

Short Communication

COMPARISON OF BOVINE ROTAVIRUS STRAINS BY PLAQUE ASSAY

L. DAGENAIS¹, A. SCHWERS¹, P.-P. PASTORET² and P. LEROY²

¹Department of Virology and ²Department of Genetics, Faculty of Veterinary Medicine, Université de Liège, 45, Rue des Vétérinaires, B-1070 Brussels (Belgium)

(Accepted 4 May 1981)

ABSTRACT

Dagenais, L., Schwers, A., Pastoret, P.-P. and Leroy, P., 1981. Comparison of bovine rotavirus strains by plaque assay. *Vet. Microbiol.*, 6: 379-382.

Using plaque assay on MA 104 cells, four strains of bovine rotavirus were compared: the attenuated American strain NCDV, and three virulent strains isolated in Canada (PQ) or in Belgium (S14 and S 77). The attenuated strain (NCDV) formed smaller plaques than the three others. The plaque size may thus be used as a marker for this strain. The Belgian strain S 77 formed the largest plaques and no significant differences could be found between the Canadian strain PQ and the second Belgian strain S 14. On BSC-1 cells, the Canadian strain PQ formed much smaller plaques than on MA 104 cells.

Verly and Cohen (1977) observed differences in RNA segments of bovine rotavirus isolates and Spence et al. (1978), using the haemagglutination inhibition test, concluded that some strains of bovine rotavirus differ from others. We have tried to differentiate four strains of bovine rotavirus by the plaque assay technique on established cultures of rhesus monkey kidney cells (MA 104 or BSC-1).

An American attenuated strain (NCDV, from Prof. Mebus, University of Nebraska) was kindly furnished by Dr. R.S. Roy and a Canadian strain PQ by Dr. M.E. Bégin, both from the Veterinary School of the University of Montreal, Quebec. Two Belgian strains (S 14 and S 77) were isolated in our laboratory using the method described by Babiuk et al. (1977) (Dagenais et al., 1980, 1981). The third passage of these rotaviruses grown on cultures of MA 104 and BSC-1 cells was used for the plaque assay. Established cultures of rhesus monkey kidney cells (MA 104 or BSC-1) were cultured in Earle's minimum essential medium (MEM), supplemented with non-essential amino acids (Flow Laboratories), 0.85 g/l of sodium bicarbonate, 10% of foetal bovine serum (Gibco), 100 units/ml of penicillin and 100 µg/ml of streptomycin. These cells were kindly furnished, respectively, by Prof. Bohl (O.A. R.D.C., Ohio) and Dr. R.S. Roy (St-Hyacinthe, Quebec).

The plaque assay technique was based on the method described by Matsuno et al. (1977) and Bohl (1979). Confluent cell monolayers grown in Petri dishes (30 mm diameter) were rinsed with a solution of MEM containing 2.5 µg/ml of trypsin (1:250, Difco), inoculated with 0.2 ml of tenfold dilutions of the virus and incubated for 1 h at 37°C. The infected cells were then covered with MEM containing 1% agarose (Pharmindustrie), 2.5 µg trypsin and 100 µg DEAE dextran (Pharmacia) per ml. A similar procedure was followed on uninfected cells as a control. The cells were fixed with formaldehyde after 4 days of incubation, then stained with May-Grünwald-Giemsa (Vinde-vogel et al., 1975; Jetteur et al., 1979).

Cell cultures inoculated with the optimal dilution producing a satisfactory number of isolated plaques (approximately 50) were examined according to a method previously described (Jetteur et al., 1979). Briefly, plaques were printed with an offset camera to obtain a final magnification of $\times 10$. For each strain cultivated on MA 104 cells, the areas of 50 plaques taken at random were measured with an OH Kempton planimeter.

The normality of the plaque size distribution was tested for skewness and kurtosis. The mean plaque size of the different virus strains was compared by a one-way analysis of variance. Student's *t*-test was used to determine which strain differed significantly from the others.

The addition of trypsin and DEAE dextran in the overlay agarose allowed the production of larger plaques from *rotavirus*, as found by Matsuno et al. (1977). There were significant differences in the mean plaque size of the four *rotavirus* strains studied ($P < 0.0001$). The mean and the standard deviation of plaque size of the four strains grown on MA 104 are given in Table I. The

TABLE I

Mean and standard deviation of plaque size of the four strains of bovine *rotavirus* grown on MA 104 cells

	PQ	NCDV	S 14	S 77
Mean	12.379	5.886	12.246	14.806
Standard deviation	0.806	0.466	0.954	0.944

Values are in mm², calculated from 50 plaques.

t-test showed that the American attenuated strain (NCDV) produced significantly smaller plaques than the other strains ($P < 0.0001$); the plaques induced by the Belgian strain S 77 were larger than those induced by S 14 and PQ. These two last strains did not differ significantly in their mean plaque size. The four strains of bovine *rotavirus* inoculated on BSC-1 cells induced much smaller plaques. Statistical analysis was only performed on the PQ strain. The mean and the standard deviation of plaque size are given in Table II. The

analysis of variance showed that the two populations were quite different (Fig. 1).

