

Non-polio enterovirus isolation among families in Ulaanbaatar and Tov province, Mongolia: prevalence, intrafamilial spread, and risk factors for infection

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SUMMARY

Studies of non-polio enterovirus prevalence and transmissibility in developing countries are limited and few studies have investigated specific risk factors for infection. An epidemiological survey of non-polio enterovirus among families in Mongolia was conducted in the late summer of 2003. Stools of 122 healthy persons were collected weekly for 5 weeks. Eight serotypes of non-polio enteroviruses (echovirus 30, 33, 12, 25, coxsackievirus A10, A2, A4, A24) were isolated from 62 persons, with an overall isolation rate of 51%, and 64% and 35% among children under 10 years and adults over age 21 years. Fifty-four per cent of isolations were due to intrafamilial infection. Analysis of risk factors for infection suggested contamination of indoor kitchen, bathroom, toilet, and waste disposal area. Hand washing after defecation was protective against infection. Our study findings stress the importance of hand washing and cleaning hygienic facilities to prevent infection by enteric viruses in the home environment.

INTRODUCTION

Though most enterovirus infections are asymptomatic, enterovirus can become a serious public health concern with a capability of causing a spectrum of clinical illnesses such as paralysis, aseptic meningitis, encephalitis, herpangina, hand, foot, and mouth disease, upper respiratory disease, cardiac disease, and acute haemorrhagic conjunctivitis [1–3]. Enterovirus prevalence and transmissibility vary by factors such as climate, geography, crowding, and socio-economic status; previous studies among healthy individuals in different countries have shown diversity in isolation

rate [4–13]. However, studies of enterovirus, especially non-polio enterovirus, in developing countries are limited and few studies have investigated specific risk factors for infection.

In Mongolia, enteric diseases are widespread, imposing a heavy burden on the population's health. Hepatitis A is considered highly endemic: 8250 cases were clinically diagnosed as hepatitis A in 2000 among the national population of 2·7 million [14]. Diarrhoea is a significant problem resulting in a high infant mortality rate which was 51·97/1000 births as of 2002 [15]. Despite our knowledge that enteric diseases are endemic, no study has been done on non-polio enterovirus prevalence and transmissibility in Mongolia.

Therefore, this study aims to determine non-polio enterovirus prevalence in urban areas of Mongolia, to attempt isolation of enterovirus from hand, to assess

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the degree of intrafamilial spread of non-polio enterovirus as a measure of its transmissibility, and to investigate risk factors for non-polio enterovirus infection such as living environment and hygiene practices.

METHODS

Study site

This study was conducted from July to August 2003 in Ulaanbaatar and Tov province in Mongolia. Four districts were chosen from Ulaanbaatar: Chingeltei, Bayangol, Bayanzurkh, and Songinokhairkhan. From Tov province, Zuum mod (provincial centre) and Bayanchandmani (provincial district centre) were selected. Areas with different living environments such as type of residence and water supply were considered for site selection.

Areas within the six districts can be classified into three distinct categories: slum area in Ulaanbaatar, apartment area in Ulaanbaatar, and remote but accessible area from Ulaanbaatar (Zuum mod and Bayanchandmani). In Ulaanbaatar, *gers* (traditional Mongolian dwelling consisting of tent-like wooden structure covered with felt, no inside hygienic facilities) and houses (structure with one or more rooms, some with piped water and/or inside hygienic facilities) co-exist on dirt roads in slum areas, whereas apartment (provided with piped water and hygienic facilities) areas have paved roads and often have local shopping centres. Zuum mod and Bayanchandmani are located 45 and 80 km from Ulaanbaatar and are surrounded by grassland and gentle hills.

Study population and oral poliovirus vaccine (OPV) administration

Four to five infants who were scheduled for OPV immunization during July 2003 and their families were consecutively sampled in each of the six areas. An infant (study child), mother, father, and one youngest sibling were included in the study from each household. For some cases, a relative living in the same household as the study child, or a contact of a sibling of the study child who lived in a different household were sampled for investigation. OPV (SB Biologicals, Rixensart, Belgium) was administered to study children under routine immunization on the first week.

