

Detection and Typing of Enterovirus in Cerebrospinal Fluid

Blair Rosen, Brett Slater, Michelle Dupuis, Rene Hull,
Rocco Ferrera, and Cinnia Huang

Wadsworth Center

New York State Department of Health

Griffin Laboratory

Albany, NY

Wadsworth Center

New York State Department of Health

Introduction

- Human enteroviruses (family Picornaviridae) cause a wide range of illnesses
 - aseptic meningitis, meningoencephalitis, and myocarditis.
 - Enteroviruses are responsible for 30,000 to 50,000 meningitis hospitalizations each year in the United States.
- The detection of enteroviruses in clinical samples is a service performed by many state health laboratories.
- Additional efforts by these laboratories to identify the virus serotypes offers potential benefits including:
 - I.D. of sporadic cases or epidemiologic links during an outbreak.
 - Information on currently circulating serotypes.
 - Determine illnesses associated with specific enterovirus serotypes.

- Traditional methods for the typing of enteroviruses are labor intensive:
 - Isolation of the viruses in cell culture.
 - Neutralization assays with antibodies.
- Molecular methods for enterovirus typing have recently been developed (RT-PCR and sequencing):
 - 3' half of genomic region encoding VP1 - molecular and serologic typing results have good agreement.
- The majority of the molecular typing studies that have been reported involve adaptation of viruses in clinical samples to cell culture prior to typing.

