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Morphological Diversity of Ruminal Bacteriophages from Sheep and Cattle

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Large numbers of bacteriophages (2×10^7 to 1×10^8/ml) were present in ruminal fluid from sheep and cattle. Twenty-six distinct types were identified and placed in three morphological groups; several phages possessed unusual structural features. The large numbers and diversity of phages observed indicates a possible role in bacterial lysis and hence in the population dynamics of the ruminal bacteria.

Although there are several reports of bacteriophages in crude ruminal fluid, only two groups (14, 15) have studied the range of morphological diversity of the bacteriophages found in the rumen. In cattle, six morphologically distinct types were found in ruminal fluid samples collected over a 12-month period (14). By comparison, in a brief report (15) on cattle and sheep, more than 40 distinct phage types from three major morphological groupings were described. These groupings corresponded to Bradley's (3) groups A, B, and C.

The discrepancy between the findings of these two studies and the paucity of detailed descriptions of ruminal bacteriophages suggested that further study would be useful in this area. If a wide variety of phage types is present in the rumen, it is possible that phages may be significant in interactions within the complex ruminal ecosystem. In the present study, large numbers of diverse bacteriophages were found to exist in ruminal contents, indicating a possible role in the population dynamics of the ruminal bacteria.

Ruminal fluid samples were obtained from two cattle (*Bos taurus*) and seven sheep (*Ovis aries*), each of which was fitted with a ruminal cannula, housed indoors in a pen, and fed once daily. The cattle received chaffed rice straw, and the sheep received oaten chaff. The diets of the cattle and of three of the sheep were supplemented with a commercial mineral and vitamin mix.

Ruminal fluid samples (approximately 20 ml each) were obtained from each animal by aspiration of ruminal contents through a nylon stocking. Ruminal fluid samples were also obtained via a stomach tube from 10 sheep grazing improved native pasture. Samples were stored at 4°C for up to 24 h. Phages in these samples were concentrated and partially purified by a simplified differential centrifugation procedure (2). Ruminal contents were centrifuged twice at 12,000 × *g* for 15 min at 4°C to remove bacteria and large debris. The supernatants were filtered through a 0.45-μm-pore-size filter (Sterifil aseptic filtering system; Millipore Corp.), and phages were then pelleted by centrifugation at 32,000 × *g* for 2 h at 4°C. The supernatants were discarded, and the pellets were each suspended in 100 μl of Hungate-type salt solution (6).

Formvar- and carbon-coated electron microscope grids were wetted with 5 μl of the phage sample by the drop technique (9). The samples were negatively stained with 1% potassium phosphotungstic acid (pH 6.5), and grids were examined for phage particles by using a Phillips 300 transmission electron microscope at a magnification of ×35,000.

The phages were classified on the basis of gross morphology by the scheme of Bradley (3). By this scheme tailed bacteriophages are placed into one of three groups: Group A, long contractile tails; Group B, long noncontractile tails; Group C, short noncontractile tails. The remaining phage types are placed into one of three other groups (3): Group D, tailless icosahedra with large capsomeres; Group E, tailless icosahedra with small capsomeres; and Group F, filamentous phages.

The method for concentrating phage resulted in 200 to 1,000 phage particles per grid square, and this phage density facilitated rapid identification of different types. Extrapolated from the numbers per grid square, the phage density was approximately 2×10^7 to 1×10^8 particles per ml of original ruminal fluid sample.

The range of forms present in sheep and cattle is described in Table 1. The range of forms present in both species appeared similar, regardless of diet, except that more very-long-tailed phages were present in the cattle. Other exceptions were phages 15, 24, and 26, which were only found once and were all from sheep.

Twenty-six morphologically distinct bacteriophages were found which, with one exception, were classified on the basis of the scheme of Bradley (3). The distribution found was as follows: Group A, 14 types; Group B, 7 types; and Group C, 4 types. Phages representative of each group are presented in Fig. 1.