Sample collection

Stool specimens were collected from all participants at weekly intervals for 5 weeks. Swab samples were

taken from palms and fingers of 46 available subjects who took care of study children on the second week. Swabs soaked in 1 ml PBS (Nissui, Tokyo, Japan) were used. All samples were carried at 4 °C and stored at -20 °C. Interviews to determine living environment and hygiene practices were conducted using a semi-structured questionnaire. Questions on living environment of each household were asked of the mother or father, and questions on hygiene behaviour were asked of all subjects except study children. For children too young to answer questions, the mothers answered for them. This did not seem inappropriate as in the case of very young children the mothers instructed them in hygienic activities or they did those activities together. The questionnaire was reviewed and revised by health workers participating in the study.

Ethical approval was obtained from the University of Tokyo Ethical Committee, and approval was given by the Ministry of Health in Mongolia. Written informed consent was collected from all participants or their guardians.

Isolation and identification of enterovirus in stools

Virus isolation was done according to the WHO recommended method [16] with the following modifications. Stool extracts were treated with PBS containing 10% chloroform and stored at -20 °C until inoculation onto rhabdomyosarcoma (RD), HEp-2 and L20B cell lines. Isolation was conducted using 24-well plates. Two sequential passages of 7 days were performed in the three cell lines before recording as negative. Samples which showed a cytopathic effect (CPE) in RD and/or HEp-2 cells after 14 days of observation were passaged to L20B cells and observed for another week. For samples which showed CPE in L20B cells, identification for poliovirus was conducted. For samples which showed CPE in RD and/or HEp-2 cells but not in L20B cells, identification for non-polioviruses was performed using enterovirus antisera (RIVM, Bilthoven, The Netherlands). For samples which were poliovirus positive in L20B cells and also showed CPE in RD and/or HEp-2 cells, samples were analysed for concurrent non-polio enterovirus infection. A total of 50 µl of diluted tissue culture fluid from RD and/or HEp-2 cells was mixed with 50 µl of antiserum to poliovirus types 1+2+3 in RD and/or HEp-2 cells respectively, and observed for 1 week. For CPE-positive samples, virus fluid was re-inoculated onto

L20B cells to confirm the absence of poliovirus and observed for another week.

RNA extraction and RT-PCR

For non-poliovirus samples for which a virus serotype could not be identified by neutralization using the RIVM kit, viral RNA was extracted from 140 μ l of infected cell culture fluid using QIAamp Viral RNA kit (Qiagen, Hilden, Germany). Extracted RNA (5 μ l) was used for genomic amplification by RT-PCR using Access RT-PCR kit (Promega, Madison, WI, USA). RT-PCR was conducted using 50 pmol of primers OL68-1 (5'-GGTAAAYTTCCACCACCA-NCC-3', antisense) and EVP4 (5'-CTACTTTGGG-TGTCCGTGTT-3', sense) in the 5'NTR-VP4-VP2 region [17]. The condition for RT-PCR was 45 min at 48 °C, 2 min at 94 °C, 35 cycles at 94 °C for 10 s, 50 °C for 10 s, 65 °C for 1 min, and 5 min at 65 °C.

Sequencing analysis

Amplified products were purified using QIAquick PCR purification kit (Qiagen). Nucleotide sequencing reaction was performed using ABI PRISM BigDye Termination Cycle Sequencing kit with 3·2 pmol of each primer (Applied Biosystems, Foster City, CA, USA). Sequence data were collected using ABI PRISM 3100 Avant Genetic Analyzer (Applied Biosystems).

For non-polio enteroviruses, clusters of each virus type were made by Clustal W software after 1000 times bootstrapping [18], using their nucleotide sequences in the 5'NTR-VP4-VP2 region identified using OL68-1 and EVP4 primers. (The same method was used for the analysis of intrafamilial spread of non-polio enteroviruses.) One sample in each virus type was chosen and its virus type was determined by RT-PCR using 187 (5'-ACIGCIGYIGARACIGG-NCA-3', sense), 188 (5'-ACIGCIGTIGARACIGG-NG-3', sense), 189 (5'-CARGCIGCIGARACIGG-NGC-3', sense), 222 (5'-CICCIGGIGGIAYRWACAT-3', antisense), 012 (5'-ATGTAYGTICCCICCGIGG-3', sense), 040 (5'-ATGTAYRTICCIMCIGGIGC-3', sense), and 011 (5'-GCICCIGAYTGTGICCAA-3', antisense) in the VP1 region [19]. A total of 20 pmol of each antisense primer and 40 pmol of sense primer were used for the RT-PCR reaction with the same cycle condition as above.