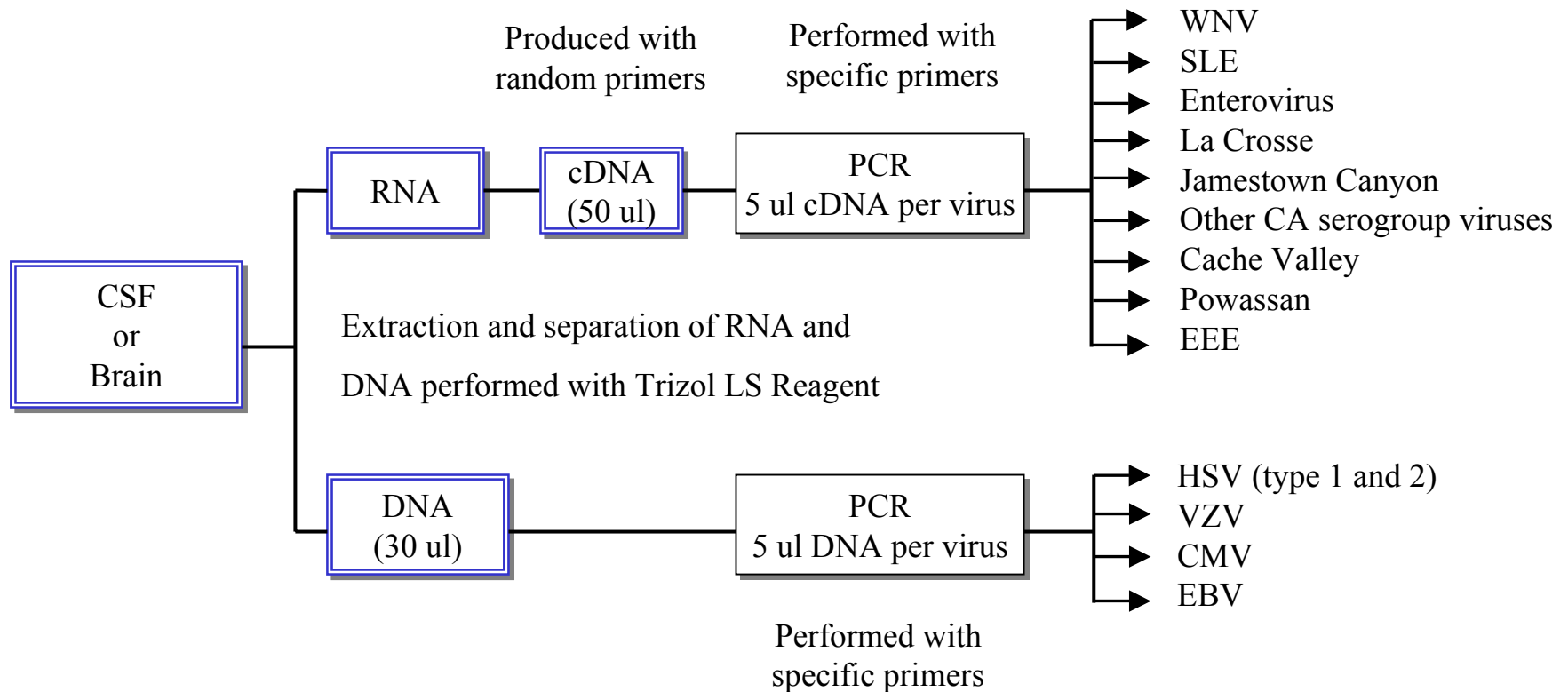
Objectives

1. Investigate the ability to type enteroviruses by PCR and sequencing with cDNA produced directly from viral RNA in clinical samples.
2. To determine the enterovirus types circulating in the New York State area.

Materials and Methods

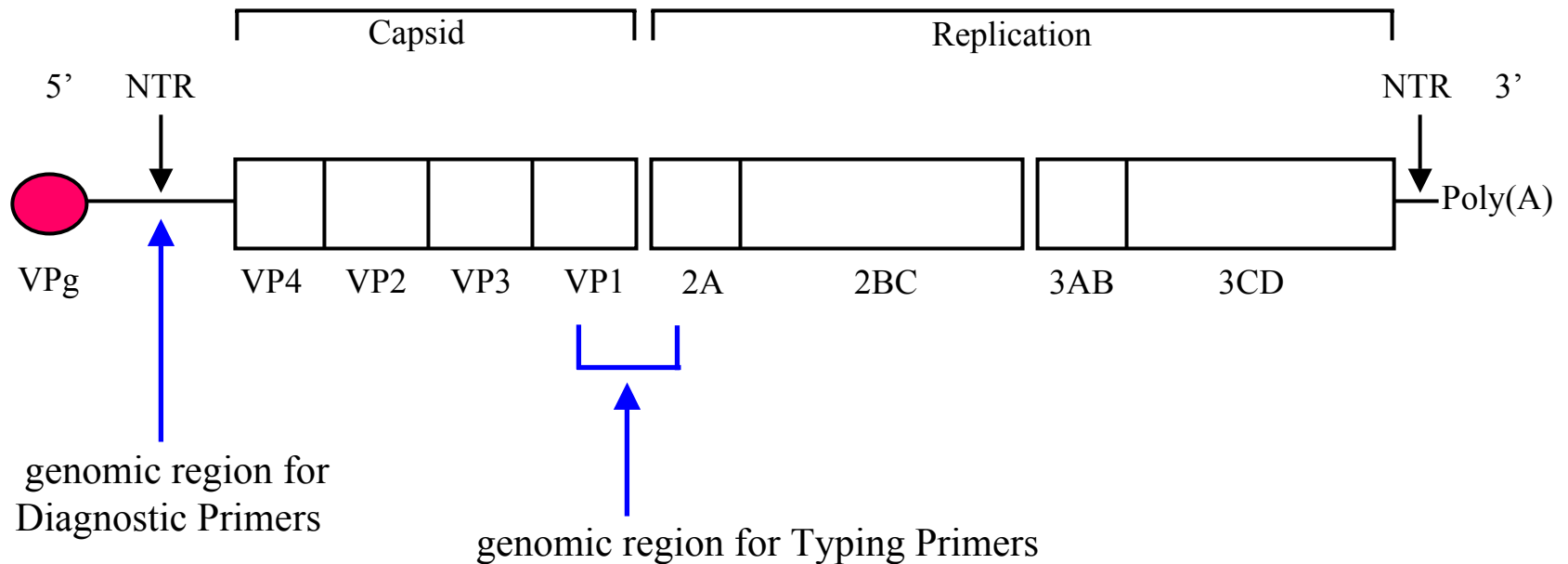
- Clinical Samples
 - Examined 1,545 cerebral spinal fluid samples, July 1997 to December 2001.
 - Samples were collected from hospitals and private clinics in New York State.
- PCR with Diagnostic Primers
 - Screening - was performed by agarose electrophoresis and ethidium bromide staining.
 - Positive Samples - were repeated (re-extraction, RT-PCR, agarose, sequencing).

