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RESEARCH ARTICLE

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Prevalence of measles virus-specific IgG antibodies according to vaccination schedule in medical students of Padua University

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Aims: The goal of the present study was to establish the efficacy of the measles vaccine and the validity of the vaccination schedule adopted in Italy. **Materials & methods:** The following procedures are adopted: analyze the compliance to the vaccination schedule; assess the seroprevalence of measles antibodies according to the year of birth and the number of doses; and investigate the persistence of positive antibodies post-vaccination. We gathered and elaborated data of both vaccination history and seroprevalence against measles in a large population of students (4195) belonging to the Medical School of Padua University (Italy). **Results:** Our results reveal a requirement for a two-dose vaccination schedule to ensure protection from the disease. Nevertheless, these results clearly indicate that the percentage of seropositivity reached using the two-dose strategy is below the percentage (95%) that ensures optimal population immunity. **Conclusion:** It is uncertain whether immune coverage persists when circulating antibodies vanish, but two vaccine doses should prevent disease outbreaks.

KEYWORDS
• antibodies • measles • vaccination • vaccination schedule

Measles vaccination has been recommended in Italy since 1979 (one-dose schedule) [1]; nevertheless compliance has not exceeded 50%, most likely due to the low consideration of facultative vaccinations [2]. The vaccine schedule (two doses, not mandatory) for measles, mumps and rubella (MMR) was introduced in 1999 [3] and the approval of the National plan for the eradication of measles and congenital rubella [4] according to the objectives of WHO [5], led to an increase in the implementation of the vaccine in individuals younger than 24 months from 74.1% in 2000, to 89.1% in 2008 [6]. At present, the vaccine coverage in Italy for measles is almost 90%, with large regional variations.

The vaccination schedule for MMR consists of a first dose in the second year of life and, according to an elimination plan [4], a second dose at 5–6 (recommended) or 11–12 years of age. As a consequence, the incidence of measles dramatically decreased from 31.6/100,000 in 2002 to 8.7/100,000 in 2008 [6] and 3.6/100,000 in 2013 [7]. Several MMR vaccine types have been used in Italy, such as Pluserix® (GlaxoSmithKline, Schwarz strain), Priorix® (GlaxoSmithKline, Schwarz strain), Triviraten® (Berna, Edmonston-Zagreb strain), Morupar® (Sclavo-Chiron, Schwarz strain) and MMRII® (Aventis-Pasteur, Edmonston 749D strain).

Despite the increase of vaccination coverage and decrease of measles incidence, several outbreaks have occurred in Italy in recent years [8–12], as in other European countries including Romania, Germany, UK and Switzerland [13].

Measles eradication is also needed because the rate of serious complications is equal to 80% in patients with immunodeficiency [14] and the death rate is 70% in oncological patients and 40% in HIV seropositive patients [15].

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The two-dose schedule is strongly recommended because it has been recognized [16] that the effectiveness of the first vaccination is greater than 95%, increasing to more than 99% after the second dose and that immunity persists for an extended period; 15 years after the second dose of MMR vaccine, the rate of measles seropositivity was approximately 95% [17].

The objectives of the present study performed in a large cohort of students belonging to the Medical School of Padua University are: to provide an analysis of vaccination history (age of vaccination, number of doses, interval between doses and vaccination schedule) and compliance to vaccination, to verify the seroprevalence of measles antibodies to establish the efficacy of the current vaccination protocol and to investigate the persistence of seropositivity after vaccination.

Materials & methods

• Setting

According to Italian law on safety and health (legislative decree 81/08), students belonging to degree courses of the Medical School of Padua University (medicine and surgery, dentistry and health professions) have been subjected to health surveillance since 2004. During this period, 5116 students had been screened for antibodies against transmissible but preventable diseases and 4195 were enrolled according to the decision flow chart represented in **Figure 1**.

In addition, the casuistry was subdivided into five age classes illustrated in **Table 1**.

During medical examination, a history of previous measles infection or vaccination has been collected and, if available, the booklet of vaccination had been included with their medical documents.