Previously reported sequences used in the comparison were obtained from GenBank. Molecular-based enterovirus typing method described by

Oberste et al. [20] was used with nucleotide sequence data of VP1 region.

Restriction fragment length polymorphism (RFLP) analysis

For samples positive for poliovirus, RFLP analysis was conducted for intra-typic differentiation to distinguish between vaccine and wild polioviruses [21]. The purified PCR products were digested with *Dde*I, *Hpa*II, and *Hae*III (Takara, Kyoto, Japan) at 37 °C for 2 h. The digested fragments were electrophoresed on 2% agarose gel.

Detection of enterovirus on hands

RNA was extracted from 980 μ l PBS in which the swab was soaked. RT-PCR was conducted using 19 μ l RNA and 80 pmol of EVP4-OL68-1 primers and UG1-UC1 (5'-GAATTCCATGTCAAATCT-AGA-3', sense) primers [21] with the same condition as with stool samples. Subsequently, nested PCR was conducted using puReTaq Ready-To-Go PCR beads (Amersham Biosciences, Piscataway, NJ, USA) with 1 μ l PCR product of EVP4 and OL68-1 and 20 pmol of OL68-1 and OL24 (5'-CTACTTTGGGTGTCCG-3', sense) primers [22] in a 25 μ l reaction. PCR products were electrophoresed in 2% agarose gels.

Statistics

Univariate analysis was conducted to screen statistically significant ($P < 0\cdot05$) variables: χ^2 tests for categorical data and Student's *t* test for continuous data were adopted. Fisher's exact test was applied for data with small frequencies and Yates' correction was used with 2×2 categorical data. Logistic regression was performed for each significant variable and odds ratios were calculated for each risk factor. Analysis of variance (ANOVA) was conducted for analysis of factors associated with degree of intrafamilial spread, and $P < 0\cdot1$ was considered significant due to the small number of households analysed. The degree of intrafamilial spread was calculated by dividing the number of family member(s) infected by the total number of family members in the household. In the analysis of association between hygiene practices and non-polio enterovirus isolation, infants were excluded from analysis due to the difficulty in acquiring accurate data on their behaviour. For the analysis of factors associated with the degree of intrafamilial

Table 1. *Enterovirus isolated from stool specimens collected from families in Ulaanbaatar and Tov province, Mongolia, late summer 2003, by age*

		Age group (years)												Total	
		0-1		2-10		11-20		21-30		31-40		41+			
Virus		30 tested		23 tested		12 tested		37 tested		12 tested		8 tested		122 tested	
Group	Type	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Polio	1	9	(30)	1	(4.3)	1	(8.3)	1	(2.7)	0	(0)	0	(0)	12	(9.8)
	2	5	(16.7)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	5	(4.1)
	3	6	(20)	0	(0)	0	(0)	0	(0)	1	(8.3)	0	(0)	7	(5.7)
Total persons shedding poliovirus*		15	(50)	1	(4.3)	1	(8.3)	1	(2.7)	1	(8.3)	0	(0)	19	(15.6)
Concurrent infections†		1 P1 + P2‡		3 P1 + P3‡		1 P2 + P3									
Echo	30	7	(23.3)	9	(39.1)	0	(0)	5	(13.5)	4	(33.3)	1	(12.5)	26	(21.3)
	33	7	(23.3)	6	(26.1)	6	(50)	9	(24.3)	1	(8.3)	0	(0)	29	(23.8)
	12	1	(3.3)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	1	(0.8)
	25	1	(3.3)	1	(4.3)	0	(0)	0	(0)	0	(0)	0	(0)	2	(1.6)
	Total*	16	(53.3)	16	(69.6)	6	(50)	14	(37.8)	5	(41.7)	1	(12.5)	58	(47.5)
Coxsackie	A10	4	(13.3)	3	(13)	1	(8.3)	0	(0)	0	(0)	0	(0)	8	(6.6)
	A2	0	(0)	1	(4.3)	0	(0)	0	(0)	0	(0)	0	(0)	1	(0.8)
	A4	0	(0)	1	(4.3)	0	(0)	0	(0)	0	(0)	0	(0)	1	(0.8)
	A24	0	(0)	0	(0)	1	(8.3)	0	(0)	0	(0)	0	(0)	1	(0.8)
	Total*	4	(13.3)	5	(21.7)	2	(16.7)	0	(0)	0	(0)	0	(0)	11	(9)
Total persons shedding non-polio enterovirus*		17	(56.7)	17	(73.9)	8	(66.7)	14	(37.8)	5	(41.7)	1	(12.5)	62	(50.8)
Concurrent infections†		1 E33 + CA10		2 E33 + CA10		1 E33 + CA4‡		1 E30 + CA10							

