Epidemiological and molecular assessment of a Rubella outbreak in North-Eastern Italy

Pierlanfranco d'Agaro, Gianna Dal Molin, Emanuela Zamparo, Tatiana Rossi, Michele Minuzzo, Marina Busetti, Daniela Santon, Cesare Campello

Journal of Medical Virology

Epidemiological and molecular assessment of a Rubella outbreak in North-Eastern Italy

Journal:	*Journal of Medical Virology*
Manuscript ID:	JMV-10-1806.R2
Wiley - Manuscript type:	Research Article
Date Submitted by the Author:	23-May-2010
Complete List of Authors:	D'Agaro, Pierlanfranco; University of Trieste, Reproductive, Developmental and Public Health Sciences, UCO Hygiene and Preventive Medicine; Institute of Child Health IRCCS Burlo Garofolo Dal Molin, Gianna; Institute of Child Health IRCCS Burlo Garofolo Zamparo, Emanuela; ASS6 Friuli Occidentale, Department of Prevention Rossi, Tatiana; Institute of Child Health IRCCS Burlo Garofolo Minuzzo, Michele; ASS6 Friuli Occidentale, Department of Prevention Busetti, Marina; Institute of Child Health IRCCS Burlo Garofolo Santon, Daniela; Institute of Child Health IRCCS Burlo Garofolo Campello, Cesare; University of Trieste, Reproductive, Developmental and Public Health Sciences, UCO Hygiene and Preventive Medicine; Institute of Child Health IRCCS Burlo Garofolo
Keywords:	Rubella, outbreak, genotyping, sequences, seroepidemiology

Epidemiological and molecular assessment of a Rubella outbreak in North-Eastern Italy

Pierlanfranco D'Agaro [1], Gianna Dal Molin [1], Emanuela Zamparo [2], Tatiana Rossi [1], Michele Micuzzo [2], Marina Busetti [1], Daniela Santon [1], Cesare Campello [1]

[1]Department of Reproductive, Developmental and Public Health Sciences, UCO Hygiene and Preventive Medicine, University of Trieste, and IRCCS Burlo Garofolo, Trieste, Italy

[2]Department of Prevention, ASL 6 of Friuli-Venezia Giulia Region, Italy

Running title. Rubella outbreak in North-Eastern Italy

Key words. Rubella, outbreak, genotyping, seroepidemiology

Correspondence to: Prof. Pierlanfranco D'Agaro

UCO Hygiene and Preventive Medicine, Via dell' Istria 65/1, 34137 Trieste, Italy. Phone +39 040 3785845, Fax +39 040 7600324.

E-mail: dagaro@burlo.trieste.it

ABSTRACT

From January to June 2008, a rubella outbreak involving 111 laboratory confirmed cases occurred in the Friuli Venezia Giulia (FVG) region of North-Eastern Italy. The outbreak occurred initially in two residential homes for young adults disabled mentally and physically. Subsequently, the epidemic spread to the general population. Young adult cohorts were mostly affected and the mean age of the patients was 26.8 years; the majority of cases were male (73.8%), with a mean age of 26.6 years in males and 27.4 in females. Three pregnant women had a primary infection and two had their pregnancies terminated. Genotyping of sixteen isolates showed the circulation of RUBV 2B, a genotype originating from Asia and South Africa and now present in Europe. In addition, molecular analysis revealed a well defined space-temporal spread of two viruses showing distinct sequences. A seroepidemiological survey carried out in a city within the same geographical area showed that the proportion of women of childbearing age still susceptible to rubella virus was 5.5%, fairly close to the figure (less than 5%) expected by 2010.

INTRODUCTION

Rubella is a mild exanthematic illness whose public health relevance is due to infection in the first trimester of pregnancy, leading potentially to the congenital rubella syndrome (CRS). CRS comprises a lengthy list of abnormalities, which include commonly deafness, ocular and cardiac defects and mental disability [Miller et al., 1982].

The rubella vaccines are highly efficient for the prevention of rubella infection and CRS worldwide [WHO, 2005a; Pandolfi et al., 2009]. In Italy, universal vaccination against rubella, measles and mumps has been recommended since the early 1990s for all children at fifteen months of age [Ciofi degli Atti et al., 2004; Bonanni et al., 2007; Spiteri et al., 2008; Pandolfi et al., 2009]. Before the 1990s, vaccination was offered actively to pre-pubertal girls with a high variability of compliance. In addition, in 2003, the National Plan for Measles and Congenital Rubella Control launched by the Italian Ministry of Health recommended strongly immunization of seronegative women of childbearing age. The plan introduced also two other strategies, i.e. universal second doses at 5-6 years and a catch-up vaccination of children attending primary school. Despite these efforts, rubella is still endemic in Italy as documented by outbreaks recorded in 1997 and 2002, while the rate of CRS is so far higher than the target established by WHO of less than one case/10^5 live births by 2010. In Italy, data on CRS dates only from 2005 when the CRS, as well as rubella infection during pregnancy, was established as a notifiable syndrome. Thus, between 2005 and 2008, seven confirmed or probable cases of CRS and 31 cases of

rubella infection in pregnant women were reported, with a sharp rise during the first months of 2008. It is worth noting that only a number of Italian regional health services report both CRS cases and rubella infection during pregnancy, hence the prevalence of CRS in Italy is underestimated [Giambi et al., 2008].