Flow Chart of Encephalitis PCR testing



Routine procedure regardless of the number of agents requested to be screened

Regions of the Enterovirus Genome used for RT-PCR



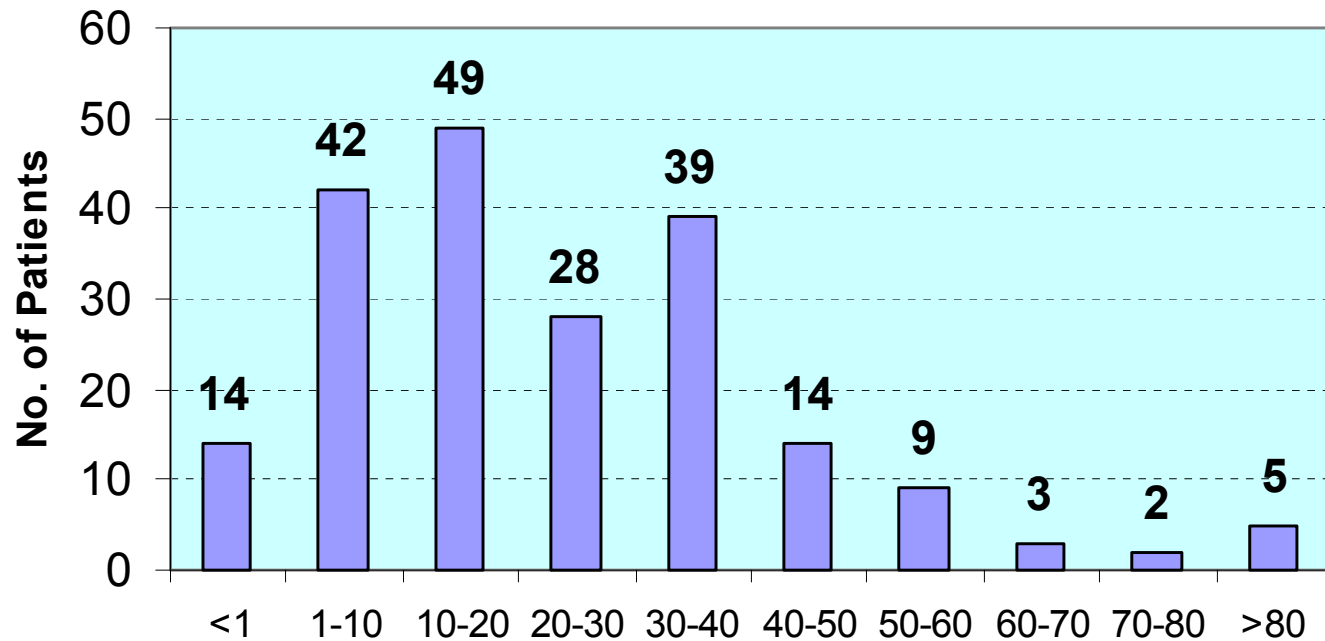
(Oberste et al., 2000, J. Clin. Micro., 38:1170-1174)

- Typing Procedure - PCR and sequencing
 - Primers and general PCR procedures have previously been described.

(Oberste et al., 2000, Comparison of classic and molecular approaches for the identification of untypeable enteroviruses. J. Clin. Microbiol. 38:1179-1174.
- Analysis of PCR Products
 - PCR products were examined in ethidium bromide stained agarose gels.
 - PCR amplicons of expected size were extracted and sequenced.