* There are individuals infected with multiple virus types during the course of 5 weeks; such individuals are counted as one.

† Number of persons concurrently yielding multiple virus types during the course of 5 weeks.

‡ P1, poliovirus type 1; P2, poliovirus type 2; P3, poliovirus type 3; E30, echovirus 30; E33, echovirus 33; CA10, coxsackie A10; CA4, coxsackie A4.

spread, one study child who lived in an orphanage was excluded. Statistic analysis was performed using SPSS 11.0.1J (SPSS Japan Inc., Tokyo, Japan).

RESULTS

Demographic characteristics

A total of 29 households involving 122 subjects participated in the study. The mean age of the study children was 3.9 months [\pm standard deviation (s.d.) 1.0 month], siblings 5.9 \pm 4.1 years, mothers 26.6 \pm 5.4 years, fathers 28.9 \pm 5.9 years, relatives 30.1 \pm 17.1 years, and contacts of siblings 29.7 \pm 23.2 years. A total of 70/122 (57%) of subjects were female.

From Chingeltei, Bayangol, Songinokhairkhan, Bayanzurkh, Zuum mod, and Bayanchandmani, 25, 17, 23, 20, 23, and 14 subjects respectively, participated.

Poliovirus and non-polio enterovirus isolation

Poliovirus isolation status is shown in Table 1. Fifteen study children (50%) excreted poliovirus, and polioviruses were isolated from four familial contacts: one sibling (age 2 years), two mothers (ages 21 and 34 years), and one uncle (age 16 years). Intra-typic differentiation revealed all polioviruses to be Sabin-like (vaccine type).

Table 2. *Non-polio enterovirus isolated from stool specimens collected from families in Ulaanbaatar and Tov province, Mongolia, late summer 2003, by sex and age*

Age group (years)	Female		Male	
	%	(No. positive /no. tested)	%	(No. positive /no. tested)
0–1	43	(6/14)	69	(11/16)
2–10	64	(7/11)	83	(10/12)
11–20	70	(7/10)	50	(1/2)
21–30	39	(9/23)	36	(5/14)
31–40	50	(3/6)	33	(2/6)
41+	17	(1/6)	0	(0/2)
Total	47	(33/70)	56	(29/52)

In the 5 weeks of observation of 122 subjects, 69 non-polio enteroviruses were isolated (Table 1). Eight serotypes of non-polio enteroviruses (echovirus types 30, 33, 12, 25, coxsackie A10, A2, A4, and A24 variant) were isolated from 62 persons with an overall isolation rate of 51% (62/122). By neutralization, only echovirus 30 was identified; therefore, molecular typing was performed for the samples for which a virus serotype could not be identified by neutralization. By analysis of the VP1 region, molecular typing identified the serotypes of all samples, which included echovirus 12, 25, 33, coxsackie A2, A4, A10, and A24. Among the serotypes, echovirus 30 and 33 predominated: 21% (26/122) and 24% (29/122) of the subjects were shedding the respective viruses. In the 5-week period, six individuals yielded multiple virus types: one study child (age 5 months) excreted echovirus 30, 33, and coxsackie A10, one sibling (age 4 years) excreted echovirus 33 and coxsackie A4, one sibling (age 2 years) excreted echovirus 30 and coxsackie A10, and one study child (age 5 months) and two siblings (ages 2 and 4 years) excreted echovirus 33 and coxsackie A10. Overall the non-polio enterovirus isolation rate was highest among the 2–10 years age group (74%) (Table 1). 57% (17/30), 69% (18/26), 45% (13/29), 36% (8/22), 12.5% (1/8), and 71% (5/7) of study children, siblings, mothers, fathers, relatives, and contacts excreted non-polio enteroviruses.

