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Occurrence of the Rumen Ciliate *Oligoisotricha bubali* in Domestic Cattle (*Bos taurus*)†

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Oligoisotricha bubali, previously observed twice in water buffalo, was detected in rumen contents of domestic cattle (*Bos taurus*) in two different areas of Tennessee. Concentrations ranged from <1 to 35% of the total protozoa in unweaned calves and up to 72% in older animals in feedlot. In contrast to the other genera of holotrichs, both total numbers and percent composition of *O. bubali* increased when animals were fed a corn silage-concentrate diet.

A new species of rumen ciliate protozoa, *Isotricha bubali*, was described by Dogiel (6) in 1928. The organism was observed in the rumen contents of two water buffalo from Russia. No additional observation of this species was recorded until 1981, when Imai (10) detected its presence in water buffalo from Taiwan. Based on several morphological differences with the genus *Isotricha*, i.e., lack of somatic cilia on the posterior one-sixth of the body surface and absence of a nucleosuspensory apparatus, Imai proposed a new genus, *Oligoisotricha*, for this species.

During the course of a 2-year study on feeder calves in Tennessee, a small, unusual rumen protozoan was observed and has subsequently been identified as *Oligoisotricha bubali*. The present study reports numbers of this species in animals fed different diets, as well as cell measurements and microphotographs.

O. bubali was observed in the rumen contents of randomly sampled feeder calves from two areas in central Tennessee. One area was in the Sequatchie Valley in the south (two adjacent farms), and the second area was about 100 miles north in Pickett County (three farms). Breeds differed between the two locations; Hereford calves were in the south, and Angus calves were in the north. At the beginning of the study, the calves were 5 to 8 months old, weighed 150 to 250 kg, and were not yet weaned. They also had access to pasture, primarily tall fescue, ladino clover, and lespedeza. The calves were all taken to commercial auctions and after processing were fed to market weight at Tennessee Experiment Station feedlots. Samples were taken from several of the animals after 150 days in the feedlot, where they were fed a diet of corn silage and concentrates. The animals from each farm were housed as separate groups in the feedlot.

Samples of rumen contents were obtained by stomach tube. The procedures used for preservation of samples, total and differential counts, morphological studies, and cell measurements have been described previously (4). Photomicrographs were taken with a Polaroid camera, model ED-10.

Measurements of 50 specimens of *O. bubali*, 10 cells from each of five animals, are presented in Table 1. For each animal, the respective mean dimensions of *O. bubali*, in micrometers, were 24.2, 21.6, 22.3, 22.2, and 23.4 in length by 15.8, 13.3, 13.8, 14.2, and 15.5 in width. A statistical analysis revealed that no one animal harbored *O. bubali* cells which were significantly longer or wider than cells from all of the other animals. The average size of the cells in this study was just slightly larger than that observed by Dogiel (6), but considerably larger than those measured by Imai (10). The length-to-width ratio was similar to that of Dogiel's specimens; both exceeded the ratio reported by Imai. The mean thickness, measured on 10 cells, was 8.9 μm and ranged from 7.7 to 9.9 μm.

Figure 1 presents photomicrographs showing the different morphological features of *O. bubali*. Note the absence of cilia on the posterior end of the cell in Fig. 1a and b. The vestibulum, lateral view, is clearly visible in Fig. 1b. The vestibular opening can be seen in Fig. 1c and is about 2.7 μm in diameter. In Fig. 1d, the camera was focused to show the cell outline. The cells shown in Fig. 1e and f were stained with methyl green and show the variation observed in shape

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TABLE 1. Dimensions of *O. bubali* from rumen contents of *Bos taurus* and *Bubalus bubalis*

Species	Dimensions[a] of *O. bubali*		
	Length (μm)	Width (μm)	Length/width ratio
B. taurus[b]	22.7 ± 2.3 (17.6–27.5)	14.5 ± 1.7 (11.0–18.7)	1.57 ± 0.11 (1.33–1.90)
B. bubalis			
Imai[c]	15.8 ± 1.9 (12–20)	12.2 ± 1.8 (8–15)	1.30 ± 0.14 (1.07–1.60)
Dogiel[d]	20 (17–22)	13 (10–15)	1.54

[a] Expressed as mean ± standard deviation (except for the results of Dogiel, for which only the mean is given). The range is given within parentheses.
[b] Measurements of 50 specimens, 10 from each of five animals.
[c] Results of Imai (10).
[d] Results of Dogiel (6).

of the macronucleus. In general, the morphological features of the present specimens agree with the descriptions of Dogiel (6) and Imai (10), except that both of these authors have shown a depression at the posterior end of the body in their drawings and descriptions. The posterior end of the body in the present specimens appears to be smoothly rounded (Fig. 1).

