

From poliovirus surveillance to enterovirus surveillance: a complete picture?

Since the poliovirus eradication programme was settled by the World Health Assembly in 1988, considerable efforts have been made to improve and standardize virus isolation procedures, typing, and subtyping of polioviruses as wild-type or vaccine-derived strains (Aylward *et al.*, 2000). Poliovirus surveillance includes systematic virus isolation from faeces samples of acute flaccid paresis cases, and in some countries also virus isolation from sewage and environmental samples (Manor *et al.*, 1999; World Health Organization, 1997).

Fortunately, these virus isolation procedures also detect non-polio enteroviruses (NPEV), either because these are the aetiology of flaccid paresis in some cases or, if not related to flaccid paresis, because they are shed with faeces as innocent bystanders. NPEV are endemic worldwide and multiple infections with various of the more than 70 types are usual. Symptoms of NPEV infection may be mild and subclinical, or the infection can present as a common cold, summer grippé, foot and mouth disease, Bornholm's diseases and haemorrhagic conjunctivitis, for example.

However, NPEV are occasionally related to more serious illnesses, for example aseptic meningitis, life-threatening myocarditis and hepatitis, and are probably associated with juvenile diabetes mellitus type 1 (Lonnrot *et al.*, 2000; Salminen *et al.*, 2003). Compared to the number of NPEV infections, these serious organ infections are rather rare events, with a frequency quite similar to that of poliomyelitis anterior, which is an infrequent organ manifestation of poliovirus wild-type infection, and an extremely rare complication of poliovirus vaccine strains (Friedrich, 1998). In contrast to our knowledge of poliovirus, our knowledge of NPEV incidence, risk factors for serious organ manifestation and the association of certain serotypes with serious clinical manifestations of NPEV infections is rather incomplete. Precise information on the epidemiology of NPEV is fundamental for understanding the association of NPEV with serious diseases. Fortunately, typing

procedures for poliovirus and NPEV have been improved significantly with the development of PCR protocols amplifying the neutralizing epitopes of the VP1 capsid protein and with the development of molecular typing by sequencing (Caro *et al.*, 2001; Norder *et al.*, 2001; Oberste *et al.*, 1999). Thus typing of most enteroviruses and the simple identification of new, as yet untyped, enterovirus types is feasible (Bailly *et al.*, 2004; Norder *et al.*, 2003; Oberste *et al.*, 2001).

Bahri *et al.* (2005) in this issue of the journal describe the epidemiology of NPEV and poliovirus in Tunisia between 1992 and 2003. Their study shows more or less constant circulation of a few serotypes (echovirus 6, 11 and 30) and occasional isolation of many other NPEV types which may have been imported and transmitted epidemically over a limited time period. The combination of such an NPEV epidemiology dataset with information on the incidence of NPEV-associated diseases such as myocarditis or diabetes mellitus may give new insights into the association of certain serotypes with disease burden.

For estimating the validity of NPEV epidemiology data generated as a side product of poliovirus surveillance, sampling strategies have to be considered. Inclusion of environmental samples is favourable (Manor *et al.*, 1999) whereas focusing on acute flaccid paresis cases will probably bias the data in such a way that neurotropic NPEV types are over-represented. Inclusion of diagnostic samples from patients with NPEV-associated diseases, for example aseptic meningitis, will also bias the results. Nevertheless, such a strategy is justified if the study investigates the association of NPEV types with a certain disease.

Besides sampling strategies, virus isolation procedures may bias the results. Several coxsackievirus A (CAV) serotypes of the species *Human enterovirus A* are hard to isolate on cell cultures and require animal experiments with suckling mice for virus

isolation. These are not routinely performed in most laboratories. Fortunately, RD cells are recommended by the World Health Organization for poliovirus surveillance. Use of RD cells and of the shell vial technique clearly improves isolation of CAV serotypes but some serotypes and strains even fail to replicate on RD cells (Perez-Ruiz *et al.*, 2003; Schmidt *et al.*, 1975). Thus poliovirus surveillance efforts may produce some data on CAV circulation but some CAV types are still overlooked by this approach, leaving the picture of enterovirus surveillance somewhat incomplete.

PCR protocols for the amplification of the conserved 5' non-translated region (5'NTR) of the genetically highly diverse and variable enteroviruses are promising as fast, sensitive and simple tools to detect these viruses directly from clinical and environmental samples. Several diagnostic 5'NTR PCR protocols have been developed since the late 1980s (Chapman *et al.*, 1990; Hyypia *et al.*, 1989; Rotbart *et al.*, 1994) that should also cover CAV. Although diversities between primer or probe sequences and some enterovirus types may yield false-negative results occasionally (Heim & Schumann, 2002; Rotbart *et al.*, 1994), enterovirus 5'NTR PCR protocols have already improved the diagnosis of aseptic meningitis from cerebrospinal fluid samples, which contain only low virus concentrations compared to stool samples (Read & Kurtz, 1999; Vuorinen *et al.*, 2003).

Nevertheless, these PCR protocols have drawbacks of their own. DNA amplified by 5'NTR PCR is not appropriate for molecular typing because of low sequence diversity in this region and frequent recombination events resulting in enteroviruses with divergent sequences between their 5'NTR and their coding regions for neutralizing epitopes (Lindberg *et al.*, 2003; Oberste *et al.*, 2004a). Sequencing of 5'NTR amplicons may help only in the search for infection chains. Therefore, positive samples in 5'NTR PCRs must be re-tested and typed by less sensitive methods: either by VP1 PCRs and

sequencing or by virus isolation and neutralization. Although sequences of all members of the species *Human enterovirus A* (including most CAV which are hard to isolate on cell cultures) are now available (Oberste *et al.*, 2004b), VP1 PCRs may fail in some cases for sensitivity reasons.

Inevitably, there will be an increasing number of 'non-typable' samples in future studies which may include 5'NTR PCRs. Thus our picture of enterovirus epidemiology will be a little clearer but not yet complete with the help of 5'NTR PCRs.

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