In addition to the general characteristics described in Table 1, the fine detail of certain structures was determinable for some phages. Of the Group A-type phages, details of head symmetry, the collar region, and/or the base plate were discernible in six phages. Phage 5 (Fig. 1a) appeared to have icosahedral head symmetry, while phage 6 (Fig. 1b) had octahedral symmetry. Phages 1, 2 (Fig. 1c), and 5 (Fig. 1a) had a clearly defined collar region, and this structure was composed of one (phages 1 and 2) or two (phage 5) discs. Phage 2 (Fig. 1c) and phages 7 and 8 (Fig. 1d) possessed distinct base plates, which in phage 2 appeared to consist of a flat plate at 90° to the tail. The base plate of phage 7 appeared as a bulbous thickening at the end of the tail, whereas that of phage 8 was more elaborate, consisting of

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TABLE 1. Phage types belonging to morphological Groups A, B, C, D, or E (3) from the rumens of sheep and cattle

Phage no.	Morphological group	Dimensions[a] (nm)			Other features
		Head	Tail	Tail sheath[b]	
1	A	150 by 150	150 by 12	170 by 35	Collar and baseplate
2	A	115 by 115	180 by 25	Uncontracted	Collar and baseplate
3	A	110 by 120	275 by 15	150 by 35	
4	A	110 by 110	410 by 10	220 by 24	
5	A	100 by 100	315 by 10	185 by 25	Collar
6	A	90 by 90	100 by 8	40 by 20	Collar and baseplate
7	A	90 by 90	90 by 20	Uncontracted	Baseplate
8	A	90 by 90	125 by 20	Uncontracted	Collar and baseplate
9	A	80 by 80	290 by 8	120 by 20	Baseplate
10	A	75 by 75	460 by 8	320 by 30	Baseplate
11	A	60 by 80	230 by 20	Uncontracted	Collar and baseplate
12	A	65 by 65	180 by 20	Uncontracted	Collar and baseplate
13	A	60 by 60	180 by 8	80 by 25	
14	A	60 by 45	165 by 5	65 by 16	
15	B	238 by 85	400 by 11		Head composed of large subunits. Baseplate
16	B	95 by 65	285 by 10		Head shape ovoid. Dark lines on head. Baseplate
17	B	95 by 65	280 by 10		Thickenings on tail
18	B	85 by 85	1,050 by 10		
19	B	65 by 65	500 by 6		Collar
20	B	65 by 65	210 by 15		Collar and baseplate
21	B	55 by 55	180 by 10		Collar and baseplate
22	C	70 by 70	35 by 10		A disc separated the head and tail. Baseplate
23	C	70 by 70	25 by 10		
24	C	65 by 65	?[c]		Fanlike tail
25	C	40 by 40	12 by ?[c]		Filamentous material about the collar region
26	D or E	17 by 17			

[a] Expressed as length by width.
[b] Only measurable when sheath was contracted.
[c] Tail dimensions were obscured by other structures.

three ovoid lobes connected to a flared plate at the base of the contractile sheath.

Structural details were visible on four of the Group B phages. Phage 15 (Fig. 1e) was very unusual; it had a head (Fig. 2) composed of large subunits (16 nm in diameter) and a T-shaped base plate (Fig. 1e). Phages 16 (Fig. 1f) and 20 also had visible tail structures. The tail of phage 16 was finely tapered, and phage 20 possessed a plate (30 nm long) set at 90° to the tail. Phages 19 (Fig. 1g) and 20 had collars; the collar of the former composed a cone-shaped structure of the head from which the tail appeared to originate. The collar of phage 20 consisted of a single disc.

Of the four Group C phages, three possessed noteworthy structures. The collar region of phage 22 (Fig. 1h) was obscured by a semicircular disc (50 nm long). The collar region of phage 25 (Fig. 1i) was obscured by filamentous material. On phage 24 (Fig. 1j), a filamentous fanlike structure originated from the collar region and obscured the tail. In addition to the forms described above, phages 1, 6, 8, 9, 10, 11, 12, and 21 appeared to possess structures, such as collars and base plates, that could not be clearly discerned.