Overall, 47% (33/70) of females and 56% (29/52) of males excreted non-polio enteroviruses (Table 2). Comparison between the two sexes indicated that there was a tendency for higher isolation rates among

Table 3. *Number of households which showed intrafamilial spread of non-polio enterovirus, Ulaanbaatar and Tov province, Mongolia, late summer 2003*

Virus Group	Type	No. of household(s) with intrafamilial spread/No. of household(s) with at least one infected individual
Echo	30	8/10
	33	8/10
	12	0/1
	25	1/1
	Total different households*	16/20
Coxsackie	A10	1/7
	A2	0/1
	A4	0/1
	A24	0/1
	Total different households*	1/10
Total different households*		16/23

* There are households with multiple virus types; such households are counted as one.

males in younger age groups and females in older age groups.

Detection of enterovirus on hands

No enterovirus was detected from any of the hand swabs of 46 subjects.

Familial spread of non-polio enterovirus

We determined intrafamilial spread through isolation of the same virus type with high sequence homology in the 5'NTR-VP4-VP2 region from two or more members in the same household. The number of households tested and households which showed intrafamilial spread of non-polio enterovirus are shown in Table 3. All isolates from members living in the same household had 100% sequence homology except two households from which sequences differed by one nucleotide. Intrafamilial spread was responsible for 54% (37/69) of non-polio enterovirus infections.

Out of 16 households in which intrafamilial spread was confirmed, mode of transmission within the household could be observed in nine households by the difference in timing of virus isolation among family members. It supports the view that siblings

Table 4. Order of transmission of non-polio enterovirus within household determined by difference in timing of virus isolation, Ulaanbaatar and Tov province, Mongolia, late summer, 2003. Modes of transmission for the 16 households which showed intrafamilial spread are shown

Household	Virus	Week 1	Week 2	Week 3	Week 4	Week 5
1	E30	S	→	M		
2	E30	V S M C	→	F		
3	E30		S	→	V M	
4	E30		S	→	V M F	
5	E30				V S	→ M
6	E30	V M F				
7	E30	S C				
8	E33	V S M F	→	C		
9	E33	V S M	→	F		
10	E33		S	→	V	
11	E33				V S	→ M F C
12	E33	V S M F				
13	E33	V S F				
14	E33		S M			
15	E25			V S		
	E30			V S		
16	E33					V M
	CA 10	V S				

V, study child; S, sibling; M, mother, F, father; C, contact.

are chiefly responsible for introducing virus into the family (Table 4). Fifty-nine per cent (32/54) of intrafamilial contacts excreted virus when the sibling was excreting virus, whereas only 9% (2/23) excreted virus when the sibling was not excreting virus ($\chi^2_{DF=1}=14.74$, $P<0.001$ with Yates' correction; odds ratio 15.27, 95% confidence interval 3.25–71.86). Echoviruses were isolated from 62% (36/58) of people in contact with siblings excreting echovirus: 72% (13/18), 61% (11/18), 47% (7/15), 33% (1/3), and 100% (4/4) of study children, mothers, fathers, relatives, and contacts of siblings respectively excreted virus when the sibling excreted virus. Coxsackie A virus seemed to be self-limiting, among 15 people who came into contact with siblings shedding virus, only one infant (7%) excreted virus.

Factors associated with non-polio enterovirus infection

Factors associated with non-polio enterovirus isolation are shown in Tables 5 and 6. Factors associated with intrafamilial spread of non-polio enterovirus are shown in Table 7. Overall, crowding, water storage, cover on stored water, years of maternal and paternal education, type of water supply (well/water distribution service/piped water), and location of toilet (inside/outside/none) showed no influence on

enterovirus isolation. However, for people living in houses, households with an inside toilet showed a significantly higher degree of intrafamilial spread compared to those with outside toilets (Table 7).

