

Electron Microscopic Characterization of a Bovine Herpes Virus from Minnesota

(Accepted 15 December 1971)

A skin disease of unknown aetiology appeared in a herd of 17 dairy cattle in northern Minnesota during late summer of 1970. The disease was characterized by small elevated nodules on all parts of the body with two animals showing teat lesions. The possibility of lumpy skin disease, a disease of cattle exotic to the United States, was considered.

Samples of excised nodules and whole blood were sent to Plum Island for investigation. Tissue cultures inoculated with these samples were passaged several times until cytopathic effects were seen. The results of the early tissue culture experiments were used to establish a preliminary diagnosis (Yedloutschnig *et al.* 1970). Electron microscopy of thin sections of such infected cells revealed a herpes virus. The present report describes the identification of this virus and compares it with other previously known bovine herpes viruses: bovine herpes mammillitis (BHM) (Martin *et al.* 1966; Pepper *et al.* 1966; Rweyemamu, Johnson & Tutt, 1966); Allerton (Polson & Kipps, 1967). Cross-reactions with ferritin-tagged antibodies were also studied. The Neethling poxvirus (Munz & Owen, 1966) was included since it also has been recovered in South Africa from cattle with lumpy-skin disease.

Portions of the blood and excised nodules from the affected cattle in Minnesota (Accession No. 243-70) were passed in primary bovine-kidney tissue cultures. Cytopathic effects were seen by the second and third passages. Electron microscopy of samples taken at subsequent passages showed a bovine herpes virus (Minnesota).

Two other bovine herpes viruses and a poxvirus were compared with the Minnesota isolate in cross-reactions using ferritin-tagged antibodies. Allerton virus (K₂/T₄/K₂), which had been stored lyophilized since its receipt from South Africa in 1958, was reconstituted and passed in bovine-kidney tissue cultures until cytopathic effects were seen. Bovine herpes mammillitis virus (TV 3/8) in lyophilized form from Dr E. P. J. Gibbs, University of Bristol, England, was similarly reactivated. Neethling poxvirus of lumpy-skin disease (strain 2490), which had been stored frozen at -70° since its receipt from South Africa in 1966, was grown in lamb testis culture cells.

Bovine antisera to each of these viruses were prepared by inoculation of virus into susceptible animals. A bovine serum (No. 4506) taken 14 days after infection from an animal in the Minnesota herd was used to confirm the identity of the virus of this isolation.

Cultures infected by the four virus samples (Minnesota, BHM, Allerton, Neethling) were observed for cytopathic changes. Partially destroyed cell layers were washed with Sorenson's buffer (SB), pH 7.3, scraped off and fixed in 1% glutaraldehyde in SB for 20 to 30 min. at 4°, washed twice with SB, postfixed in 2% osmium tetroxide, dehydrated with alcohols and embedded in Epon (Luft, 1961).

An indirect ferritin-tagging method was used in the tests. Rabbit anti-bovine serum both from Plum Island and commercially prepared (Mann Research Laboratories, New York, U.S.A.) were conjugated to ferritin (Breese, 1970). Infected cells showing extensive cytopathic effects were scraped from each prescription bottle into a conical test-tube. Cells and debris were centrifuged at 900g to form a loose pellet of approximately 0.05 ml. In each experiment infected cells were treated with 0.2 ml. of 1/2, 1/4, and 1/8 dilutions of the