The large numbers of phage particles found on the electron microscope grids, although they could not be accurately quantified, indicated that the number of phages (2×10^7 to 1×10^8/ml) in ruminal contents is large. Schwan and Hartman (19) found that filters (Millipore GS) similar to those used in the present study (Millipore HA) bound phage and thus reduced counts. If this occurred in our study, then the estimated numbers of phages may be low. Other workers have estimated numbers of phages in the rumen to be 5×10^7 (14) and in excess of 10^9 (15) per ml of ruminal fluid.

The morphological forms of bacteriophage found in the present study were of a diversity similar to that previously reported (15). Although these workers found a wider variety of types than were found in the present study, their discrimination between phages was in part based on differences in the fine structure of the base plate. This structure was not clearly discernible in many of the phages examined in the present study, so it is possible that the range of diversity was greater than that reported by us. In cattle, many more morphologically distinct types were found than were previously described (14), and none of the phages described previously were morphologically similar to those found in the present study.

The Group A-type phages appeared to be the most numerous and had the greatest morphological diversity. Much of this diversity was due to small differences in gross size and to the relative dimensions of major structural components. The Group B-type phages, almost as numerous as the Group A-type phages, had fewer morphological types but greater differences between types. Within this group, phage 15 (Fig. 1e), although a typical Group B-type phage, had a very large head composed of large subunits (Fig. 2). These subunits appeared too large to be capsomeres and may represent an additional level of complexity in the structure of the head. Ritchie et al. (15) presented an electron micrograph of a similar type of phage, also from ruminal fluid. However, no reference to phage of this type is contained in general reviews on bacteriophage morphology and structure (3, 7).

Phage 18 (Fig. 1k) was the longest phage so far recorded from the rumen; it had a total length of 1,135 nm (1,050 nm was tail length). The Group C-type phages were less numerous than types A and B and tended to have smaller heads, but they did possess considerable diversity of form. Two of