DISCUSSION

Limitation

Generalization should be made carefully for the following reasons: our results reflect non-polio enterovirus isolation under routine immunization of OPV, and interference may have occurred between polio and non-polio enterovirus, serological analysis on each serotype was not conducted, therefore, prior infection was not considered, enterovirus prevalence varies by year and by season, therefore the period of 5 weeks only provides a snapshot of the situation in Mongolia, a larger study population is required to assure generalizability, and a lack of throat swabs may have decreased the isolation rate as well as the detection of certain serotypes.

Impact of molecular typing

For non-poliovirus isolates, only echovirus 30 was identified through neutralization. The use of

Table 5. Associations between demographic characteristics and non-polio enterovirus isolation, Ulaanbaatar and Tov province, Mongolia, late summer 2003

Variables	No.	OR	95% CI
Age group (years)			
0–1	30	9.1	1.0–83.8
2–10	23	19.8	2.0–195.9
11–20	12	14.0	1.3–156.3
21–30	37	4.6	0.5–38.3
31–40	12	5.0	0.5–54.4
41+	8	1.0	
Family status			
Study child	30	9.1	1.0–83.8
Sibling	26	15.7	1.7–149.8
Mother	29	5.7	0.6–52.2
Father	22	4.0	0.4–38.6
Relative	8	1.0	
Area			
Ulaanbaatar slum area	49	7.9	2.9–21.6
Ulaanbaatar apartment area	32	1.6	0.6–4.3
Tov province	37	1.0	
Sex (age ≤ 10 years)			
Male	28	2.8	0.9–8.8
Female	25	1.0	
Sex (age ≥ 10 years)			
Male	24	0.6	0.2–1.8
Female	45	1.0	

OR, Odds ratio (calculated by univariate logistic regression); CI, confidence interval.

molecular typing in addition to neutralization allowed the identification of the serotypes of all samples including the coxsackie A group which is generally difficult to identify through neutralization. The effectiveness of the use of molecular typing to identify non-polio enteroviruses was confirmed in our study.

Isolation rate of non-polio enterovirus

Although there is difficulty in comparing data due to differences in specimens sampled, specimen sampling frequency, selection of subjects, and virus isolation and identification methods, the observed overall isolation rate of 51 and 64% among children under 10 years of age is by far the highest isolation rate reported. Previous studies conducted among young children in the United States [8] and Hungary [12] reported an isolation rate of 21 and 26% respectively,

and an isolation rate of 61% was reported among children under 10 months of age in Ghana [13].

Enterovirus infections peak in the warm months and are least frequent in the cold months [23, 24]. The warm season in Mongolia is very short, and after the severe winter season, enterovirus infections may become extremely prevalent in the summer. Additionally, the sharp increase of tourists during the summer season, as well as the holding of Naadam, a national sports festival to which Mongolians gather from all over the country, in the beginning of July in Ulaanbaatar, may have created an environment which facilitated virus introduction as well as person-to-person transmission, therefore influencing the incidence of infection.

Age, sex and isolation rate of non-polio enterovirus

In our study, the 21–40 years age group showed a high isolation rate of 39%. Data on the isolation rate of non-polio enteroviruses among healthy adults is limited, and to our knowledge, this is the highest isolation rate reported among adults. Our study demonstrates that infection usually occurs in childhood, but asymptomatic infection can be remarkably high among adults, depending on previous infection history with a given serotype in a given locale.

Overall, more males were shedding non-polio enteroviruses than females. This supports the findings that enteroviral diseases occur more frequently in males [8, 25, 26] and indicates that the higher morbidity among males is due to a higher infection rate among males. However, although the sample size in the age groups was small, there appeared to be higher infection rate among females above age 11 years. This is consistent with a finding that there was predominance of female patients due to enteroviral disease in a group over the age of 10 years [8]. It has been suggested that biological reasons are responsible for the higher infection rate among males such as longer duration of virus excretion and higher virus titre in stools of males [27]. However, our finding of mother > father > relative infected within the same household suggests that higher infection rate among adult females may reflect higher contact frequency and intimacy with infected children.

