Original article

Vaccination status and prevalence of enteric viruses in internationally adopted children. The case of Parma, Italy

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Abstract. On age basis, internationally adopted children may have begun or fully completed all required vaccinations, but official documentation from original Countries is frequently insufficient. Aims of this study were to evaluate the seroprotection rate for tuberculosis, hepatitis B, poliomyelitis and tetanus according to immunization cards in 67 children recently adopted and to test the prevalence of enterovirus on faecal specimens. Seroprotection and vaccination status were frequently inconsistent and these results confirm that immunitary surveillance is a cornerstone for the prevention of diseases for which a vaccination is available. (www.actabiomedica.it)

Key words: internationally adopted children; immune protection; enteric viruses prevalence

Introduction

Internationally adoption in Italy

Every year, North America and several European countries receive a migratory flow of adopted children from East-Europe, South-America, Asia and Africa.

International adopted children are a multi-ethnic and multi-cultural ensemble that is rapidly evolving due to the growing number of involved countries. By 2005, the slow accreditation procedures in Russian Federation and Ukraine and the empowerment of new laws in Belarus (2004) were associated with a growing number of Asian origin adoptions in Italy . Between 2004 and 2007, homeland countries of international adoptees grew up from 58 to 76 and included Armenia, Kazakistan, Latvia, Lebanon, Mali, Mauritius, Central African Republic, Dominican Republic, Senegal and Uruguay, Kyrgyzstan, Tanzania and Bosnia-Herzegovina. By 1999, International Adoption flows are monitored by the National Commission for International Adoptions (NCIA) (Presidential Decree n. 492 of December 1st 1999), whose reports eventually encompass all international adoptions admitted to Italy between November 2000 and December 2007.

NCIA report describes 2007 as the year with the highest entry flow ever (3,420 children); despite ranking 9th among countries with high adoption rate, Italy is presently the only country recording a still growing adoption flow. More precisely, Italian adoption flow grew by 10% between 2005 and 2006, and by 7.3% during the following year. Moreover, the number of adopted children per couple showed a similar growing trend, reaching the peak of 1.27 during 2007 and becoming as very close to the national ratio of natural child per woman (presently ranging between 1.03 in Sardinia and 1.54 in Trentino Alto-Adige). Emilia Romagna records 1.32 biological children per couple and 1.19 requests of international adoption per couple.

Overall, 58% of children admitted to Italy are males, the mean age being 5.2 years. The age appears to be strongly correlated with the country of origin:

younger children (≤ 4 years) are more frequently from Asia (35% of Asian International Adoptees is less than 1 year old, and 39.9% less than 4 years old), whereas older children (> 4 years) are typically of South American and East European countries (1). Thus, in Italian regions with high presence of Asian adoptees, the average newly adopted children age is lower (mean adoption age: 3.72 years for Piemonte, 3.87 for Val d'Aosta, 4.05 for Trentino Alto Adige).

Adopted Children and Immunity

Nearly all children coming to Italy as adopted or following their immigrant families come from countries where life conditions and poor healthcare systems might impair their health status. Moreover, their interaction with welcoming population could potentially constitute a risk (2-4). Accurate and scrupulous clinical evaluations are therefore mandatory, aiming to an early diagnosis of nutritional deficiency or infectious diseases (5), and obviously requiring immediate identification of performed vaccinations.

On age basis, adoptees may have begun or fully completed all required vaccinations, although with some scheduling difference (6), but official documentation from original Countries is frequently scarce, or insufficient (7). As an example, the survey performed by Schulpen et al. (8) on adopted children from Africa, Asia, South America and Easter Europe (13 countries overall) identified the 30% of Chinese-origin children as incompletely vaccinated against poliomyelitis, diphtheria and tetanus, despite their vaccination schedule was recorded as fully completed.

In order to protect the child and his adoptive family, immunitary status of adopted children should be regularly evaluated, providing all vaccinations required by our health regulation when otherwise necessary (9). The public health approach to this problem should consider all adopted children as actually "not-vaccinated", thus providing all requested vaccinations to each single adopted child or, more appropriately, vaccinating only those with a serologically confirmed "no-vaccination status".

Aim of this study is then to evaluate health and particularly vaccination status in international adopted children.

Materials and Methods

Between March 2004 and September 2007, 67 children adopted by families residing in Parma and counties nearby were evaluated by the "Center for First Sanitary Evaluation of International Adoptions". Admission protocol required an accurate evaluation of physiological and pathological conditions of the Adoptee, achieved through a deep physical examination and by laboratory tests.