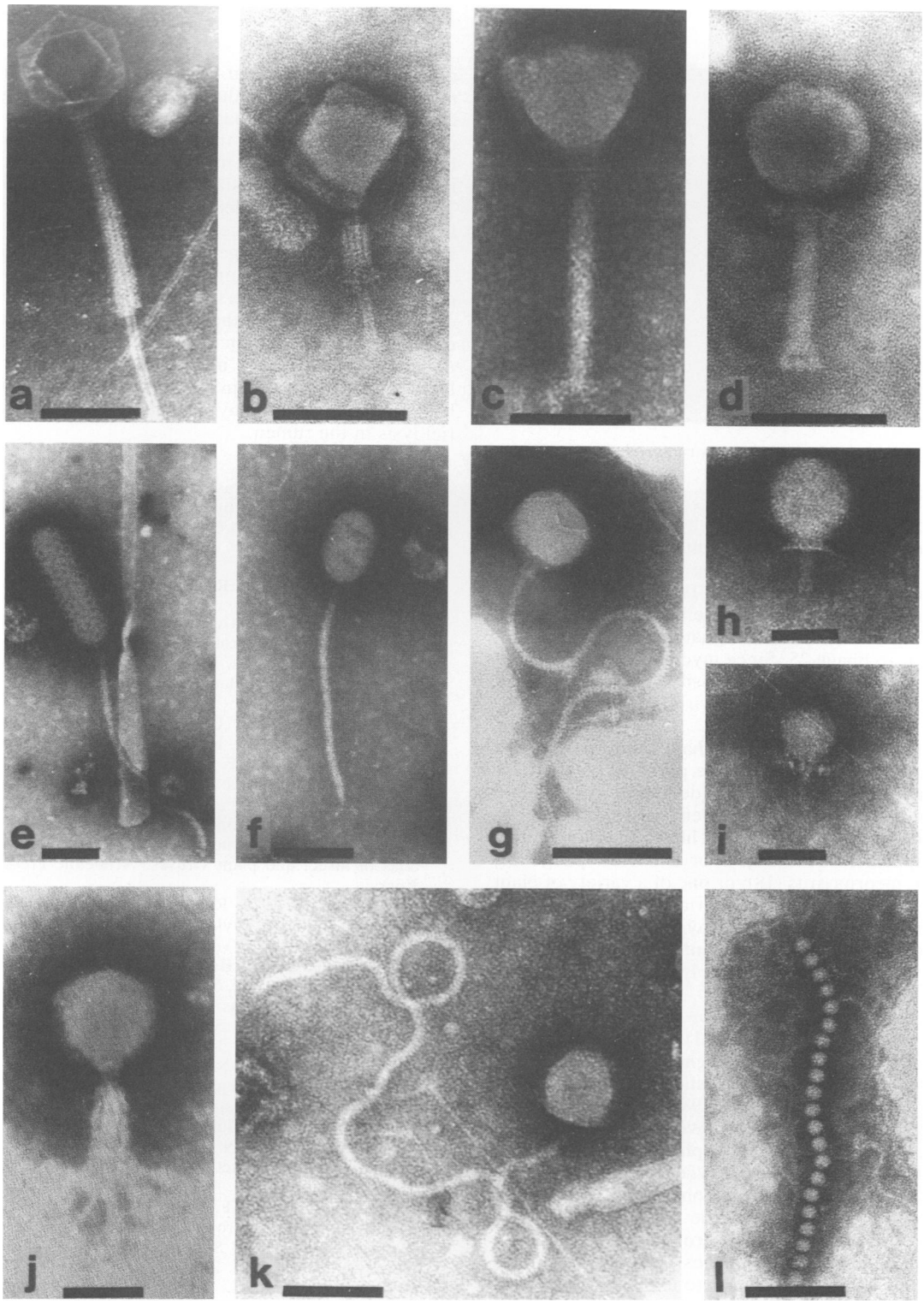

FIG. 1. (a) Phage 5. Group A-type morphology with sheath contracted. Head symmetry appears to be icosahedral, and a collar is present. Bar = 100 nm. (b) Phage 6. Group A-type morphology with the tail contracted. The head appears to possess octahedral symmetry, and the tail is relatively short. Bar = 100 nm. (c) Phage 2. Group A-type morphology with the contractile sheath uncontracted. Both collar and base plate are present. Bar = 100 nm. (d) Phage 8. Group A-type morphology with the contractile sheath uncontracted. The base plate is composed of three lobes attached to the flared base of the contractile sheath. Bar = 100 nm. (e) Phage 15. Group B-type morphology. The head is elongated and comprises large subunits (16 nm in diameter). A T-shaped base plate is present. Bar = 100 nm. (f) Phage 16. Group B-type morphology. The head is elongated, and the tail is finely tapered terminally. Bar = 100 nm. (g) Phage 19. Group B-type morphology. A typical long-tailed group B phage. A funnellike collar is present joining the head to the tail. Bar = 100 nm. (h) Phage 22. Group C-type morphology. The collar has a semicircular disc (50 nm long) attached, and the tail terminates in a bilobed plate. Bar = 50 nm. (i) Phage 25. Group C-type morphology. The collar region is obscured by filamentous material. Bar = 50 nm. (j) Phage 24. Group C-type morphology. The tail is obscured by a filamentous fanlike structure originating from the collar region. Bar = 50 nm. (k) Phage 18. Group B-type morphology. This phage has a very long (1,050 nm) tail and lacks both a collar and a base plate. Bar = 100 nm. (l) Virus 26. A chain of icosahedra, possibly group D or E bacteriophage. Bar = 100 nm.