OPV interference by non-polio enterovirus

A review of immunity among children in developing countries has indicated low rates of seroconversion

Table 6. Associations between hygiene behaviours and non-polio enterovirus isolation, Ulaanbaatar and Tov province, Mongolia, late summer 2003

Variables	No.	OR	95% CI
Hand washing after defecation			
Always	55	1.0	
Not always	37	2.5	1.1–5.8
Hand washing before eating			
Always	46	1.0	
Not always	46	1.3	0.6–3.0
Hand washing before cooking			
Always	57	1.0	
Not always	13	0.8	0.2–2.8
Bathing frequency			
More than 2 times a week	41	1.0	
Weekly or less frequent	51	0.6	0.3–1.4
How bottom is cleaned			
Toilet paper	83	1.0	
Other (cotton, newspaper)	6	0.5	0.1–2.7
How many times hands washed*			
		Mean (s.d.)	<i>P</i> value
Negative	47	4.1 (0.3)	<i>P</i> = 0.394
Positive	45	3.7 (0.3)	

OR, Odds ratio (calculated by univariate logistic regression); CI, confidence interval; s.d., standard deviation.

* *t* test. Question was asked for subjects to recall how many times they washed their hands the day before the questionnaire survey was conducted.

to poliovirus types 1 and 3 after administration of three doses of OPV [28]. Factors such as sub-optimal vaccine potency, breaks in the cold chain, sub-optimal vaccine schedule, and concurrent enteric infections have been postulated as reasons for the poor seroconversion rate. The effect of concurrent enteroviral infections in reducing seroconversion rate has been repeatedly suggested [29–31], and low rates of seroconversion have been reported more frequently during summer than in winter [32, 33].

Our study showed that there was high transmission by multiple types of non-polio enteroviruses in the summer season in Mongolia. Mongolia has reduced its OPV schedule to birth, 2, 3, and 4 months of age from 2003. To avoid interference by non-polio enteroviruses during OPV immunization, attention must be paid to children who will be receiving most of their OPV in the summer season. We discovered infants concurrently yielding multiple non-polio enteroviruses, and the efficacy of OPV administered to such infants is doubtful. A study of immunity to poliovirus in children receiving OPV in the summer season may be required. This is considered important to maintain high herd immunity

and to avoid the risk of vaccine-derived poliovirus infection.

Detection of enterovirus on hands

The finding that persons who do not always wash their hands after defecation are 2.5 times more likely to be infected compared to those who answered that they always washed their hands highlights the importance of hand contact in the transmission pathway. This was consistent with studies of echovirus 30 which showed that hand washing was protective against infection [34, 35]. However, despite the high isolation rate of non-polio enteroviruses from stools, no enterovirus was detected from any of the hand swabs. The lack of detection may have been associated with the hands being rubbed onto many other objects or body parts, therefore, the chance of isolating virus from hands at random times in a real-life setting was very small. The PCR method was adopted due to the limited amount of samples and due to its high sensitivity for virus detection; however, the detection limit of the method may have overlooked the presence of virus due to a small virus load.

Table 7. Associations of living environment and demographic character with degree of intrafamilial spread of non-polio enterovirus in Ulaanbaatar and Tov province, Mongolia, late summer 2003

Variables	No. of households	Mean (s.d.)†	95% CI	P value
Residence				
Ger	5	0.73 (0.25)	0.42–1.05	$P=0.097^*$
House	14	0.55 (0.42)	0.31–0.80	
Apartment	10	0.30 (0.35)	0.05–0.55	
Kitchen				
Sole use	17	0.40 (0.42)	0.18–0.62	$P=0.095^*$
Shared	3	0.92 (0.14)	0.56–1.28	
None	9	0.55 (0.30)	0.32–0.77	
Bathroom				
Sole use	10	0.38 (0.41)	0.08–0.67	$P=0.041^{**}$
Shared	4	0.94 (0.13)	0.74–1.14	
None	15	0.46 (0.36)	0.26–0.66	
Waste				
Tube	10	0.68 (0.43)	0.37–0.98	$P=0.053^*$
Special hollow outside	12	0.51 (0.37)	0.28–0.75	
Outside, no special place	7	0.21 (0.23)	0.01–0.42	
Toilet‡				
Inside	5	0.95 (0.11)	0.81–1.09	$P=0.003^{***}$
Outside	9	0.33 (0.35)	0.06–0.61	
Income (Togrog)§				
1–50 000	7	0.71 (0.39)	0.35–1.08	$P=0.136$
50 001–100 000	17	0.48 (0.39)	0.28–0.68	
100 001–150 000	2	0.50 (0.35)	–2.68–3.68	
150 001–200 000	3	0.08 (0.14)	–0.28–0.44	

CI, confidence interval.