A number of epidemics were recorded in Italy in 2008, one of which is described below together with the genetic characterisation of rubella virus (RUBV) strains. In addition, since the main strategic solution for prompt control of congenital rubella syndrome is focused on the vaccination of susceptible women of childbearing age, the results of a seroprevalence survey carried out in women in the same geographical area sometime before this outbreak are also reported.

MATERIALS AND METHODS

Patients

In Italy, rubella is a mandatory notifiable disease. Since January, 2008, any notified case with fever, typical rubella-like rash and lymphoadenopathy was registered as a suspected case at the Prevention Department of the Health District ASL 6, which corresponds roughly to the administrative province of Pordenone with an area of about 300,000 inhabitants. The suspected case was interviewed over the telephone or asked to complete a questionnaire on demographic data and history of exanthemata, vaccinations and contacts. Following informed consent, the patients provided clinical samples for laboratory investigation.

A confirmed case was defined as a patient with documented seroconversion, with or without detection or isolation of virus from clinical samples. No possible case, *i.e.* a

clinically suspected case with exposure to rubella but without laboratory evidence, was considered in this survey.

Between January and October, 2006 a survey on the prevalence of rubella antibody was carried out on women of childbearing age (mostly pregnant women) from Trieste attending the Maternal-Children Hospital for TORCH serology evaluation. Since in this area almost all pregnant women are screened for *Toxoplasma gondii*, all sera were also tested for rubella antibody, irrespective of a specific request. In this way a representative sample of all women of childbearing age living in a limited urban area was enrolled.

Serology

Diagnostic serology was carried out with a Rubella IgG and IgM EIA commercial kit (Dia-Sorin, Saluggia, Italy). Rubella infection was confirmed when IgM antibody was present unequivocally. In the event of an equivocal result, a second serum sample was required to ascertain seroconversion. For the seroprevalence survey, rubella susceptibility was confirmed when serum had an IgG titre less than 10 U.I./mL.

Virological and molecular tests

Virological and/or molecular tests were carried out on throat swabs, urine and peripheral blood in EDTA. Nucleic acids were extracted with the NucliSens easyMag System (Biomerieux Italia, Firenze, Italy) and rubella RNA detection was carried out with a commercial nested RT-PCR and using biotin-labelled probes on streptavidin-coated microplate wells (ProDect Rubella, Bcs Biotech, Cagliari Italy). Pharyngeal

swabs and urine were seeded on VERO cells, incubated for 10 days and submitted to one blind passage. Supernatants of cell cultures with or without cytopathic effects were submitted to RNA extraction and subsequent RT-PCR. The set of primers used for virus detection in cell cultures were E1.5 (+), E1.6R (-) for the first round of amplification and E1.7 (+) and E1.8R (-) for the nested PCR (Jin and Thomas, 2007). The sequences of the 281 bp amplicon in the 3' end of the E1 region included 225 bp (9245-9469) in the 739 nt window required for genotyping [WHO, 2005b]. The amplicons were concentrated and desalted using the Microcon 100 device (Amicon, Beverly, MA), and were sequenced by Big dye terminator chemistry, v. 3.1, under standard conditions (Applied Biosystems, Foster City, CA) using E1.7 and E1.8R primers. Reaction products were analysed by the ABI 310 Genetic analyser (Applied Biosystems, Foster City, CA). The assemblage of sequences was carried out by the Sequencer package 4.5 of Gene Codes Corporation (Ann Arbor, MI). Phylogenetic and molecular analyses were carried out using MEGA version 4 [Kumar et al., 2004]. A phylogenetic tree was constructed by the Neighbor-Joining method on amino acid sequences; the Kimura two-parameter method was used to calculate nucleotide substitutions, and a bootstrap of 500 replicates confirmed the significance of tree topology. A search for highly similar sequences was performed with the Megablast Algorithm [Zhang et al., 2000]. Sequences generated in this study were deposited in GenBank under the following accession numbers: HM0511264-HM051279. Rubella reference strains [WHO, 2007] and previously reported sequences of Rubella E1 region from GenBank were included in the phylogenetic tree.

Statistics

Data are presented as frequency, proportion or mean, as appropriate. The Chi square and Fisher exact tests were used to test differences in frequencies and the Student-t test to assess differences in mean. The differences were considered significant when $P<0.05$.

RESULTS

Overall, 133 cases were notified, all were submitted to laboratory testing and 111 cases were confirmed. Figure 1 shows the distribution by age of notified cases of rubella together with the number and the proportion of laboratory confirmed cases. Only one case of rubella was confirmed in the highly vaccinated cohort of children (<10 years), while 91% of confirmed cases occurred in the older cohorts. It is worth noting that an alternative viral etiology was established in eight patients: three were positive for HHV6, three positive for parvovirus B19 and two were EBV-positive. Thus, only laboratory-confirmed cases have been used to describe the outbreak and to analyse it in relation to time, place and personal features.

