Eudiplodinium	0.9 (2.7)	1.1 (3.2)
Metadinium	0.7 (1.2)	0.8 (2.4)
Ostracodinium	1.2 (2.0)	2.2 (5.2)
Polyplastron	0.6 (1.5)	1.0 (2.7)
Epidinium	10.6 (27.4)	7.4 (23.5)
Ophryoscolex	0.8 (1.8)	1.0 (3.0)

[a] These values were different from those for the omasum at $P < 0.05$.

dry matter in all species (Table 4). The amount of grain in the diet influenced the omasal dry matter. In cattle fed high-grain finishing diets, the omasal laminae were compacted with grain and had dry matter contents of 44.7 to 51.5%, whereas omasal dry matter contents in cattle fed all-forage diets ranged from 18.4 to 24.2%. Comparative ruminal dry matter contents ranged from 12.7 to 20.7% for cattle on the high-grain diet and 9.8 to 16.5% for cattle on the all-forage diet. Correlation analysis indicated no relationship ($r = 0.14$) between omasal dry matter and omasal protozoan numbers.

Omasal and ruminal pHs were similar in all three species (Table 4). The number of omasal laminae was comparable between cattle and bison and approximately double that found in sheep (Table 5). Likewise, both cattle and bison possessed relatively large quantities of digesta in the omasum as compared with sheep (Table 5). Large variations in omasal size were observed among individuals within each species, and total omasal contents of one Angus × Hereford steer weighed 10.5 kg.

DISCUSSION

Although the omasum possesses significantly fewer protozoa than the rumen, it does harbor relatively high protozoan numbers. The average concentration of protozoa residing within the omasum of sheep was somewhat higher than that in omasal effluent (15, 17, 29). Regardless of diet, the ratio of total protozoan numbers in omasal versus ruminal contents was similar for all animals, suggesting a proportional passage out of the rumen. Differences in protozoan concentrations between the rumen and the omasum have been attributed to sequestration of protozoa in the rumen (7, 13, 29). By adhering to feed particles, protozoan species could forestall ruminal washout and be selectively retained within the rumen (5, 21). Apparently, most ciliated protozoa never leave the rumen (7, 13). Protozoans that flow through the reticulo-omasal orifice, however, can be trapped and detained in the omasal laminae.

Within the omasum, ciliated protozoa may persist for extended periods. Although Czerkawski (6) hypothesized that protozoa from ruminal efflux were lysed in the omasum, we rarely observed disintegrated or distorted protozoan cells in any omasal sample. Microscopic examination of intermittent omasal contents always revealed viable protozoa.

The omasal pH was similar to the ruminal pH in all species and agreed with previously reported comparisons (2, 24, 29). In animals in which the ruminal pH and protozoan numbers were low, the omasal pH and protozoan numbers were correspondingly low. Thus, if adverse dietary conditions that lower pH and eliminate ruminal protozoa persist, they also may eliminate omasal protozoa.

The number of omasal laminae found in the sheep was somewhat higher than the 53 reported for Merino sheep (16). In cattle, however, the number of laminae was similar to that noted in other reports (2, 16). Differences in omasal size and morphology among ruminant species can be interrelated to their feeding habits. Nonselective roughage grazers (e.g., cattle and bison) have a large ruminal volume and a large omasum with numerous well-developed laminae. In comparison, selective grazers (e.g., sheep) have a small rumen and a relatively small omasum (9, 12).

TABLE 2. Ciliated protozoa in ruminal and omasal contents of 15 bison

Parameter or genus	Mean % (SD) in:	
	Rumen	Omasum
Total protozoa (10^3/g)	1,748.4[a] (834.4)	303.8 (194.3)
Protozoan volume (10^8 μm^3/g)	897.4[a] (520.3)	142.0 (117.4)
Isotricha	1.5 (1.3)	1.3 (2.9)
Dasytricha	0.9 (1.4)	0.4 (0.7)
Entodinium	90.6 (6.9)	93.4 (4.8)
Eudiplodinium	1.3 (2.3)	0.9 (1.7)
Metadinium	0.6 (0.9)	0.5 (0.9)
Ostracodinium	0.1 (0.2)	0.1 (0.3)
Elytroplastron	0.1 (0.4)	0.1 (0.4)
Polyplastron	0.2 (0.3)	0.3 (0.5)
Epidinium	4.7 (6.0)	3.0 (3.7)
Ophryoscolex	0.1 (0.2)	<0.1 (0.1)

[a] These values were different from those for the omasum at $P < 0.05$.