* $P<0.1$, ** $P<0.05$, *** $P<0.01$.

† ANOVA. Degree of intrafamilial spread was calculated by number of family member(s) infected divided by total number of family members in the household.

s.d., standard deviation.

‡ Only households residing in house are included.

§ \$1.00 = 1140 Togrog as of July 2003.

Intrafamilial spread and transmissibility of non-polio enterovirus

Examination of the order of virus excretion among family members revealed that siblings or young children aged 2–10 years were chiefly responsible for introducing virus into the household. Our study provides direct evidence that young children are the most important transmitters of enteroviruses compared to studies in which statistics were used to associate number of young children and number of infected family members.

Transmissibility of echovirus was high, while coxsackie A virus infection was self-limiting. The low

transmissibility of coxsackie A virus may be explained by multiple reasons: herd immunity may have been established due to previous infection, supported by an indication that coxsackievirus infections peaked in early summer whereas echovirus tended to be restricted to late summer [8]; transmissibility of coxsackie A virus may be lower than coxsackie B virus; the dry climate of Mongolia may have limited the transmissibility of coxsackie A virus; relatively low sensitivity of the used cell lines towards coxsackie A virus [1] may have limited its detection; and coxsackie A virus may spread mainly through the respiratory route rather than the faecal–oral route, therefore lack of throat swabs may have decreased

our detection rate. Additionally, it is indicated that coxsackie A24 variant spreads primarily by direct or indirect contact with eye secretions [36], thereby limiting its detection. There were limitations in comparing transmissibility of echovirus and coxsackie A virus, however, echoviruses showed considerably higher transmissibility than coxsackie A viruses when stool specimens were examined using the L20B, Hep-2, and RD-A cell lines.

Risk factors of non-polio enterovirus infection

We observed difference in the degree of intrafamilial spread by type of residence. *Ger* dwellers had the highest risk of intrafamilial infection. Although not statistically evident, low accessibility to water and high density and intimacy among family members due to small housing space [4.3 m² (1.10 m² per person)] appeared to be the major reasons for the high infection rate.

For those living in a house or apartment, multiple families sharing a kitchen and/or bathroom was a significant risk factor for promoting intrafamilial spread of non-polio enterovirus. Additionally, for those living in a house, a higher risk of intrafamilial infection was associated with an inside toilet rather than an outside toilet. A virological study of families whose infants were recently vaccinated with OPV showed that 15 and 10% of swab samples taken from bathroom/toilet and kitchen respectively were positive for poliovirus [37]. The absence of such facilities inside the residence may have decreased the chance of contracting virus, and on the other hand, having and sharing such facilities increased the introduction of virus into the household as well as the chance of infection. Cleaning of kitchens, bathrooms, toilets, and waste disposal areas as well as hand washing, especially after defecation, are probably the most important factors to prevent infection in the home environment.

Our findings contradict previous studies of pathogens which are transmitted by the faecal–oral route. In a study of *Helicobacter pylori*, infection among those who used outdoor toilets was significantly higher than those who used indoor toilets [38]. In a study of hepatitis A, the absence of a toilet [39] and a kitchen [40] were associated with a higher prevalence of anti-hepatitis A virus antibodies. The difference in study finding may reflect differences in hygiene practices and knowledge of hygiene among the different populations. Urban areas of Mongolia have gone through a transition of housing during the 70

years of Soviet dominance. Soviet-style apartments providing a toilet, bathroom, kitchen, and piped water were built, and the lifestyle changed drastically for those who moved into apartments from a *ger*, lacking such facilities. The reasons for contradiction with previous studies may be because Mongolians living in urban areas are going through a transition in lifestyle; however, the knowledge of hygiene and hygiene practices has not yet reached the standards required for the new lifestyle.

Access to improved sanitation is one of the millennium goals targeted by the United Nations. Our study findings show that the mere presence of hygienic facilities is not sufficient to decrease infection with enteric viruses. Hygiene education, stressing the importance of cleaning hygienic facilities, and hand washing after defecation is required. A comprehensive sanitation programme encompassing provision of sanitary facilities as well as education in hygiene is indispensable for improved health.

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