Serological Comparison of Hemagglutinating Encephalomyelitis Viruses Isolated from Different Outbreaks

A. S. Greig and A. Girard*

SUMMARY

Examination by the HI test of 20 representative HEV isolates reveals that only one of them, HEV-18 differs quantitatively from the others. The difference is confirmed qualitatively by the SN test. Both the HI and SN tests demonstrated that all the other HE viruses, though isolated from oubreaks of disease in widely separated areas of Canada over a period of eight years, represent a single strain.

RÉSUMÉ

On a étudié 20 échantillons du virus hémagglutinant de l'encéphalomyelite porcine. Les réactions d'inhibition de l'hemagglutination et de séro-neutralisation ont permis de conclure que tous les échantillons, excepté le HEV-18, semblent appartenir antigéniquement à une seule souche. En effet, ces résultats coincident avec l'isolement répété d'une seule souche HEV en des régions du Canada très éloignées les unes les autres.

INTRODUCTION

Hemagglutinating Encephalomyelitis Virus (HEV) was first isolated in 1960 (3) from the brain of a piglet suffering a viral encephalomyelitis. Since then HEV has been isolated from more than 30 different outbreaks of encephalomyelitis in Canadian pigs. Identification of the agent in each case was based on three criteria, namely, the formation of multinucleated giant cells (polykaryocytes) in primary pig kidney (PK) cell cultures, production of hemagglutinins for chicken erythrocytes in culture fluids and the inhibition of hemagglutination by specific antiserum.

As the identity of each isolated virus was confirmed, it was assigned a serial number starting with HEV-1, and placed in storage either as frozen or lyophilyzed culture fluids.

The criteria used in identification served to demonstrate hemagglutinins common to each of the viruses but did not define the degree of antigenic relationship. A study was therefore undertaken to compare more closely a selected number of the stored viruses by means of cross hemagglutination-inhibition tests (HI) and reciprocal cross neutralizations tests. Since HEV is not widely known, a brief review of clinical and pathological findings precedes the results of the serological tests reported here.

CLINICAL AND PATHOLOGICAL REVIEW

HEV was first isolated from the brain of a pig originating on a farm in Eastern Ontario and was named HEV-1. All the following isolations up to serial number HEV-13 were from various parts of Southern Ontario. HEV-14 was isolated from an outbreak in Quebec province, near Ottawa. In 1964 an isolation was made from a specimen submitted from the province of

*Animal Pathology Division, Health of Animals Branch, Canada Department of Agriculture, Animal Diseases Research Institute, P.O. Box 1400, Hull, Quebec.

Alberta. Thereafter, with the exception of HEV-21 all subsequent virus isolations have been made from disease outbreaks in Alberta.

Clinical histories with one exception were the same in both the Eastern and Alberta outbreaks (5). Generally entire litters under two weeks of age were affected, but only one or two litters on any one premises. A few days after birth affected piglets stopped nursing, huddled in a corner, shivered and were reluctant to move. This was followed by signs of nervous disorder including vomition, ataxia, hyperaesthesia, incoordination of movements, weakness, paddling movements while lying on the side and finally death two or three days after the onset of symptoms. Sometimes pigs died with few preceding signs of illness. On a few occasions the sow was observed to go off feed for a day or two but no other external signs of disease involvement were seen; however, she rapidly developed antibodies to HEV and these were detectable in the serum and in colostrum of neighboring sows on the premises subsequent to the initial outbreak.

Histological examination of the brains of affected piglets showed a viral type of encephalomyelitis involving the mesencephalon, pons, medulla oblongata and spinal cord. The lesions consisted of perivascular cuffing with mononuclear cells, neuronal degeneration and the formation of glial nodes.

The one exception to these findings was in the pig which yielded HEV-18. In this case the pig was four weeks of age, and histopathological examination of the brain failed to reveal evidence of encephalomyelitis.

Experiments on infectivity have shown that HEV cultivated in primary PK cell cultures will produce viral encephalomyelitis in susceptible pigs less than two weeks of age when administered orally or intracranially. Older pigs show no signs of disease but develop high levels of humoral antibodies (1).