The research is based on data gathered during health surveillance, then an evaluation by an ethical committee was not required.

• Measurement of measles antibodies

The measurement of antimeasles IgG antibodies was carried out in all 4195 enrolled students using commercial ELISA Enzygnost (Dade Behring, Marburg, Germany). Antibody levels were reported as positive (higher than 300 IU/l),

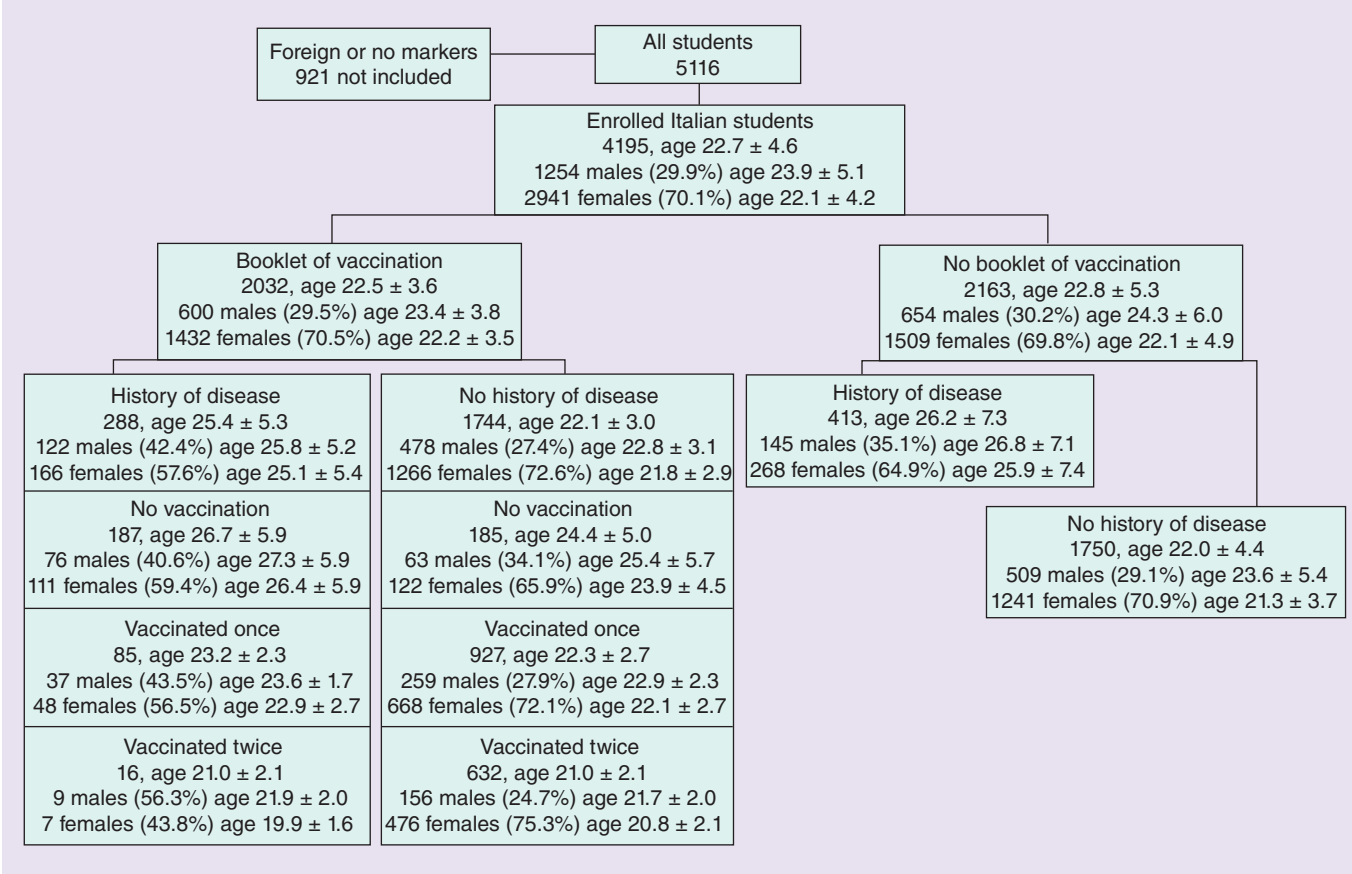


Figure 1. Enrollment and outcomes. Percent of males and females to the total individuals of the specific group.