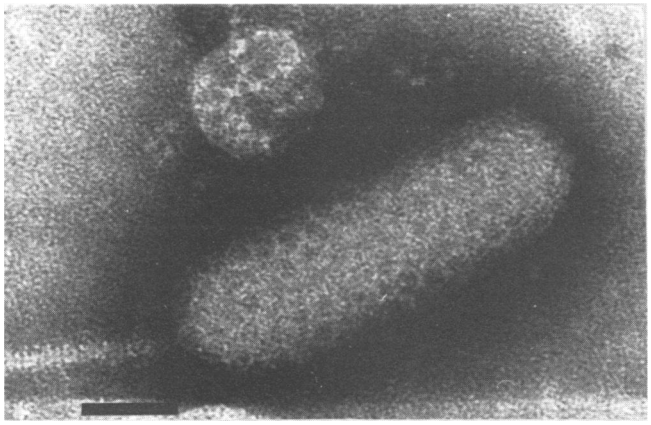

FIG. 2. Head of phage 5; the subunit structure is shown. Bar = 50 nm.

the phage-like and viruslike particles appear to merit more-detailed discussion because of their unusual appearances.

Phage 25 (Fig. 1i) was a small phage with a head diameter of 40 nm and may be a small Group C-type phage. However, viruses which attack spiroplasmas are bacteriophage-like in morphology (5), and the virus designated SVC3 is morphologically similar to phage 25. Since mycoplasmas are known to exist in the rumen (16, 17), it is possible that phage 25 and similar phages may be mycoplasma viruses and not bacteriophages.

Viruses similar to virus 26 (Fig. 1l) have not been reported previously from the rumen. Viruslike particles with similar morphology are common from a wide range of plant and animal sources and thus cannot definitely be linked to ruminal microorganisms. The most likely identity of this virus would appear to be Group D or E bacteriophage (3), parvovirus (8), picornavirus (18), or one of a variety of plant viruses. Only if virus 26 were a bacteriophage would it be of interest, as parvoviruses and picornaviruses would only be likely to be present as infectious agents of the host animal, and plant viruses would be inevitably derived from feedstuffs.

Bacteriophages that infect *Streptococcus durans* (4), *Streptococcus bovis* (1, 10, 11, 21), *Serratia* spp. (1), *Bifidobacterium ruminale* (12), *Magnoovum eadii* (13), a *Methanobrevibacter* sp. (L. Baresi and G. Bertani, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, I-74, p. 133), and *Fusobacterium necrophorum* (20) have been isolated from ruminal fluid. However, apart from phage 2BV of *Streptococcus bovis* (10) and the phage of *Magnoovum eadii* (13), all of the others are small Group B-type phages similar to phage lambda of *Escherichia coli*. Thus, the morphological diversity of phages observed with the electron microscope has not been reflected in the range of phages isolated on ruminal bacteria. Three explanations appear to explain this discrepancy. The majority of phages in the rumen may exist in harmony with their hosts in a state of lysogeny or pseudolysogeny. The phages may have very narrow host ranges, and susceptible hosts may not yet have been found. Of the bacteria that have been used as hosts for phage isolation, only *Streptococcus bovis*, *Magnoovum eadii*, and *Methanobrevibacter* spp. are common ruminal inhabitants. It is possible that the lack of phage diversity found in infection studies reflects the limited range of ruminal bacteria used so far to isolate lytic phages.

At present, there is considerable interest in the genetic manipulation of ruminal bacteria from the standpoint of increased ruminant productivity or biotechnology. If suitable ruminal bacteriophages can be isolated, such phages may serve as vectors for the transfer of recombinant DNA in ruminal bacteria. Furthermore, the phage pool of the rumen may represent a useful source of genetic material.

This study has extended the range of the known morphological diversity of phagelike particles found in the rumen and has identified a large number of morphological types. The presence of large numbers of phages adds an additional dimension to our understanding of the complex ruminal ecosystem. The role of these viruses in the rumen and their effect on the population dynamics of the bacteria is not understood. However, in view of the large number of bacteriophages present in the rumen, it seems possible that bacteriophage action may contribute significantly to bacterial lysis in the rumen.

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