The distribution of cases by month and origin is shown in Figure 2. The epidemic started in a home for young adults disabled mentally and physically . The first case of rubella was notified on 10 January, 2008. He was a 25 years old unvaccinated male resident in a protected care setting. The exposure of the index case to a rubella source could not be discovered. The outbreak involved another disabled home attended by some patients and health care workers of the first home. Eighteen cases were

recorded among subjects attending the care facilities. The mean age was 36.6 years and the male/female ratio was 2 (Table I). No one had been vaccinated previously against rubella. The last case of institutional rubella was observed on 23 March, 2008.

From 2 February 2008 a community-acquired outbreak took place involving a total of 93 subjects: 69 males with a mean age of 25 years and 24 were females with a mean age of 24.5 years. All but two subjects were unvaccinated. Almost all cases occurred in high schools and in industrial settings. Three pregnant women were infected at different gestational weeks. Two pregnancies were terminated and tissues of one fetus were positive for rubella virus. A clear relationship between the two subsequent waves could not be established on the grounds of epidemiologic assessment. The epidemic ended on 3 June, 2008 following the vaccination of susceptible subjects attending the residences for the disabled (56 subjects) and general control measures launched in the entire region.

A phylogenetic tree of the sixteen sequences obtained from the 281 bp amplicons in the E1 region is shown in Figure 3 together with some reference sequences available in Genbank. All viruses were related to the 2B genotype with a highly significant bootstrap value (>70). Only one strain (RVi/ FVG.ITA/06.08/2) proved distinct showing a non-synonymous mutation in nucleotide 2884 of the ORF2 sequence, corresponding to F962G mutation. In addition, the nucleotide sequence analysis revealed two viral populations showing synonymous mutations in positions 2904 and 2955 (Table II). During the first epidemic phase, viruses had a Cytosine both in

position 2904 and 2955, while eight strains isolated from subjects in the general population during the second wave, had Thymine in the same positions. The CC pattern was significantly more frequent in institutionalized patients than in the general population (Fisher exact test: P=0.006) and in the first half of the epidemic than in the second one (Fisher exact test: P=0.005). Interestingly, one sequence (RVi/FVG.ITA/08.08) had a mixture of the two patterns (YY). The Blast analysis of the 280 bp nucleotide sequence with the CC pattern revealed a complete identity with the RVs/London.GRB/9.08 acquired in Italy (GenBank FJ774989) while the TT pattern was very similar to the strain isolated in London in 2006 (RVs/London.GBR/07.06) and in Milan, Italy, in 1994 (RVi 5298-ITA94).

Table III shows the results of the seroprevalence survey in women of childbearing age living in Trieste. From 20 to 44 years of age, the prevalence of rubella antibody was distributed evenly in all age groups (range 92.9-95.3% of seropositivity). The overall prevalence rate in 1416 women of childbearing age was 94.5%.

DISCUSSION

This is one of the largest rubella outbreaks recorded in a highly vaccinated population of a limited area in Italy with about 300,000 inhabitants. It is worth noting that the description of the outbreak is based only on laboratory confirmed cases since the predictive value of the clinical diagnosis of rubella is unreliable, mainly in children with a high immunisation cover. According to findings obtained in similar highly

vaccinated populations, in the youngest cohorts exanthemata of viral or other origin could be confused frequently with rubella infection [Guy et al., 2004].

The pattern of this epidemic was somewhat unusual. The outbreak epicentres were two residential homes for the disabled, and this wave was stopped by vaccination of susceptible subjects. Subsequently, rapid involvement of the general population occurred. Considering the high rate of asymptomatic rubella infections, it is conceivable that the circulation of the virus was extensive. Another epidemiological feature was the marked age-shift of infection. Children were affected very rarely, whereas the age group most affected was that of 20-29 years. Overall, the proportion of females infected was low, probably owing to previous selective vaccination by gender. A different exposure by gender could be excluded, at least in schools. The pattern is consistent with that described in other European countries where rubella vaccination was offered to pre-pubertal girls before universal child immunisation and where opportunistic rubella screening and vaccination are currently offered to women of childbearing age [Jin and Thomas, 2007; Nardone et al., 2008; Schmid et al., 2009]. Despite the low proportion of infected women of childbearing age, three pregnant women were infected, two voluntary abortions were performed and one rubella virus was detected. A revision of CRS registry of this region did not show any additional case of CRS or primary rubella infection in pregnancy until June, 2009.