Table 1. Subdivision according to age (year of birth classes) and gender of students enrolled to study measles seroprevalence.

Year of birth classes		Total	Booklet		Vaccination		
			No	*Yes*	*No*	*Once*	*Twice*
<1980	All	544	456	88	75	13	0
	Males	235	198	37	33	4	0
	Females	309	258	51	42	9	0
1980–1982	All	518	386	132	56	67	9
	Males	170	123	47	27	18	2
	Females	348	263	85	29	49	7
1983–1985	All	1304	663	641	135	393	113
	Males	404	190	214	48	128	38
	Females	900	473	427	87	265	75
1986–1988	All	1242	619	623	68	285	270
	Males	350	130	220	26	112	82
	Females	892	489	403	42	173	188
>1988	All	587	39	548	38	254	256
	Males	95	13	82	5	34	43
	Females	492	26	466	33	220	213

negative (lower than 150 IU/l) or equivocal (150–300 IU/l measles). Equivocal results were statistically processed as positive according to a position paper by the WHO [18].

• Statistics

χ^2 test 2 by 2 (Yates correction) was the prevailing statistical method used to compare the seroprevalence of measles antibodies. Parametric (unpaired t-test) and nonparametric (Mann–Whitney U test) tests were employed to compare the means. Other statistical analyses, such as the mean ± standard deviations (SD) and the median are descriptive. Significance is considered when $p < 0.05$. Statsdirect 2.7.7 version (Statsdirect Ltd, UK) was used for the statistical analyses.