The concentrations of *O. bubali* in calves before weaning and after 150 days in the feedlot on a corn silage-concentrate diet are shown in Table 2. In the Hereford calves, the mean values for both numbers and percentages of *O. bubali* were higher when the animals were in the feedlot; however, total protozoan numbers were slightly lower. These differences were not statistically significant, as might be expected based on the low number of animals and the large variation among animals. A considerably larger number of Angus calves were sampled, and all three parameters were significantly higher ($P < 0.01$) for those animals in the feedlot. These data suggest that *O. bubali* occurs in higher concentrations in animals fed a diet with more available

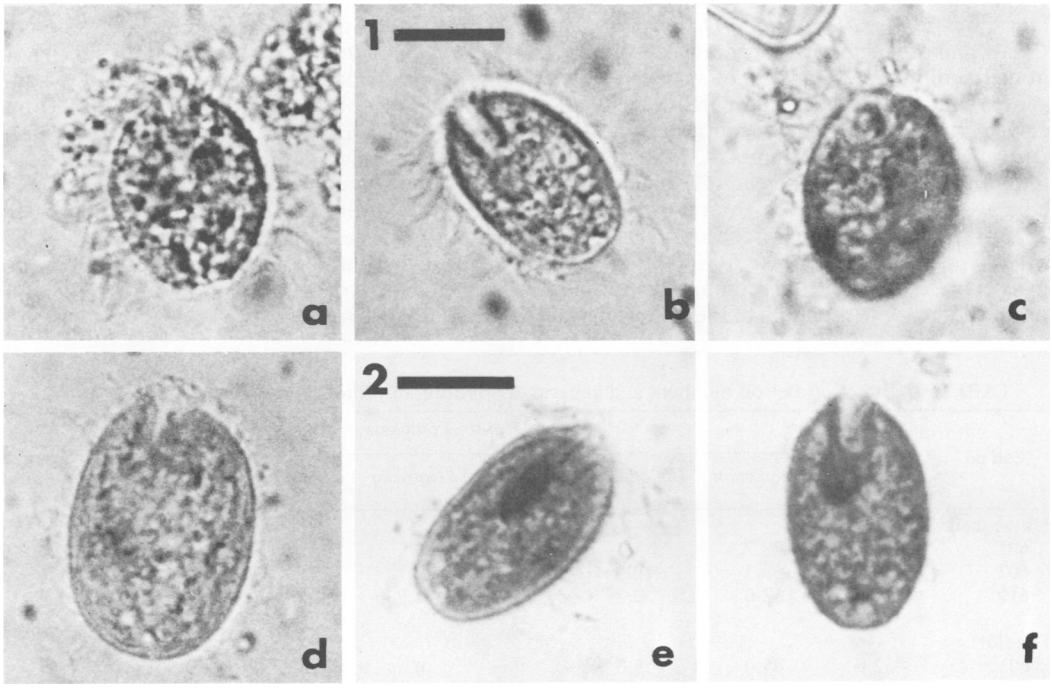

FIG. 1. Photomicrographs of *O. bubali*. (a and b) Cells stained with iodine, with focus on cilia; (c) cell stained with methyl green, with the photo taken from the anterior end of the cell; (d) cell stained with methylene blue, with focus on cell wall outline; (e and f) cells stained with methyl green, with focus on the macronucleus. Bars, 10 μm (bar 1 applies to panels a–d; bar 2 applies to panels e and f).

TABLE 2. Concentration of *O. bubali* in rumen contents of calves (*B. taurus*)

Calves	No. of farms	No. of animals	No. of protozoa ($\times 10^4$) per ml of rumen contents[a]		*O. bubali* (% of total protozoa)
			O. bubali	Total	
Hereford					
Unweaned[b]	2	4	1.1 ± 1.0 (0.3–2.4)	28.7 ± 12.3 (21.0–47.0)	4.5 ± 4.1 (1.0–9.9)
Feedlot[c]	1	3	3.6 ± 4.2 (0.8–8.4)	21.2 ± 16.6 (5.2–38.4)	20.4 ± 19.2 (4.2–41.7)
Angus					
Unweaned	3	21	2.0 ± 2.6 (0.2–9.3)	22.2 ± 11.8 (5.9–55.5)	8.4 ± 9.2 (0.8–35.4)
Feedlot	1	10	10.7 ± 21.0[d] (0.8–68.8)	36.0 ± 33.2[d] (11.6–99.6)	21.0 ± 25.7[d] (0.7–72.0)

[a] Expressed as mean ± standard deviation. The range is given within parentheses.
[b] Unweaned, Nursing with access to pasture.
[c] Feedlot, Sampled after 150 days on a corn silage-concentrate diet.
[d] Significantly higher ($P < 0.01$) than values for unweaned Angus calves.

energy. Although this apparent increase in concentration could also result from a decrease in the volume of the total rumen contents with concentrate feeding, the increased percentage of *O. bubali* indicates that a shift occurred in the proportions of protozoan species.