- Identification of the Enterovirus Strain.
 - Comparisons were made with sequences in GenBank using NetBlast
(Wisconsin Package Version 10.2, GCG).
 - The sequences were confirmed as those of enteroviruses.
 - Criterion for matching the enterovirus serotype.
 - Sequence identity of at least 75% with an enterovirus of known serotype.
 - The identity score of the next closest matching serotype is less than 70%.

Distribution by Age of PCR-Positive Enterovirus-Infected Patients



Distribution by Sex

Males = 113 patients

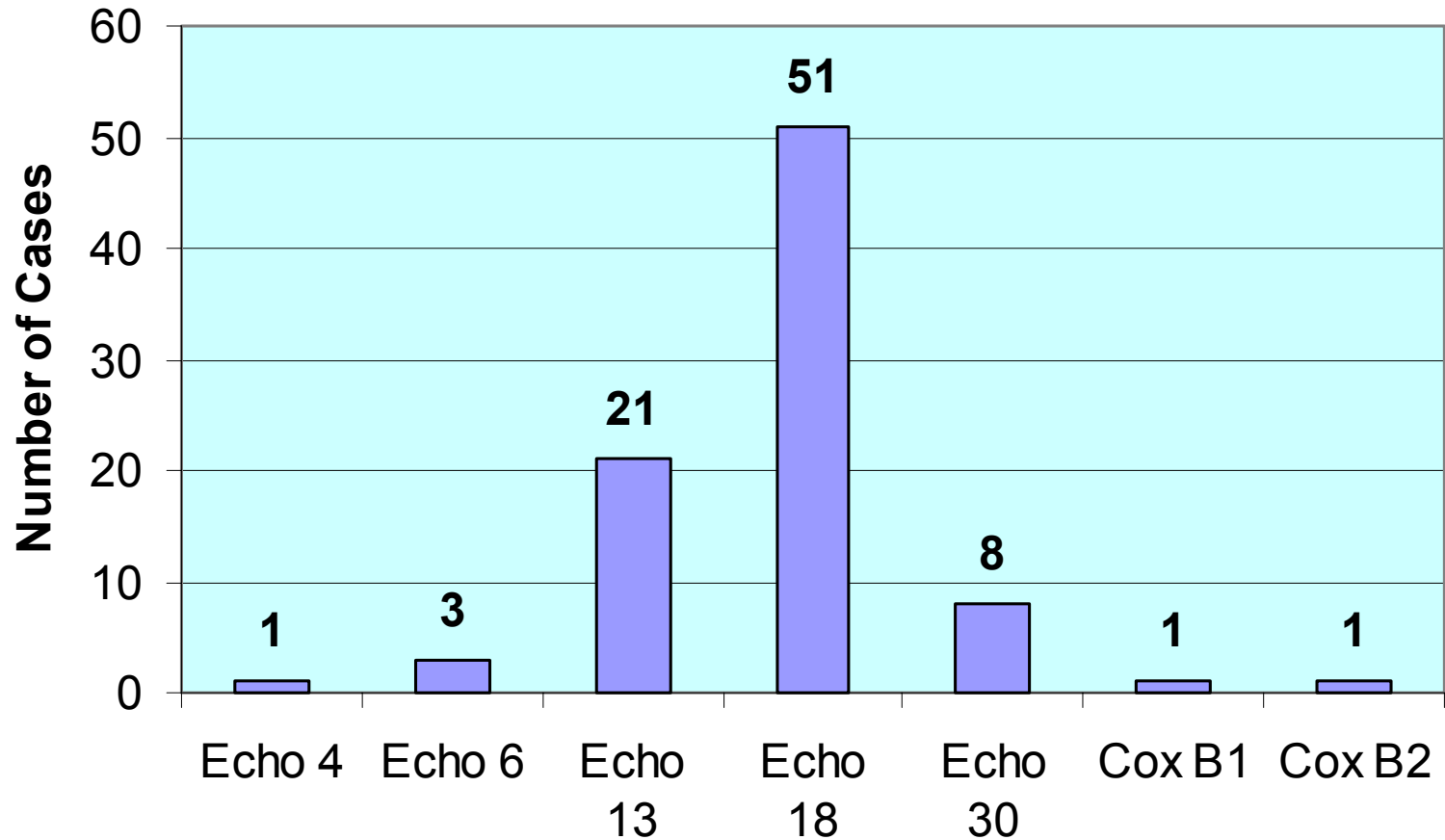
Females = 94 patients

Total Number of PCR-Positive

Enterovirus-Infected Patients = 207