Genotyping of the viruses isolated showed that the outbreak was sustained by a strain classified as RUBV 2B genotype virus. Genomic characterisation was performed on sixteen isolates by amplification of a screening window of 256 nt inserted in the E1

region, as suggested by WHO Standardisation Committee [WHO, 2005b]. Although the length of amplification fragments was sub-optimal in comparison to that proposed, it has been demonstrated that this way of genotyping is still reliable [Jin and Thomas, 2007]. Phylogenetic analysis showed that the rubella viruses isolated during the present outbreak were related to the reference strain RVi/Seattle.USA/16.00. Although the viruses circulating in both the care facilities and the general population proved to be similar, being identical in all but one aminoacidic sequences, the nucleotide sequences presented two synonymous mutations apparently with a non-casual pattern. The CC pattern was significantly more frequent in patients in institutions during the first half of the epidemic while the TT pattern was present in community acquired rubella during the second wave. This space-temporal pattern may be justified by an evolution of the virus during the epidemic but intermediate CT or TC sequences were not found and a simultaneous mutation in both sites seems unlikely. Thus, molecular tracing by sequencing suggests an independent and subsequent introduction of two distinct variants of RUBV genotype 2B. WHO Laboratory Network for measles and rubella viruses surveillance reported the global distribution of RUBV genotypes, showing that in Europe genotype 1E and 1G are predominant [WHO, 2006; Hubschen et al., 2007]. More recently, the circulation of genotype 1j was documented in Spain, possibly introduced by persons from Latin America [Martinez-Torres et al., 2009]. Genotype 2B is autochthonous in the Far East, namely Japan and Korea, and in South Africa, although it is now considered an imported European genotype [Jin and Thomas,

2007; Novo et al. 2009]. In Italy, genotyping of RUBVs isolated during three consecutive outbreaks (1991-97) showed that clade 1 viruses were predominant, while only two strains belonging to clade 2 were detected during the epidemic of 1994 [Zheng et al., 2003]. Interestingly, 2B genotype sequences detected in this study clustered with one of these old isolates, suggesting an autochthonous origin of this outbreak. Unfortunately, no temporal series of Italian rubella genotypes is available to support this hypothesis. Otherwise, genotype 2B may have been imported, either from countries where this virus was circulating or from the United Kingdom where the genotype had been endemic since 2006. An interchange between Italy and the United Kingdom has been demonstrated for rubella virus as well as for measles virus [Ansaldi et al., 2009].

The epidemic described could be attributed mainly to the vaccine cover in children, still suboptimal, and due to the high rate of seronegative young adults, particularly adult males. In this region, the current MMR vaccine cover of children at two years is about 90%, thus missing the target rate of 95%. The offer of the second dose of the MMR to children aged six-twelve years was launched in this region from 2003 and it is estimated that the current cover based on the 2001 cohort is around 80%. The rate of seronegative adults, notably women of childbearing age, is not well known. Several efforts have been made in Europe to establish a standardized method for attaining comparable data on seroepidemiology [Tischer et al., 2007]. A recent multi-country European survey showed that in several areas the proportion of rubella immune women of childbearing age was far from the optimal figure of more than

95% suggested by the WHO. Twelve out of seventeen countries studied did not attain the required rate of immunity to rubella [Nardone et al., 2008]. In Italy, a cross-sectional nation-wide survey was carried out in 2004 on sera from subjects of all age groups, both male or female [Rota et al., 2007]. The most significant results were the proportion of seronegative males higher than that of female. This is probably due to selective vaccination of pre-pubertal girls and adult women which was undertaken widely, if irregularly, in the past and is in line with the finding of a higher morbidity in males during the outbreak described above. With regards to immunity in women of childbearing age, the proportion of seropositive women ranged from 92% to 95%, with a marked difference between northern and southern areas of Italy. This figure should be compared with that obtained by a cross-sectional survey carried out in 2006, just before the epidemic, and involving more than 1400 women of childbearing age coming from a limited urban area of the same region. The data show that the required proportion of rubella susceptible women (less than 5%) is now close to being reached. Hence, apart from the need to improve universal vaccination by the two-dose strategy, efforts should be made to identify seronegative women for immunization

Acknowledgements

We are very grateful to Mrs. Nadia Giannini and Giusi Carnelos who collected samples and epidemiological data and we thank Mrs. Claudia Biagi, Fabia Petronio and Elena Samar for their excellent technical assistance

REFERENCES

Ansaldi F, Orsi A, Altomonte F, Bertone G, Parodi V, Carloni R, Moscatelli P, Pasero E, Comaschi M, Oreste P, Orengo G, Durando P, Icardi G. 2009. Syndrome surveillance and molecular epidemiology for early detection and tracing of an outbreak of measles in Liguria, Italy. J Med Virol 81:1807-1813.

Bonanni P, Bechini A, Boccalini S, Peruzzi M, Tiscione E, Boncompagni G, Mannelli F, Salmaso S, Filia A, Ciofi degli Atti M. 2007. Progress in Italy in control and elimination of measles and congenital rubella. Vaccine 25:3105-3110.

Ciofi degli Atti M, Filia A, Revello MG, Buffolano W, Salmaso S. 2004. Rubella control in Italy. Euro Surveill 9:19-21.

Giambi C, Filia A, Ciofi degli Atti M, Rota MC, Salmaso S. 2008. Alarm rubella: promote actionsfor capable vaccinate women of childbearing age. BEN Not Ist Super Sanità 21:2.