Only 3 of the 21 Angus calves sampled before weaning were subsequently sampled after 150 days on the corn silage-concentrate diet. Total numbers (Table 3) increased in two of the three calves, which would be expected from previous studies (1, 8, 9, 13). The percentage of *Entodinium* spp. increased in all animals, and with the exception of *Diplodinium* in calf 601, *Diplodinium, Epidinium, Isotricha, Dasytricha*, and *Charonina* organisms disappeared after 150 days in the feedlot. Low numbers of species of these genera did occur in several of the other feedlot calves, but in general their occurrence was quite sporadic. As with total numbers, such a shift in the protozoal population generally occurs when animals are changed to a high-energy diet (5, 9, 13, 14). In contrast, the percentage of *Oligoisotricha* spp. increased markedly in all three animals. One of the reasons commonly proposed for the decrease in numbers of the holotrichs and higher genera of entodiniomorphs in animals fed concentrates is inhibition by the low rumen pH characteristic of this type of diet (2, 7, 14, 15). Based on the present data, *Oligoisotricha* organisms did not appear to be seriously affected by a low-pH environment.

In some animals, *O. bubali* comprised a high percentage of the total protozoa. However, this may be misleading as regards the contribution of *O. bubali* to the total protozoa volume or mass. Using the data of Kofoid and MacLennan (12), which give length, width, and thickness, mean values for these dimensions were calculated for 20 species of *Entodinium*. The mean values corresponded quite closely to the dimensions of *E. biconcavum*, a fairly common species of rumen protozoa. One animal in this study had a generic distribution of 46.4% *Entodinium* and 53.6% *Oligoisotricha*. If the mean *Entodinium* size is considered to be similar to that of *E. biconcavum*, volumes can be calculated by using the ellipsoid formula (17), and the percent distribution can be expressed on this basis. The percent distribution of protozoa by volume for this animal would be 85.5% *Entodinium* and 14.5% *Oligoisotricha*, as compared with the

TABLE 3. Effect of diet on numbers and generic distribution of protozoa in Angus calves (*B. taurus*)

Calf no.	Total protozoa ($\times 10^4$) per ml	% of total protozoa in genus:						
		Entodinium	*Diplodinium*	*Epidinium*	*Isotricha*	*Dasytricha*	*Charonina*	*Oligoisotricha*
Unweaned[a]								
601	9.4	79.7	3.4	0	0	3.4	10.2	3.4
602	14.7	66.3	10.9	19.6	0	2.2	0	1.1
619	27.7	52.0	9.2	17.3	1.2	19.1	0	1.2
Feedlot[b]								
601	32.0	85.0	5.0	0	0	0	0	10.0
602	99.6	95.2	0	0	0	0	0	4.8
619	22.8	93.0	0	0	0	0	0	7.0

[a] Unweaned, Nursing with access to pasture.
[b] Feedlot, Sampled after 150 days on a corn silage-concentrate diet.

46.4% *Entodinium* and 53.6% *Oligoisotricha* composition by numbers.

The occurrence of *O. bubali* in domestic cattle (*Bos taurus*) in Tennessee, which had been observed only twice previously and both times in water buffalo (from Russia and Taiwan, respectively), is difficult to explain. We know of no direct connection between these two distinctly separate geographic areas. However, one important conclusion which can be drawn from this study is that the species *O. bubali* is not host specific for water buffalo.

Based on length and width measurements, *O. bubali* is about the same size as the smallest species of *Entodinium* that has been observed, *E. nanum* (3). *Charonina ventriculi*, another species of holotrich not frequently observed, is just slightly larger (5, 11, 16). However, all three of these species have a similar cell volume, i.e., about 1.5×10^3 μm^3. For comparison, the average-sized *Entodinium* species (*E. biconcavum*) mentioned above has a volume of about 10.5×10^3 μm^3, whereas the larger species of *Diplodinium*, such as *Polyplastron multivesiculatum*, are around 700×10^3 μm^3.

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