Summary of Enteroviruses Detected in Year 2001- New York State

n = 86



Overall Enterovirus Typing Results for New York State - a project still in progress

	2001	2000	1999	1998	1997	Total
Echo 4	1					1
Echo 6	3					3
Echo 11			1			1
Echo 13	21	5	1			27
Echo 18	51		2	1		54
Echo 30	8	5	2	4		19
Cox A9		1				1
Cox B1	1					1
Cox B2	1					1
Cox B3			1			1
Cox B4				1		1
Cox B5		4				4
TOTAL	86	15	7	6	0	114

Enteroviruses 13 and 18 - Distribution by Age

Echovirus type 13 - United States, 2001, MMWR, 2001, Vol. 50, No. 36

Echovirus type 13 isolated from 76 patients in 13 states; March-June, 2001

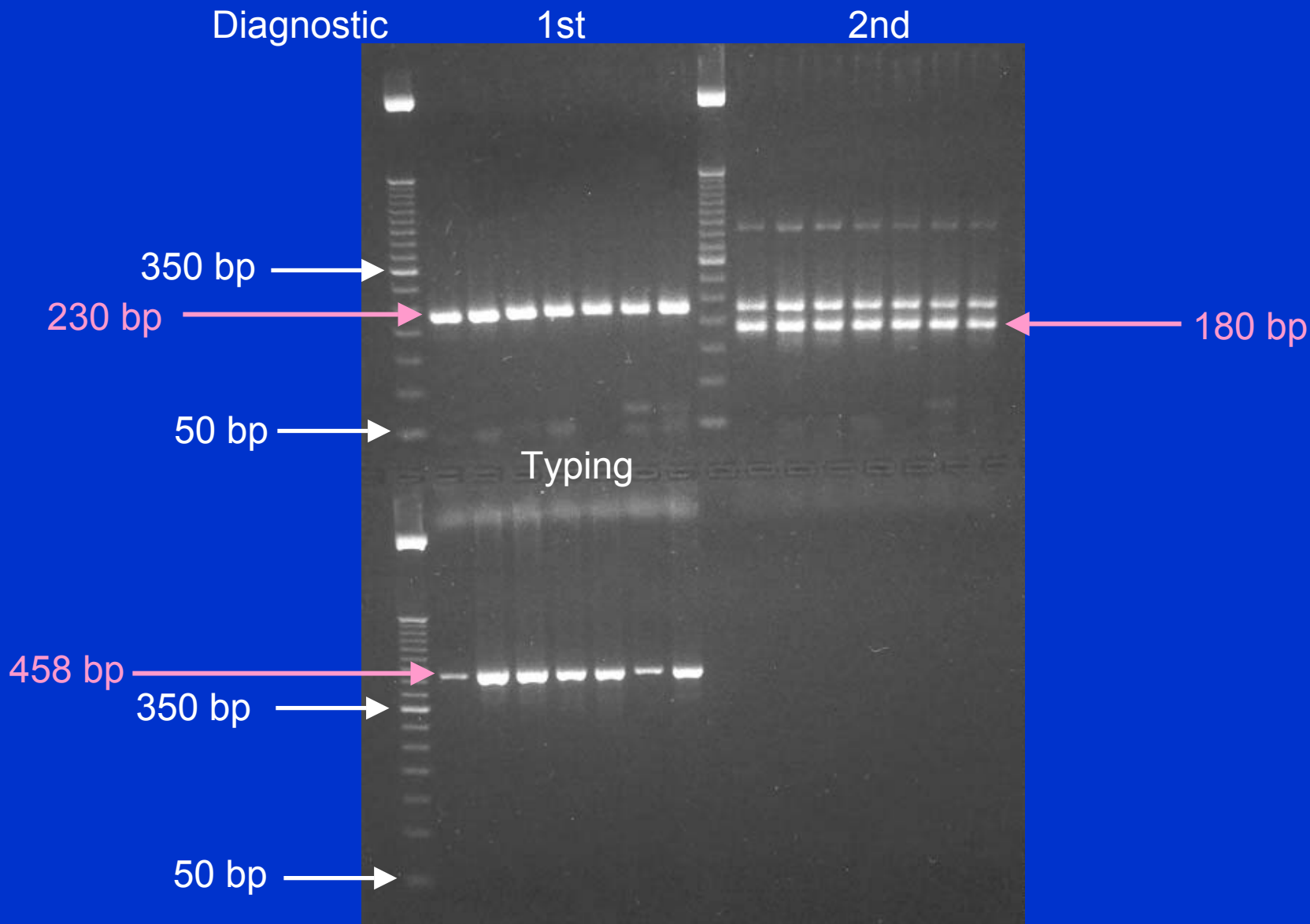
< 1 year old n = 41 (54%)