Sanitary history and full information about previously undergone vaccinations of every child was retrieved. Tubercular sensitivity were also established. Unsatisfactory nutritional status, original living environment (family or institutes), time spent in Italy before first medical evaluation were also retrieved for every child.

Faecal specimens were collected in order for enteric viruses and polioviruses determination, and blood samples were collected in order to evaluate immune status for the three polioviruses, tetanus and hepatitis B.

For poliovirus identification, fecal specimens were treated with chloroform (in order to remove bacteria, fungi and cytotoxic lipids) centrifuged and supplemented with antibiotics. The supernatant was recovered and stored at -20°C. Three cellular lines were applied in order to isolate cytopathogenic viruses: Hep2, RD and L20B. Isolate characterization was performed through infectivity neutralization reaction with standard sera (WHO methodics (10)). Every specimen was double-checked by means of RT-PCR, with Enterovirus-specific primers (11).

Infectivity-neutralizing antibodies were identified for the three polioviruses through a long incubation neutralization assay (12). The antibody titer of the three polioviruses was defined as the reciprocal value of the highest serum dilution showing a complete neutralization of the cytopathic effect. Antibody titers lower than 1:2 were defined as seronegative whereas levels equal or higher than 1:8 as protective. Anti-tetanus antibody determination was performed by means of an ELISA kit (RIDASCREEN® Kit Tetanus IgG, Biopharm®), considering titers ≤ 0.1 UI/ml as negative, average protection between 0.11 UI/ml and 0.9 UI/ml, and strongly protective ≥ 1 UI/ml.

Immune-enzymatic methods with particles capture (MEIA) were applied in order to perform Hepatitis B evalutation (AUSAB – Abbot AXSYM SYSTEM®). Anti-HBc antibodies were evaluated through CORAB® Kit (Abbott).

Statistical analysis

Continuous variables were summarized as mean, standard deviation (SD) and range of values. Categorial variables were represented in frequency tables. For poliomyelitis, data were stratified for sex, age (1-2 years old, 3-5 years old, ≥ 6 years old), likely vaccination schedules and WHO region of origin. Differences in geometrical mean titers (GMTs) were evaluated through Analysis of Variance (F test and Student's t test) on regional origin basis, nutritional status, sex, age class. P value < 0.05 was considered statistically significant. All statistical analyses were performed by SPSS 14.01.

Results

Sample characteristics

A total of 67 adopted children were included in the study: 29 females (43.3%) and 38 males, with a mean age of 4.3 *(2.9)* years (range 1-14 year old) from all WHO regions, but mainly from WHO European Region. The mean time gap between arrival to Italy and medical evaluations was 8.3 weeks (range 1-34 weeks). The mean age of children at adoption appeared as progressively increasing, however not reaching statistical significance (3.5 years in 2004, and 4.8 years in 2007; linearity test for trend: p = 0.057). A statistically significant difference was observed when mean age of children coming from for different WHO regions of origin was evaluated. Eighty-one per cent (54/67) of children were previously living in residential institutions, among which 24% suffered of unsatisfactory nutritional status. No correlation was found among original institution, sex and nutritional status.

Virological analysis

Enteric viruses were identified in 52% of cases (26/50), of which 40% were non-polyomielitis enteroviruses (Coxsackievirus) and 14% were adenoviruses. Mean age was significantly lower in positive than in negative children (3.3 years; $CI_{95\%}$ 2.6-4.1 vs 5.5 years; $CI_{95\%}$ 4.1-7.0). Furthermore, residence in Italy of positive children appeared as significantly longer (10.2 weeks; $CI_{95\%}$ 6.8-13.5 vs 6.2 weeks; $CI_{95\%}$ 4.5-7.9; p = 0.043).

Immunization records and vaccine serology

In figure 1, vaccination status of adopted children is reported. An official certificate of performed tuberculosis vaccination was available for 31 of 67 children (46.3%). However, Mantoux test, performed at the first medical evaluation on 43 children, was positive in 4 vaccinated children (12.9%) and in 4 children lacking sanitary documentation (11.1%). A vaccination for MMR was certified in 30 children (44.8%), whereas 50 (74.6%) resulted previously vaccinated against tetanus/diphtheria, 47 (70.1%) for HBV and 54 (80.6%) for poliomyelitis. Serologic data about tetanus showed a highly protective status (> 1 UI/ml) in 35 of 67 children (52.2%), in 30 an intermediate status (range 0.11 - ≤ 1 UI/ml) and an insufficient status in 2 (3%, respectively 6 and 7 year-old). No significant differences in mean serologic values were identified among geographical origins or age of children (Table 1).