Guy RJ, Andrews RM, Kelly HA, Leydon JA, Riddell MA, Lambert SB, Catton MG. 2004. Mumps and rubella: a year of enhanced surveillance and laboratory testing. Epidemiol Infect 132:391-398.

Hubschen JM, Yermalovich M, Semeiko G, Samoilovich E, Blatun E, De Landtsheer S, Muller CP. 2007. Co-circulation of multiple rubella virus strains in Belarus forming novel genetic groups within clade 1. J Gen Virol 88:1960-1966.

Jin L, Thomas B. 2007. Application of molecular and serological assays to case based investigations of rubella and congenital rubella syndrome. J Med Virol 79:1017-1024.

Kumar S, Tamura K, Nei M. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Brief Bioinform 5:150-163.

Martinez-Torres AO, Mosquera MM, Sanz JC, Ramos B, Echevarria JE. 2009. Phylogenetic analysis of rubella virus strains from an outbreak in Madrid, Spain, from 2004 to 2005. J Clin Microbiol 47:158-163.

Miller E, Cradock-Watson JE, Pollock TM. 1982. Consequences of confirmed maternal rubella at successive stages of pregnancy. Lancet 2:781-784.

Nardone A, Tischer A, Andrews N, Backhouse J, Theeten H, Gatcheva N, Zarvou M, Kriz B, Pebody RG, Bartha K, O'Flanagan D, Cohen D, Duks A, Griskevicius A, Mossong J, Barbara C, Pistol A, Slacikova M, Prosenc K, Johansen K, Miller E. 2008. Comparison of rubella seroepidemiology in 17 countries: progress towards international disease control targets. Bull World Health Organ 86:118-125.

Novo A, Huebschen JM, Muller CP, Tesanovic M, Bojanic J. 2009. Ongoing rubella outbreak in Bosnia and Herzegovina, March-July 2009--preliminary report. Euro Surveill 14.

Pandolfi E, Chiaradia G, Moncada M, Rava L, Tozzi AE. 2009. Prevention of congenital rubella and congenital varicella in Europe. Euro Surveill 14:16-20.

Rota MC, Bella A, Gabutti G, Giambi C, Filia A, Guido M, De Donno A, Crovari P, Ciofi Degli Atti ML. 2007. Rubella seroprofile of the Italian population: an 8-year comparison. Epidemiol Infect 135:555-562.

Schmid D, Kasper S, Kuo HW, Aberle S, Holzmann H, Daghofer E, Wassermann-Neuhold M, Feenstra O, Krischka C, Allerberger F. 2009. Ongoing rubella outbreak in Austria, 2008-2009. Euro Surveill 14.

Spiteri G, Fenech Magrin AM, Muscat M. 2008. A cluster of rubella in Malta, December 2007--January 2008. Euro Surveill 13.

Tischer A, Andrews N, Kafatos G, Nardone A, Berbers G, Davidkin I, Aboudy Y, Backhouse J, Barbara C, Bartha K, Bruckova B, Duks A, Griskevicius A, Hesketh L, Johansen K, Jones L, Kuersteiner O, Lupulescu E, Mihneva Z, Mrazova M, De Ory F, Prosenc K, Schneider F, Tsakris A, Smelhausova M, Vranckx R, Zarvou M, Miller E. 2007. Standardization of measles, mumps and rubella assays to enable comparisons of seroprevalence data across 21 European countries and Australia. Epidemiol Infect 135:787-797.

WHO. 2005a. Eliminating measles and rubella and preventing congenital rubella infection: WHO European Region strategic plan 2005-2010. WHO Library Cataloguing in Publication Data Copenhagen: WHO Regional Office for Europe; 2005, update reprint 2006 ISBN 92-890-1382-6 Available from: http://wwweurowhoint/Document/E87772pdf.