< 15 years old n = 73 (96%)

New York Study, 2001

Echovirus type 13		Echovirus type 18	
<u>Age (yr)</u>	<u>No. of people; n = 26</u>	<u>Age (yr)</u>	<u>No. of people; n =54</u>
<1	5 (19.2%)	<1	0
< 15	16 (61.5%)	1 to 10	9
≥15	10 (38.5%)	11 to 20	12
		21 to 30	16
		31 to 40	15
		>41	2
		people less than 15, n=15 (27.8%)	
		people 21 or older, n = 33 (61.1%)	

Example of PCR Products Obtained with Diagnostic and Typing Primers



Echoviruses 13 and 18 - Summary and Discussion

Echovirus 13 - recent trends

United States

- MMWR, Year 2001 - reported an increase in the detection of echovirus 13.
- Years 1970 to 2000 - Echovirus 13 accounted for 65 of approximately 45,000 enterovirus isolates reported to the CDC.

Worldwide

- Year 2000 increase in number of reports of echovirus 13 in Europe
- Year 2001 additional reports in Europe. Reports in Australia

Echovirus 18

New York State - first reported increase in echovirus 18

MMWR - October 2000, 49:913-916

1997 - 5.5%

1998 - 1.8%

1999 - 0.6%

Questions?

- Local geographic phenomena?
- Wider diagnostic problem - under reported?
 - Problems in adapting this strain to cell culture?
 - Harder to detect by serology?

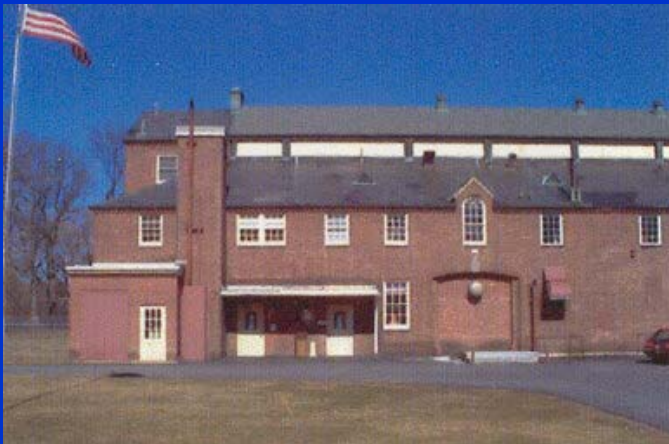
Acknowledgments

CDC, Division of Viral and Rickettsial Diseases, Respiratory and Enteric Viruses Branch

Steven Oberste

Mark Pallansch

CDC, EIP (Emerging Infectious Program) Grant



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