HBs antibodies at level ≥ 10 mUI/ml were identified in 67.2% of adopted children (45/67), 41 among vaccinated (41/47; 87.2%) and 4 among not vaccinated or lacking documentation. A 3 year-old girl from

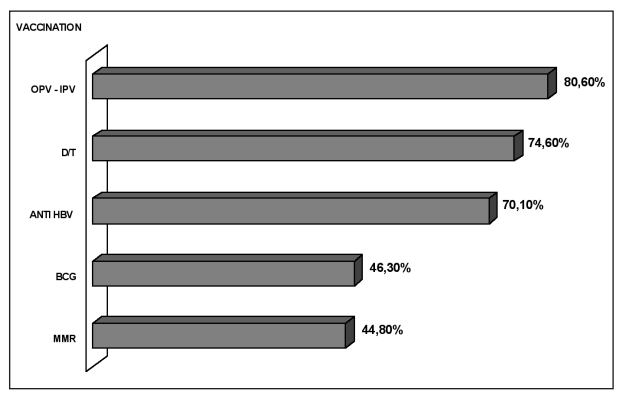

Figure 1. Immunitary status reported in sanitary documentation

Table 1. Tetanus and Hepatitis B: protective titers level frequencies

	Vaccinated No. Subjects *(%)*	Not vaccinated No. Subjects *(%)*	Data not available No. Subjects *(%)*	Total No. Subjects *(%)*
Tetanus				
0 - 0,1 UI/ml	0	1 *(33.3)*	1 *(7.1)*	2 *(3)*
0.11 - 1 UI/ml	25 *(50)*	2 *(66.7)*	3 *(21.4)*	30 *(44.8)*
> 1 UI/ml	25 *(50)*	0 *(66.7)*	10 *(71,4)*	35 *(52,2)*
Total	50 *(74.6)*	3 *(4.5)*	14 *(20.9)*	67 *(100)*
Hepatitis B				
0 - 9.99 mUI/ml	6 *(12.7)*	15 *(93.7)*	1 *(25)*	22 *(32.8)*
10 - 99.99 mUI/ml	16 *(30.1)*	0	1 *(25)*	17 *(25.4)*
> 100 mUI/ml	25 *(53.2)*	1 *(6.3)*	2 *(50)*	28 *(41.8)*
Total	47 *(70.2)*	16 *(23.9)*	4 *(5.9)*	67 *(100)*

India resulted Hepatitis B surface antigen positive and anti-HBc positive; anti-HBc antibodies were identified in 2 non vaccinated subjects (Table 1).

For poliomyelitis seropositivity (level ≥ 1:2) and seroprotective (level ≥ 1:8) frequencies were evaluated. Two children appeared as seronegative (3%) for polio 1, five (7.5%) for polio 2 and three (4.5%) for polio 3. A triple serum-negativity was identified in a 14 months-old not vaccinated child from Vietnam, and 3 double seronegativity in 2 children from Eastern Europe (one of them not vaccinated) and in a 3-year old vaccinated Vietnamese child (polio 2/polio 3 negative, and polio 1/polio 2 negative, respectively). Serum levels of protective antibodies ≥ 1:8 were identified in 97% of children for polio 1, in the 89.6% and in the 77.6% for polio 2 and 3, respectively (Table 2).

The GMTs for polio 1, polio 2 and polio 3, calculated on the total number of seropositive children, were 88.20 ($CI_{95\%}$ 85.88 - 90.52), 34.77 ($CI_{95\%}$ 32.40 - 37.13) and 18.30 ($CI_{95\%}$ 15.95 - 20.65) respectively. No significant differences were identified between the subgroups of vaccinated and not vaccinated children.