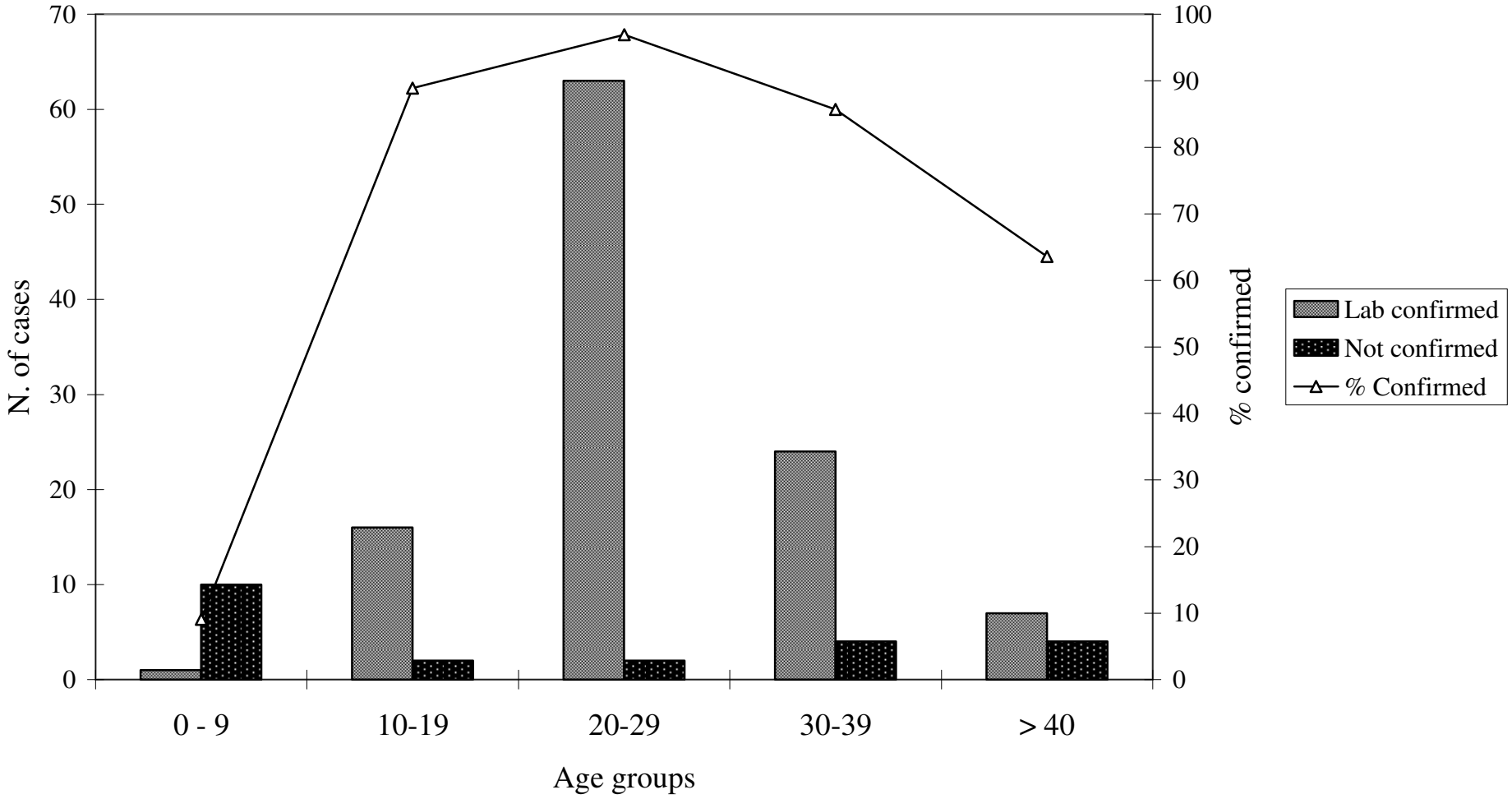
WHO. 2005b. Standardization of the nomenclature for genetic characteristics of wild-type rubella viruses. Wkly Epidemiol Rec 80:126-132.

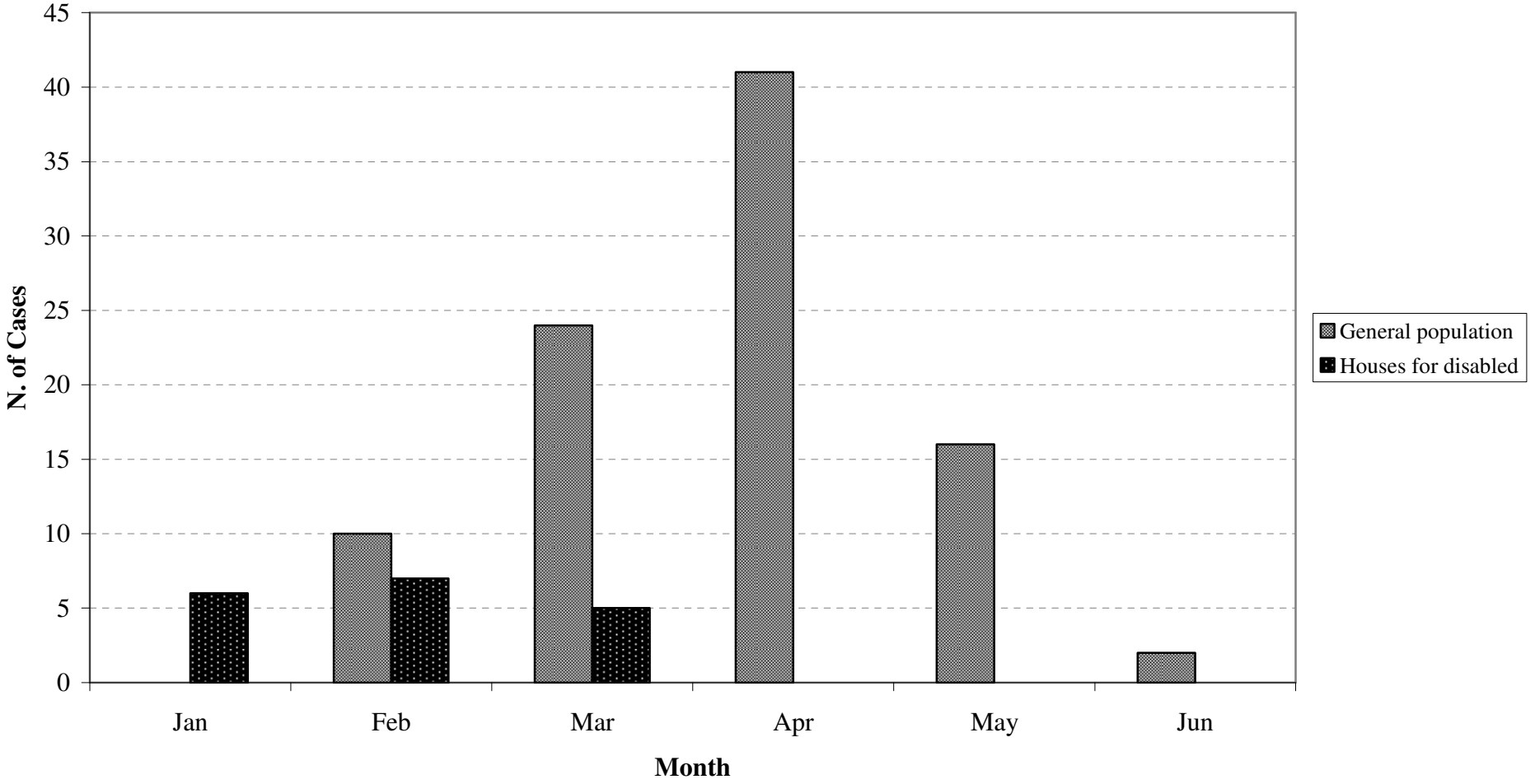
WHO. 2006. Global distribution of measles and rubella genotypes--update. Wkly Epidemiol Rec 81:474-479.