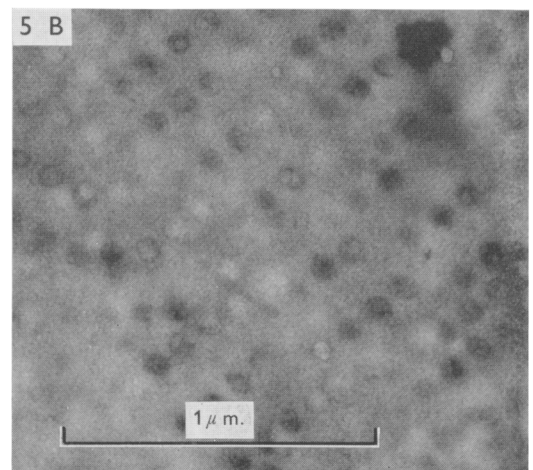
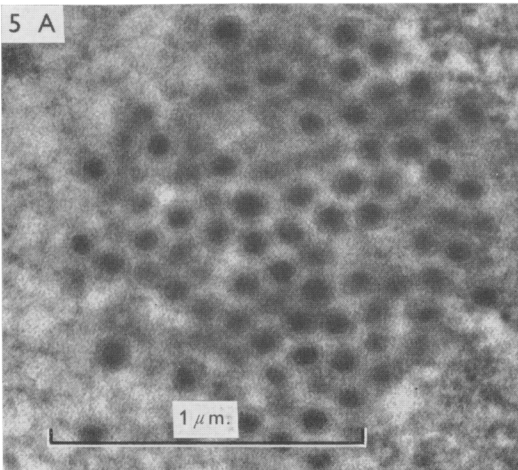
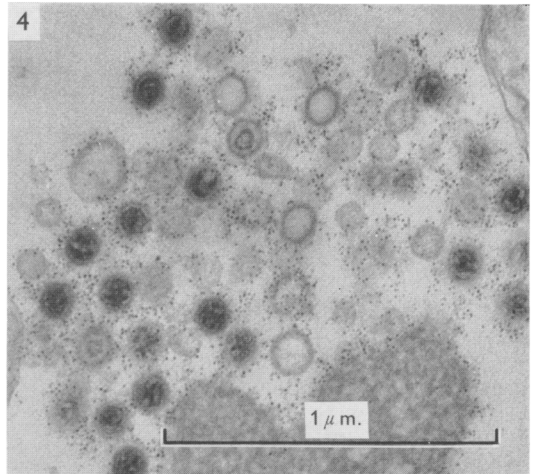
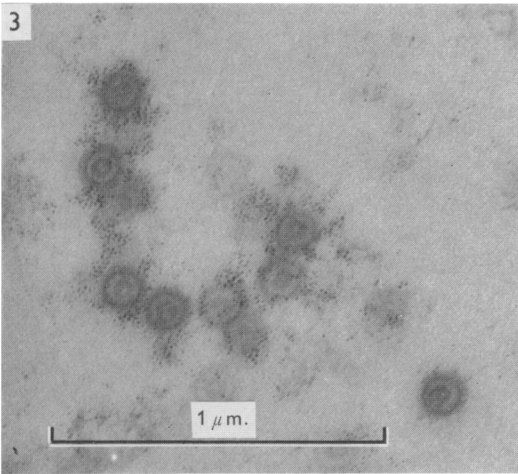
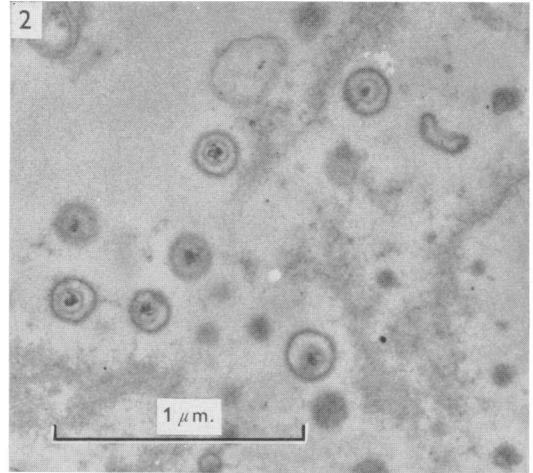
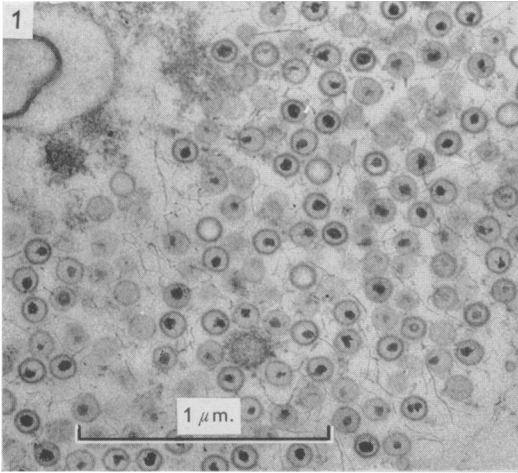


Table 1. Cross-reactions between bovine herpes viruses and antibodies labelled with ferritin

Antivirus serum and dilution	Bovine herpes virus in test				
	Minnesota	Mammillitis	Allerton	Neethling (pox)	
Minnesota	1/2	+	+	+	-
	1/4	+	-	±	-
	1/8	+	-	-	-
Bovine herpes mammillitis	1/2	+	+	+	-
	1/4	+	+	±	-
	1/8	-	+	-	-
Allerton	1/2	+	+	+	-
	1/4	+	-	+	-
	1/8	-	-	+	-
Neethling	1/2	-	-	-	-
	1/4	-	-	-	-

virus-specific antisera shown in Table 1. These mixtures were allowed to stand at room temperature for 40 to 45 min. and washed twice with SB. Treatment with 0.2 ml. of rabbit anti-bovine serum ferritin at $\frac{1}{2}$ dilution was followed by incubation at room temperature for 40 to 45 min., three washings in SB, fixation in 1% glutaraldehyde, embedding as described above, thin sectioning and examination in the electron microscope.