MATERIALS AND METHODS

Sera

Five positive sera and one negative control serum were used in this study. The negative serum was from a three month old specific pathogen free (SPF) pig. The five positive sera were produced by the inoculation of SPF pigs with virus grown in cell culture. These sera were selected because of certain characteristics of their associated viruses. HEV-1 and 21 had been used extensively in pathogenicity studies. HEV-21A and 21B differed only in originating in pigs of different ages. HEV-21A was a sow while 21B was a three month old animal. HEV-2 represented the type strain for HA antigen production. HEV-18 had a slightly different history of origin than the others. They are identified as Hemagglutinating Encephalomyelitis Antisera (HEA) — 1, 2, 18, 21A and 21B.

Antigens

Twenty antigens were produced from the supernatant fluids of cell cultures infected with HEV as previously described (4). The only difference was that Eagle's basal medium without serum was employed for cell maintenance rather than the Lépine's medium used previously. In most cases it was possible to use the fluids directly as hemagglutinating antigen (HA) after appropriate dilution. Occasionally insufficient titres required that the antigens be concentrated by centrifugation of the fluids at 50,000 g for two hours in an angle rotor and resusension of the precipitate in Eagle's basal medium to about one-tenth original volume.

Hemagglutinaton Inhibition

Hemagglutination-inhibition (HI) tests were carried out as previously described (2). Briefly, 0.25 ml amounts of antigen, diluted to contain four HA units were added to equal amounts of two-fold dilutions of serum from 1:10 to 1:2560. After 30 minutes of incubation at room temperature, 0.5 ml of 1:200 washed chicken erythrocytes were added to each serum-virus mixture. The result was read within an hour according to the pattern of settling.

Serum Neutralization Test

The four viruses for which specific immune sera were available were used in reciprocal serum neutralization (SN) tests. The same sera as used in the HI test served for SN with no further treatment. Two-fold serum dilutions from 1:5 to 1:320 were employed and mixed with suspensions of virus calculated to contain a final 100 cell culture infectious doses ($CCID_{50}$) per ml measured by the 50 per cent end-point

method. Three PK culture tubes were inoculated with each serum-virus mixture, after an incubation period of one hr at 37°C. The results were read following three days incubation at 37°C on the basis of cytopathic effects. End-points were checked by measuring the presence or absence of HA in the culture fluids.

RESULTS

The results of the HI tests are recorded in Table I. It is evident that reactions occurred to a greater or lesser degree in practically every serum-virus mixture. The only exceptions were between HEA 2 and antigen HEV-9 and between HEA 18 and antigen HEV-4. In general it was confirmed that a common antigen existed among all of the viruses tested.

Examination of the figures on the table horizontally shows that in most instances the homologous serum-virus mixtures resulted in higher titres than did the heterologous mixtures. However, antigen HEV-1 was an exception to this rule since a markedly higher titre occurred with HEA 21 than with HEA 1.

Vertical examination of the figures, on the other hand, shows that with HEA 1, 2, and 21A and B, there are heterologous titres equal to or greater than the homologous titres. Only in the case of HEV-18 is the homologous titre greater than any of the heterologous reactions. HEV-18 also reacted in a low dilution with the control serum.

The serum-virus neutralization test results are shown in Table 2. Here it is very plain that HEV-18 is antigenically different to the other virus serials. Again it is noted that heterologous titres often exceeded the homologous.

DISCUSSION

The results of the HI and SN tests reveal a number of significant facts. They show that of the 20 selected viruses, only one is in any way different. This difference between HEV-18 and the other virus serials is quantitative in the HI test but clearly qualitative in the SN test. Of greater interest is the demonstration that the other 19 virus serials are closely related antigenically and undoubtedly consist of a single virus strain. These facts confirm the evidence suggested by clinical and histopathological observations that porcine encephalomyelitis in suckling pigs in Canada has a specific etiology common to disease outbreaks in widely separated areas, and has occurred over a period of at least eight years. In the pig-raising areas of Alberta and Ontario HEV may be considered an

TABLE I Comparative HI Titres of Six Sera Tested with Twenty HEV Antigens.