WHO. 2007. Update of standard nomenclature for wild-type rubella viruses, 2007. Wkly Epidemiol Rec 82:216-222.

Zhang Z, Schwartz S, Wagner L, Miller W. 2000. A greedy algorithm for aligning DNA sequences. J Comput Biol 7:203-214.

Zheng DP, Zhu H, Revello MG, Gerna G, Frey TK. 2003. Phylogenetic analysis of rubella virus isolated during a period of epidemic transmission in Italy, 1991-1997. J Infect Dis 187:1587-1597.


70
60
50
40
30
20
10
0
N. of cases
100
90
80
70
60
50
40
30
20
10
0
% confirmed
0 - 9
10-19
20-29
30-39
> 40
Age groups
Lab confirmed
Not confirmed
% Confirmed


45
40
35
30
25
20
15
10
5
0
N. of Cases
Jan
Feb
Mar
Apr
May
Jun
Month
General population
Houses for disabled

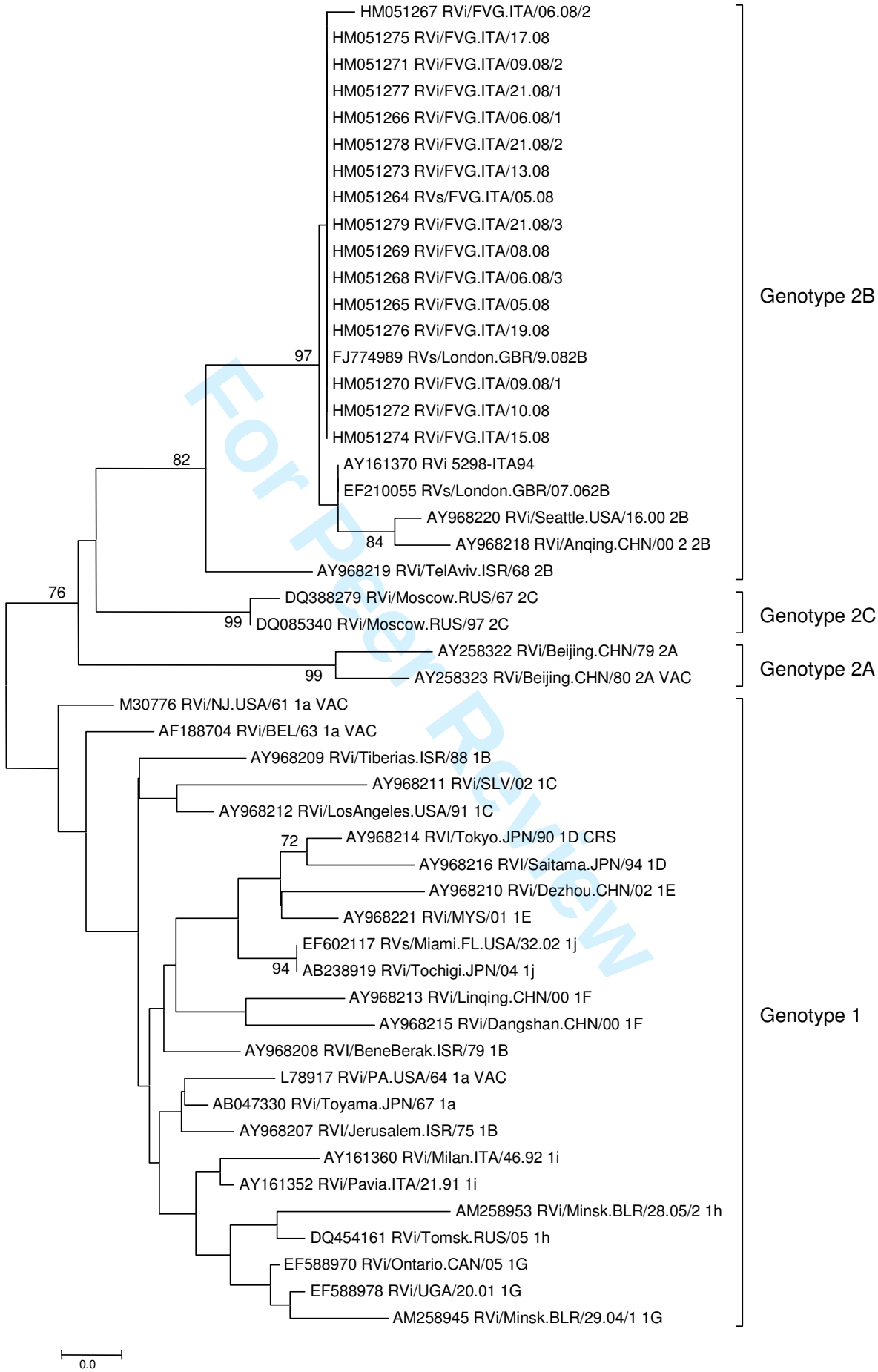


Table I.- Personal features of rubella confirmed cases from the disabled outbreak and the general population outbreak.

Setting	N. of cases	Male			Female			Total		
		N. (%)	Age mean	SD	N. (%)	Age mean	SD	N.	Age mean	SD
Disabled Houses	18	12 (*66.7*)	35.4	9.27	6 (*33.3*)	38.9	13.92	18	36.6	10.75
Community	93	69 (*74.2*)	25.0	6.02	24 (*25.8*)	24.5	9.29	93	24.9	6.96
Total	111	81 (*73.8*)	26.6	7.51	30 (*26.2*)	27.4	11.67	111	26.8	8.78

Tab. II - Epidemiologic and molecular features of 16 rubella strains representative of the outbreak

Rubella strain	Patient age	Sex	Exanthema onset	Source	Genotype	Mutations*		
						2884 [a]	2904 [b]	2955 [b]
RVs/ FVG.ITA/05.08	25	F	28/01/08	Institution	2B	T	C	C
RVi/ FVG.ITA/05.08/2	30	M	28/01/08	Institution	2B	T	C	C
RVi/ FVG.ITA/06.08/1	38	M	30/01/08	Institution	2B	T	C	C
RVi/ FVG.ITA/06.08/2	29	M	03/02/08	Institution	2B	G	C	C