The plaque assay technique is a valuable method for the study of variants of viruses as well as for the effect of some antiviral compounds (Jetteur et al., 1979; Pastoret et al., 1979; Schwerts et al., 1980). Estes and Graham (1980) observed differences in plaque size produced by *rotaviruses* of three mammalian species; simian, bovine and porcine. Our results with the plaque assay technique indicate that the American attenuated strain of *rotavirus* (NCDV) differs strongly from the three other strains. The Canadian strain (PQ) and the Belgian strain S 14 are similar, but the strain S 77 differs to a lesser degree from S 14 and PQ. Thus, the American attenuated strain (NCDV), which is used as a vaccine, induces smaller plaques than the other strains tested. This characteristic may be used as a marker to detect the NCDV strain. We were able to demonstrate differences in plaque size of four uncloned strains of *rotavirus* isolated from the same species (cattle). Our results, added to those of Verly and Cohen (1977) and Spence et al. (1978), agree with the hypothesis that differences do exist between strains of bovine *rotavirus* in nature. In our hands, BSC-1 cells proved to be less sensitive for *rotavirus* growth since the titres on these cells were lower and the plaques smaller.

TABLE II

Mean and standard deviation of plaque size of bovine rotavirus strain PQ grown on MA 104 cells and BSC-1 cells

	PQ	
	MA 104	BSC-1
Mean	12.379	2.648
Standard deviation	0.806	0.254

Values are in mm^2 , calculated from 50 plaques and 36 plaques from PQ on MA 104 and PQ on BSC-1 cells, respectively.

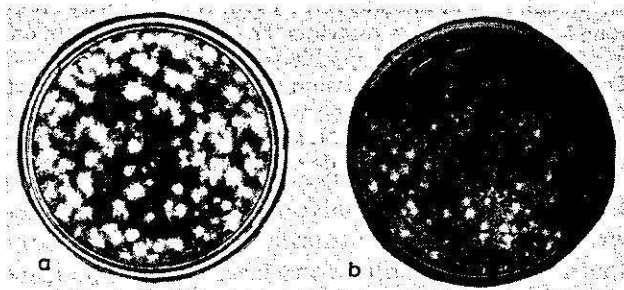


Fig. 1. Plaques formed by the Canadian strain PQ of bovine rotavirus: (a) on MA 104 cells; (b) on BSC-1 cells.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to Dr. R.S. Roy and Dr. M.E. Bégin (Veterinary School of the University of Montreal, St-Hyacinthe, Quebec), Dr. C.A. Mebus (Nebraska) for the *rotavirus* strains, Prof. E.H. Bohl (O.A.R. D.C., Ohio) for the MA 104 cells, Mr. Francis Claeys for the photo, and Mr. Michel Gobert, Miss Annick Verheyden and Mr. Sébastien Cesa for their assistance in this project. This project was supported by grants from the Institute for Encouragement of Scientific Research in Industry and Agriculture (I.R.S.I.A.). L. Dagenais is a scientific host of the University of Liège.

REFERENCES

- Babiuk, L.A., Mohammed, K., Spence, L., Fauvel, M. and Petro, R., 1977. Rotavirus isolation and cultivation in the presence of trypsin. *J. Clin. Microbiol.*, 6: 610-617.
- Bohl, E.H., 1979. Diagnosis of diarrhea in pigs due to the transmissible gastroenteritis virus or rotavirus. Colloquium of INSERM; Viral Enteritis of Humans and Animals, INRA, pp. 341-343.
- Dagenais, L., Lansival, B., Pastoret, P.-P., Massip, A. and Kaeckenbeeck, A., 1980. Absence de rotavirus dans le méconium des veaux. *Ann. Med. Vet.*, 124: 303-307.
- Dagenais, L., Pastoret, P.-P., Massip, A., Kaeckenbeeck, A. and Lansival, B., 1981. Failure to isolate rotavirus in bovine meconium. *Vet. Rec.*, 108: 11-13.
- Estes, M.K. and Graham, D.Y., 1980. Identification of rotaviruses of different origins by the plaque-reduction test. *Am. J. Vet. Res.*, 41: 151-152.
- Jetteur, P., Pastoret, P.-P., Aguilar-Setién, A., Leroy, P., Godart, M. and Schoenaers, F., 1979. Différenciation de souches du virus de la rhinotrachéite infectieuse bovine (Bovid Herpesvirus 1) fondée sur la dimension moyenne des plages. *Ann. Med. Vet.*, 123: 115-121.
- Matsuno, S., Inouye, S. and Kono, R., 1977. Plaque assay of neonatal diarrhea virus and the neutralizing antibody in human sera. *J. Clin. Microbiol.*, 5: 1-4.
- Pastoret, P.-P., Jetteur, P., Aguilar-Setién, A., Leroy, P., Schwes, A., Godart, M. and Schoenaers, F., 1979. Effet de la dexaméthasone sur le titre et la taille des plages du virus de la rhinotrachéite infectieuse bovine (Bovid Herpesvirus 1, IBR). *Ann. Med. Vet.*, 123: 397-402.
- Schwes, A., Pastoret, P.-P., Vindevogel, H., Leroy, P., Aguilar-Setién, A. and Godart, M., 1980. Comparison of the effect of trisodium phosphonoformate on the mean plaque size of pseudorabies virus, infectious bovine rhinotracheitis virus and pigeon herpesvirus. *J. Comp. Pathol.*, 90: 1-9.
- Spence, L., Fauvel, M., Petro, R., Gillani, A. and Babiuk, L.A., 1978. A preliminary study of antigenic relationships among isolates of bovine rotavirus. *Proc. 2nd. Int. Symp. on Neonatal Diarrhea*, University of Saskatoon, Canada, pp. 263-272.
- Verly, E. and Cohen, J., 1977. Demonstration of size variation of RNA segments between different isolates of calf rotavirus. *J. Gen. Virol.*, 35: 583-586.
- Vindevogel, H., Pastoret, P.-P., Burtonboy, G., Gouffaux, M. and Duchatel J.-P., 1975. Isolement d'un virus herpès dans un élevage de pigeons de chair. *Ann. Rech. Vet.*, 6: 431-436.