## Human astrovirus serotypes

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## SUMMARY

Two serotypes of human astroviruses are described. It is proposed that these should be called serotype 1 and serotype 2. Using antisera to these two types, 13 of 15 other community-acquired strains were able to be typed, 12 as serotype 1 and one as serotype 2.

Astroviruses are recognized causes of gastroenteritis (Kurtz, Lee & Pickering, 1977; Ashley, Caul & Paver, 1978). Recently, successful serial propagation of this virus in tissue culture has been achieved by incorporating trypsin in the maintenance medium (Lee & Kurtz, 1981) and this has simplified the raising of antisera to the virus in experimental animals. This paper reports the results of typing astrovirus strains derived from community-acquired cases using two anti-astrovirus sera raised in rabbits.

For antiserum production, a continuous line of rhesus monkey kidney cells (LLMCK<sub>2</sub>) were grown to confluence in two winchester bottles. They were heavily infected with the cell-adapted astrovirus and maintained in serum-free 199 medium containing 100 units/ml penicillin, 100 units/ml kanamycin, and  $10 \mu g/ml$  trypsin at 35 °C for 48 h. The cells and fluid were then harvested, frozen once and thawed, sonicated, and centrifuged at 2200 g for 30 min to deposit the cellular material. The virus was pelleted by further centrifugation at 160000 g for 2 h and resuspended in 0.5 ml phosphate buffered saline (PBS). Titration of astrovirus suspension showed that there were 10<sup>8</sup> infectious particles per ml. After treatment with trichlorotrifluoroethane (BDH Chemicals Ltd.) the suspension was layered on to a caesium chloride gradient (0.625 g/ml) and centrifuged at 120000 g for 18 h. The virus containing fractions were pooled, dialysed against PBS and made up to 3 ml. A rabbit was immunized by three weekly intravenous inoculations of 1 ml of this material. Seven days after the third dose the rabbit was bled and the serum tested for astrovirus antibodies by an indirect immunofluorescence technique. Acetone fixed astrovirus infected LLMCK2 cells or human embryo kidney cells were used as antigen.

Antisera against two strains of astrovirus, DM and JS, were produced in this way. They were then titrated against the homologous and heterologous strains. The table gives the reciprocal titres of these sera and demonstrates an antigenic difference between the two strains. Also shown in the table are the results of typing 15 other community-derived strains of astrovirus, collected between 1976 and 1982, with these two antisera. Antiserum-DM had a titre of 6400–12800 against 12 of the strains and a titre of 80 against two others. The 15th strain failed to react with antiserum-DM at a dilution of 1 in 20 but it reacted with antiserum-JS to

Astrovirus strain	Antiserum	
	DM	$\overline{\rm Js}$
$\mathbf{DM}$	12800*	< 20
JS	< 20	3200
1	12800	< 20
<b>2</b>	6400	< 20
3	$\boldsymbol{6400}$	< 20
4	12800	< 20
5	$\boldsymbol{6400}$	< 20
6	$\boldsymbol{6400}$	< 20
7	12800	< 20
8	$\boldsymbol{6400}$	< 20
9	6400	< 20
10	$\boldsymbol{6400}$	< 20
11	$\boldsymbol{6400}$	< 20
12	$\boldsymbol{6400}$	< 20
13	80	< 20
14	80	< 20
15	< 20	3200

Table 1. Titre of antisera to 17 astrovirus strains

\* Titre of antiserum.

a titre of 3200. Antiserum-JS did not react with the other 14 strains at a dilution of 1 in 20.

In order to see whether either of these two strains – DM or JS – was antigenically related to an animal strain, antisera to the bovine astrovirus (kindly provided by Dr J. D. Bridger, A. R. C. Compton) and the ovine astrovirus (kindly provided by Dr D. Snodgrass, Moredun Institute, Edinburgh) were titrated against the two human strains. Neither antiserum reacted with either human strain.

It is clear that there are at least two serotypes of human astrovirus. Further study of more astroviruses including the two strains mentioned above that did not go to titre with either antiserum-DM or antiserum-JS, will most probably reveal the existence of additional serotypes. In the meantime it may be useful to number the serotypes herein described as serotype 1 (strain DM) and serotype 2 (strain JS). This, it is hoped, will avoid the confusion of many classifications arising as new strains are found.

## REFERENCES

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