RVi/ FVG.ITA/06.08/3	34	M	04/02/08	General pop.	2B	T	T	T
RVi/ FVG.ITA/08.08	57	F	16/02/08	Institution	2B	T	Y	Y
RVi/ FVG.ITA/09.08/2	15	M	19/02/08	General pop.	2B	T	C	C
RVi/ FVG.ITA/09.08/1	30	M	21/02/08	Institution	2B	T	C	C
RVi/ FVG.ITA/10.08	54	F	01/03/08	Institution	2B	T	C	C
RVi/ FVG.ITA/13.08	31	M	22/03/08	General pop.	2B	T	T	T
RVi/ FVG.ITA/15.08	28	M	03/04/08	General pop.	2B	T	T	T
RVi/ FVG.ITA/19.08	14	M	03/04/08	General pop.	2B	T	T	T
RVi/ FVG.ITA/17.08	1	F	20/04/08	General pop.	2B	T	T	T
RVi/ FVG.ITA/21.08/1	15	M	21/05/08	General pop.	2B	T	T	T
RVi/ FVG.ITA/21.08/2	21	F	21/05/08	General pop.	2B	T	T	T
RVi/ FVG.ITA/21.08/3	24	M	23/05/08	General pop.	2B	T	T	T

* nucleotides are indicated according to the numeration of the rubella ORF2 sequence coding for structural protein;
[a] non synonymous mutation F962G, [b] synonymous mutations

Table III - Seroprevalence of Rubella IgG antibody (≥10 UI/ml) in women of childbearing age of Trieste.

Age group	N. tested	Rubella IgG positive	
		N.	%
15-19	28	26	92.9
20-24	138	129	93.5
25-29	294	280	95.2
30-34	483	454	94.0
35-39	383	365	95.3
40-44	90	84	93.3
Total	1416	1338	94.5

Fig. 1- Distribution of notified and confirmed cases of rubella by age.

Fig. 2.- Distribution of confirmed rubella cases by month and origin.

Fig. 3. Phylogenetic tree of the 280 nt Rubella E1sequences obtained in the study (in bold), in comparison to reference viruses [WHO, 2007] and sequences obtained from GenBank. Reference strains and sequences from GenBank are indicated with the accession number followed by the strain name.