Virus Number	Date of Isolation	Origin	HEA-1 Serum	HEA-2 Serum	HEA-18 Serum	HEA-21A Serum	HEA-21B Serum	Normal Serum
HEV-1	June 1961	O	*80*	160	40	640	160	0
HEV-2	Aug. 1961	O	640	*640*	80	640	640	0
HEV-3	Nov. 1961	O	640	160	20	640	1280	0
HEV-4	Nov. 1961	O	320	160	0	320	1280	0
HEV-9	July 1963	O	640	0	40	1280	1280	0
HEV-10	Sept. 1963	O	320	40	10	640	640	0
HEV-11	Nov. 1963	O	320	320	80	640	640	0
HEV-12	Nov. 1963	O	160	160	40	640	640	0
HEV-14	Feb. 1964	Q	320	320	10	640	1280	0
HEV-15	Aug. 1964	O	160	160	10	640	640	0
HEV-16	Aug. 1964	A	80	160	40	640	320	0
HEV-18	Nov. 1964	A	160	80	*320*	80	160	20
HEV-19	July 1965	A	640	1280	160	640	160	0
HEV-21	June 1966	O	320	640	160	*1280*	*1280*	0
HEV-23	Aug. 1966	A	160	320	10	320	160	0
HEV-24	Dec. 1966	A	80	160	10	320	320	0
HEV-25	Dec. 1966	A	320	320	80	1280	320	0
HEV-26	Apr. 1967	A	640	1280	80	640	160	0
HEV-27	Sept. 1967	A	160	320	20	320	320	0
HEV-29	Oct. 1967	A	80	80	20	80	320	0

Numerals refer to the reciprocal of the highest serum dilution showing complete inhibitory properties. Italicised numerals show homologous titres. Origin: O — Ontario, Q — Quebec, A — Alberta

TABLE II Comparative Titres of Reciprocal Serum-virus Neutralization Tests.

Virus	Antisera				
	HEA-1	HEA-2	HEA-18	HEA-21A	Normal Serum
HEV-1	*160*	160	NEG	320	NEG
HEV-2	160	*320*	NEG	1280	NEG
HEV-18	NEG	NEG	*40*	NEG	NEG
HEV-21	640	640	NEG	*1280*	NEG

Numerals refer to reciprocals of highest serum dilutions showing complete neutralization of virus. Italicized numerals indicate homologous reactions.

important minor disease.

Critical examination of the HI results suggest that the titres from this test are not suitable in assessing degrees of antigenic relationship. A wide range of titres were obtained which revealed no obvious pattern. In some instances heterologous titres equalled or exceeded homologous titres. This also occurred in the SN test, which however, exhibited more specificity in regards HEV-18. However, the very lack of specificity, indicated by the variations in titre found among the 19 virus serials, is an indication of their close antigenic similarity.

By hindsight it is easy to visualize that HEV-18 could differ from the others on consideration of clinical findings. It was isolated from pigs older than usual in which clinical signs and histopathological changes were less suggestive of an encephalomyelitis. The virus isolated, however, fitted the original criteria for identification as HEV. The significance of HEV-18 in any disease syndrome has yet to be determined.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the technical assistance of Mr. R. Hogan. They are indebted also to Dr. R. J. Avery and Dr. G. L. Bannister for suggestions on preparation of the manuscript.

REFERENCES

1. **APPEL, M., A. S. GREIG,** and **A. H. CORNER.** Encephalomyelitis of swine caused by a hemagglutinating virus. IV. Transmission studies. Res. vet. Sci. **6**: 482-489, 1965.
2. **GIRARD, A., A. S. GREIG,** and **D. MITCHELL.** Encephalomyelitis of swine caused by a hemagglutinating virus. III. Serological studies. Res. vet. Sci. **5**: 294-302, 1964.
3. **GREIG, A. S., D. MITCHELL, A. H. CORNER, G. L. BANNISTER, E. B. MEADS,** and **R. J. JULIAN.** A hemagglutinating virus producing encephalomyelitis in baby pigs. Can. J. comp. Med. **26**: 49-56, 1962.
4. **GREIG, A. S.** and **A. GIRARD.** Encephalomyelitis of swine caused by a hemagglutinating virus. II. Virological studies. Res. vet. Sci. **4**: 511-517, 1963.
5. **MITCHELL, D.** Encephalomyelitis of swine caused by a hemagglutinating virus. I. Case histories. Res. vet. Sci. **4**: 506-510, 1963.

Book Review

Problems in Hospital Law. *Published by Aspen Systems Corporation, Pittsburgh, Pennsylvania. 1968. 203 pages. Price $10.00.*

This treatise is devoted to the legal aspects of the operation of a modern hospital. It covers the legal responsibilities of the governing board, the administrators, the medical staff and other staff from nurses to janitors, including auxiliary and volunteer activities. The problems which may occur are well illustrated by legal cases which have occurred and have been settled in courts in various states of the United States of America.

The book is exceptionally well organized and written in layman's language. It will serve a very useful purpose as a handy desk reference for hospital administrators. *W. Henderson.*