Table 2. Poliomyelitis: seropositivity and seroprotective level frequencies

	Vaccinated			GMT	Not vaccinated			GMT	Total			GMT
Titer	≤ 1:2	1:2 - 1:4	≥ 1:8		≤ 1:2	1:2 - 1:4	≥ 1:8		≤ 1:2	1:2 - 1:4	≥ 1:8	
P1	1 (1.8%)	0	53 (98.2%)	89.36	1 (7.7%)	0	12 (92.3%)	83.55	2 (3%)	-	65 (97%)	88.20
P2	3 (5.5%)	1 (1.8%)	50 (92.7)	37.82	2 (15.4)	1 (7.7%)	10 (76.9)	24.51	5 (7.5%)	2 (3%)	60 (89.5%)	34.77
P3	1 (1.8%)	11 (20.4%)	42 (77.8%)	19.65	2 (15.4%)	1 (7.7%)	10 (76.9%)	13.63	3 (4.5%)	12 (17.9%)	52 (77.6%)	18.30

The 3-5 year group showed the highest GMTs for polioviruses: 161.27 ($CI_{95\%}$158.99 - 163.54) vs polio 1, 43.98 ($CI_{95\%}$ 41.46 - 46.49) vs polio 2 e 23.29 ($CI_{95\%}$ 20.69 - 25.88) vs polio 3. However, differences among age groups reached statistical significance ($p = 0.03$) only relatively to polio 1. No significant differences in GMTs for poliomyelitis virus were observed when the sample was stratified for region of origin.

Discussion

Emergence and re-emergence of sanitary disorders such as infectious diseases almost absent at national level is a critical problem interlinked with migratory flow and recent diffusion of international adoptions. This phenomenon demands accurate and appropriate interventional public health programs like targeted screening among this population (13, 14).

The sample we evaluate in this study was originally homogenous for body weight, institution of origin and health status. Our observations are also consistent with national data about international adoptions (despite a lower mean age) and confirm the capital role for an accurate health status evaluation associated with a meticulous surveillance of immune status.

One quarter of the evaluated children showed an unsatisfactory nutritional status and 50% was positive for enteric viruses (non poliomyelitis enteroviruses and adenoviruses). On the other hand, a percentage between 20% (OPV-IPV) and 50% of subjects resulted not-vaccinated (25% for TD, 30% for HBV and 50% for MMR and BCG) or lacking documentation about previous vaccinations.

Seroprotection and vaccination status appeared frequently inconsistent, as recently reported by Shulte JM et al(15), and Cilleruelo et al (7). For example, only 67.2% of subjects who received the HBV vaccination had a serum level of antibodies higher than 10 mUI/ml, and only 17.4% of subjects with a previous history of BCG vaccination showed a positive tuberculine test. Moreover, in the 54% of cases data about vaccination were not available despite vaccination schedule of the original country included BCG vaccination. Among these, the 11.9%, a percentage similar to that found by Mandalakas AM et al. (16), was found positive to the Mantoux test. Finally, the presence of vaccinated children with markers of infection (HbsAg+) should not to be underestimated.

For poliomyelitis, available data about previous vaccination and actual seroprotection appear adequate, but a substantially low prevalence of seroprotection against type 3 poliovirus (77.7%) was observed, and 4 children appeared not vaccinated against 2 serotypes, despite available documents attested that 2 them had been regularly vaccinated.

Protection against tetanus was high (97%), despite the available documentation was insufficient, attesting that vaccination practices are far from homogeneous in the original countries.

Virological survey performed on stool samples put under the spotlight a high prevalence of positivity for non poliovirus enteroviruses and for adenoviruses (52%), which was remarkably higher than the national average (10.4%) and than the value observed in a recent survey carried out in the schools of Parma (17).

The trend observed in this study regarding enteral viruses along age groups (1-2; 3-5 and ≥ 6 years old) is in agreement with the trend observed for the Italian children population (18). In other, positivity appeared more frequently among children with a later access to the referral centre (19).

The risk of reintroducing to Italy seronegative or infected subjects should not be underestimated. The same should be said about reintroducing vaccine polioviruses in the environment, with the associated possibility of mutations restoring viral pathogenic activity, as practically all countries but the highly developed countries (i.e. USA (20), Italy (21), France, Germany, Sweden, Finland, Iceland, Holland, and Andorra) still use OPV for routine vaccination.

However, a low-medium level of seroprotection towards type 2 or type 3 polioviruses, irrespectively to the geographical region of origin (lower GMTs for all polioviruses in children from African and West pacific regions) suggests a "non ideal" immune status at least until the complete eradication of poliomyelitis in all WHO regions (22).

The results of this study confirm that, in the context of internationally adoption, immunitary surveillance is a cornerstone for prevention of diseases for which a vaccination is available.

The aim of improving the system is, as underlined by regional reference centres involved in this ac-

tivity, twofold: i) to avoid the excessive antigenic stimulation caused by repeated vaccinations in subjects lacking vaccine documentation and ii) to fully integrate these children in the social and sanitary setting of the welcoming country.

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Accepted: October 30th 2011
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