Some cell cultures infected with the Minnesota isolate were embedded in glycol methacrylate, thin sectioned, differentially stained, and used to characterize the DNA nucleoid of the virus particles. The differential staining was for 1 min. in saturated uranyl acetate, 10 to 15 min. in EDTA in H₂O, and 1 min. in lead citrate (Bernhard, 1969).

Electron micrographs of the thin sections from cells infected with the Minnesota isolate showed particles typical of herpes virus in both nuclear (Fig. 1), and cytoplasmic and extracellular areas (Fig. 2). In similarly prepared thin sections this virus could not be distinguished morphologically from bovine herpes mammillitis or Allerton viruses.

Table 1 shows the cross-reactions between the three bovine herpes viruses using the indirect ferritin method. All three interact with one another to some extent according to the individual sera. Fig. 3 shows the positive ferritin-tagging with the Minnesota virus and its homologous antiserum, while Fig. 4 shows a similar reaction with the field serum.

The classification of a virus as a member of the herpes group can be made on morphological grounds by comparison with other known herpes viruses (Morgan *et al.* 1954) and by demonstration of the nucleic acid core as DNA. Fig. 5 shows (a) the standard staining and (b) the differential staining of cells infected with the Minnesota isolate and embedded in glycol methacrylate; the bleaching and disruption of the cores indicates that the virus has a

Fig. 1. Intranuclear bovine herpes virus particles (Minnesota isolate.)

Fig. 2. Extracellular encapsulated bovine herpes virus particles (Minnesota isolate).

Fig. 3. Ferritin-tagging of bovine herpes virus (Minnesota isolate) by its homologous antiserum.

Fig. 4. Ferritin-tagging of bovine herpes virus (Minnesota isolate) by serum from an animal in the outbreak 14 days after onset of disease.

Fig. 5. Bovine herpes virus (Minnesota isolate) in tissue culture cells embedded in glycol methacrylate before (A) and after (B) differential staining to determine DNA core.

DNA nucleoid (Bernhard, 1969). It is concluded that the disease in cattle in Minnesota was caused by a strain of bovine herpes virus of the mammillitis group.

Plum Island Animal Disease Laboratory
Veterinary Sciences Research Division
Agricultural Research Service
U.S. Department of Agriculture
Greenport, New York, 11944
U.S.A.

S. S. BREESE, JUN.
 A. H. DARDIRI

REFERENCES

- BERNHARD, W. (1969). A new staining procedure for electron microscopical cytology. *Journal of Ultrastructure Research* **27**, 250.
- BREESE, S. S., JUN. (1970). An indirect ferritin-tagged antibody system for foot-and-mouth disease virus. *Journal of General Virology* **8**, 153.
- LUFT, J. H. (1961). Improvements in epoxy resin embedding method. *Journal of Biophysical and Biochemical Cytology* **9**, 409.
- MARTIN, W. B., HAY, D., CRAWFORD, L. V., LE BOUVIER, G. L. & CRAWFORD, E. M. (1966). Characteristics of bovine mammillitis virus. *Journal of General Microbiology* **45**, 325.
- MORGAN, C., ELLISON, S. A., ROSE, H. M. & MOORE, D. H. (1954). Structure and development of viruses as observed in the electron microscope. I. Herpes simplex virus. *Journal of Experimental Medicine* **100**, 195.
- MUNZ, E. K. & OWEN, N. C. (1966). Electron microscopic studies on lumpy skin disease virus type 'Neethling'. *Onderstepoort Journal of Veterinary Research* **33**, 3.
- PEPPER, T. A., STAFFORD, L. P., JOHNSON, R. H. & OSBORNE, A. D. (1966). Bovine ulcerative mammillitis caused by a herpesvirus (letter). *Veterinary Record* **78**, 569.
- POLSON, A. & KIPPS, A. (1967). Zone electrophoresis and electron microscopy of Allerton virus. *Archiv für die Gesamte Virusforschung* **20**, 198.
- RWEYEMAMU, M. M., JOHNSON, R. H. & TUTT, J. B. (1966). Some observations on herpes virus mammillitis of bovine animals. *Veterinary Record* **79**, 810.
- YEDLOUTSCHNIG, R. J., BREESE, JUN., S. S., HESS, W. R., DARDIRI, A. H., TAYLOR, W. D., BARNES, D. M., PAGE, R. W. & REUBKE, H. J. (1970). *Proceedings of the 74th Annual Meeting of the U.S. Animal Health Association*. Bovine herpes mammillitis-like disease diagnosed in the United States. Page 208.

(Received 10 November 1971)