TABLE 3. Ciliated protozoa in ruminal and omasal contents of 40 sheep

Parameter or genus	Mean % (SD) in:	
	Rumen	Omasum
Total protozoa (10^3/g)	1,809.0[a] (1,678.3)	301.1 (315.8)
Protozoan volume (10^8 μm^3/g)	958.1[a] (960.7)	165.5 (179.0)
Isotricha	17.5 (35.7)	16.6 (33.8)
Dasytricha	0.2 (0.7)	0.1 (0.5)
Entodinium	64.0 (41.5)	63.6 (42.2)
Metadinium	0.4 (1.6)	0.3 (1.2)
Ostracodinium	<0.1 (0.03)	<0.1 (0.1)
Enoploplastron	<0.1 (0.08)	0
Polyplastron	0.2 (0.7)	0.3 (0.9)
Epidinium	12.4 (24.9)	11.3 (23.1)
Ophryoscolex	0.3 (0.8)	0.3 (0.8)

[a] These values were different from those for the omasum at $P < 0.05$.

TABLE 4. Dry matter and pH of omasal and ruminal contents in cattle, bison, and sheep

Animal	% Dry matter in:		pH in:	
	Rumen	Omasum	Rumen	Omasum
Cattle	13.80[a]	30.14	6.46	6.27
Bison	15.87[a]	27.22	5.96	5.65
Sheep	14.40[a]	23.53	6.28	6.37

[a] Different from the omasum ($P < 0.05$).

TABLE 5. Number of omasal laminae and weight of omasal contents in cattle, bison, and sheep

Animal	*n*	Mean no. of omasal laminae (SD)	*n*	Mean digesta wt in grams (SD)
Cattle	8	149 (14.8)	20	3,534 (2,027)
Bison	7	145 (8.9)	7	2,645 (1,321)
Sheep	10	74 (5.1)	14	103 (41)

Anatomical differences between the bovine and ovine omasa likely affect ruminal defaunation attempts. Conventional procedures to eliminate ciliated protozoa from ruminants involve dosing ruminal contents with an antiprotozoal detergent (1, 4, 20). Despite their widespread use, however, chemical defaunating agents are not always successful, and the persistent reappearance of protozoa following ruminal defaunation is often acknowledged (3, 4, 25, 28). A possibly more efficacious defaunation technique involves total ruminal evacuation followed by washing of the rumen with water and then rinsing of the interior ruminal walls with dilute formaldehyde (11). However, after comparing various defaunation procedures, Lovelock et al. (14) concluded that all of the techniques were ineffective in permanently removing ruminal ciliates. We also have been unable to achieve sustained defaunation in cattle using numerous putatively successful techniques.

Historically, the overwhelming majority of reportedly successful defaunation trials have been performed with sheep, whereas reports of sustained defaunation in cattle are comparatively rare. Although the reappearance of protozoa in defaunated rumens is often blamed on exogenous contamination or ineffective chemicals, we submit that sustained defaunation is unsuccessful because of residual omasal protozoa. Because antiprotozoal detergents must saturate the particulate material to be effective (20), the small omasum of sheep is potentially easier to defaunate than the large omasum of cattle. Thus, differences in omasal morphology can explain why the antiprotozoal detergent used by Bird and Leng (3) was effective in defaunating sheep but ineffective in eliminating protozoa from cattle. Apparently, omasal backflow (26, 27) carrying viable protozoa is the source for subsequent ruminal reinoculation. Differences in defaunation success between cattle and sheep have not been previously correlated to protozoan survival in anatomically dissimilar omasa. Likewise, the possibility of omasal protozoa being responsible for reinoculating transiently defaunated rumens has not been reported in the literature.

In a novel attempt to dislodge omasal protozoa and attain complete defaunation, we ruminally evacuated a cannulated Holstein steer (242 kg) that had fasted for 24 h and discarded the ruminal contents. The omasum was flushed with tepid tap water by inserting a hose nozzle into the reticulo-omasal orifice. After the rumino-reticulum backfilled with water, it was completely emptied. The omasum was flushed again, and the rumen was subsequently drained two more times. After the last emptying, 1,000 ml of dioctyl sodium sulfosuccinate solution (containing 4 g of Aerosol OT [Fisher Scientific]) was sprayed on the rumino-reticulum walls and into the reticulo-omasal orifice. Although the animal was confined in an indoor facility and isolated from other ruminants, live ciliated protozoa were found in the ruminal contents 1 day after treatment. Apparently, the flushing water was passing directly through the omasal canal without dislodging digesta deeply embedded between the omasal laminae. Oyaert and Bouckaert (23) observed that a large portion of the liquid leaving the reticulum flowed directly into the abomasum. Consequently, secluded omasal protozoa were not exposed to Aerosol OT, and subsequent omasal backflow reinoculated the rumen. Flushing the omasum repeatedly on three successive days also was ineffective in preventing subsequent ruminal reinoculation. We have successfully defaunated cannulated Holstein steers with eight omasal flushes in combination with Aerosol OT on three alternate days. However, because animals can die from water intoxication, omasal flushing is not a viable defaunation technique. Additionally, it is unlikely that flushing will consistently clean all digesta and ciliated protozoa from between the omasal laminae.

The relatively high concentrations of resident omasal protozoa appear to be responsible for reinoculating defaunated rumens, and until a technique that completely eliminates omasal protozoa is discovered, ruminal defaunation, particularly in cattle, will continue to be unreliable.

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