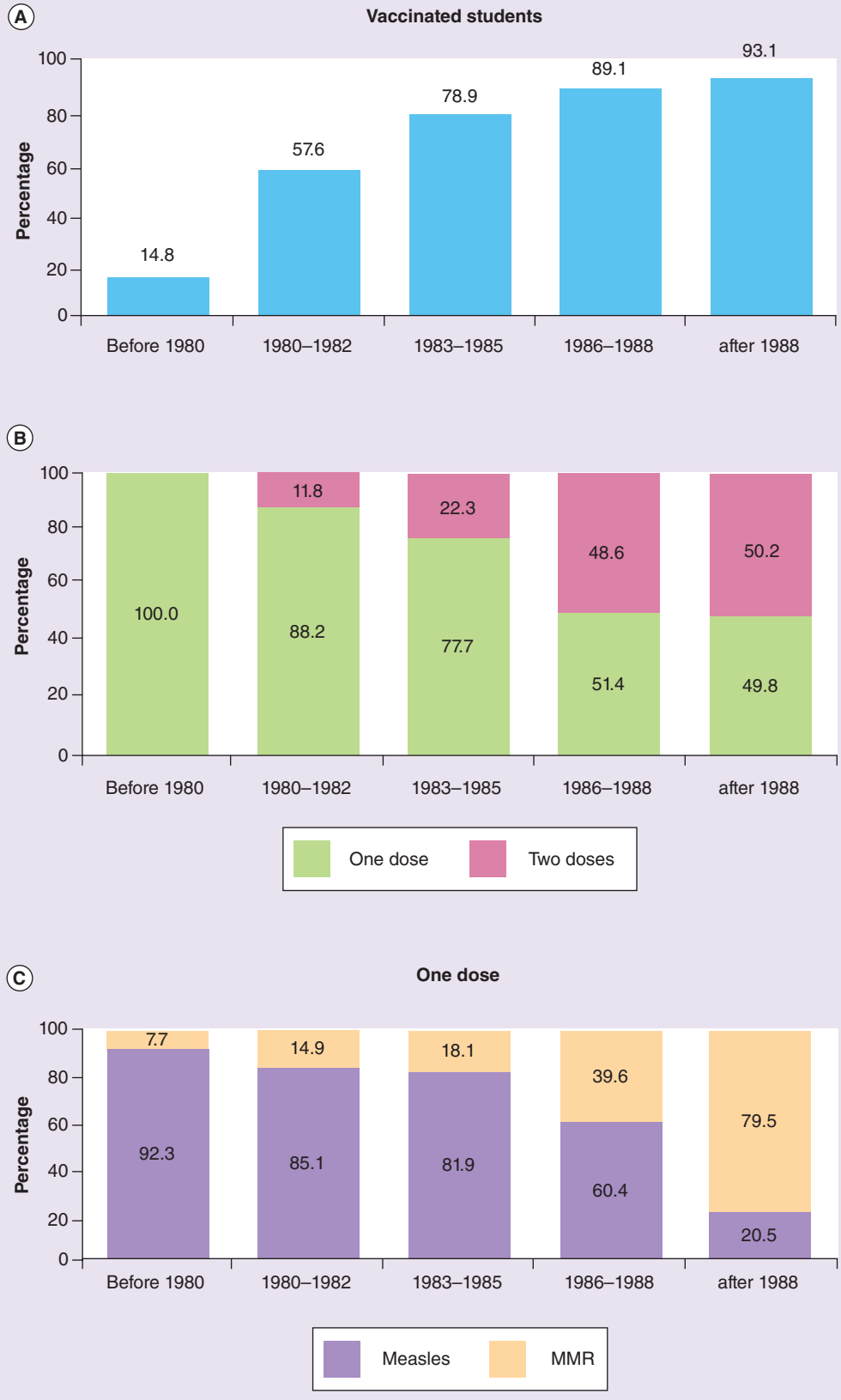
Results

The cohort with the booklet of vaccination (2032 students) reveals an overall compliance to vaccination of 81.7% that progressively increased in subjects born after 1980 and reached a peak (93.1%) in those born after 1988 **(Figure 2A)**. Females (83.7%) were significantly ($\chi^2 = 13.089$, $p = 0.0003$) more favorable to vaccination than males (76.8%) (data not shown). As illustrated by **Figure 2B**, the majority of children born before 1985 were vaccinated with one dose, whereas those born after this year were vaccinated almost equally with one and two-dose schedule. A further analysis reveals that among individuals treated once, those born before 1985 were vaccinated against measles alone, whereas a progressively increasing number of children born since 1985 received a combined MMR vaccine **(Figure 2C)**. Differently, among individuals vaccinated twice, those born before 1985 received at least one dose of the MMR vaccine and most subjects born after 1988 received two doses of the MMR vaccine **(Figure 2D)**.

The vaccination was significantly delayed ($p < 0.0001$) in subjects vaccinated once (mean age 4.07 years) compared with those vaccinated twice (mean age 2.54 years) **(Table 2)**. Moreover, this significant delay persists after subdividing the subjects according to years of birth **(Table 3)**. In the twice-vaccinated students, the interval between the doses was 9.96 years (average), with no gender differences, and the second vaccine dose was administered during adolescence (mean age 12.48 years).

The prevalence of positive measles antibodies measured in all enrolled students (4195) was 84.8%, consistent with what has been previously observed (86.3%) [19]. No significant difference was found according to gender. Students declaring a history of disease showed 93.3% seropositivity, significantly higher ($\chi^2 = 9.153$, $p = 0.0025$) than the 85.5% detected among the vaccinated individuals, independent on their disease history (data not shown).

A further analysis of seroprevalence has been performed by splitting the entire cohort into two subpopulations of individuals according to whether they were born before (544 students)


A
Vaccinated students
Percentage
100
80
60
40
20
0
14.8
57.6
78.9
89.1
93.1
Before 1980
1980–1982
1983–1985
1986–1988
after 1988
B
Percentage
100.0
11.8
88.2
22.3
77.7
48.6
51.4
50.2
49.8
One dose
Two doses
C
One dose
Percentage
7.7
92.3
14.9
85.1
18.1
81.9
39.6
60.4
79.5
20.5
Measles
MMR

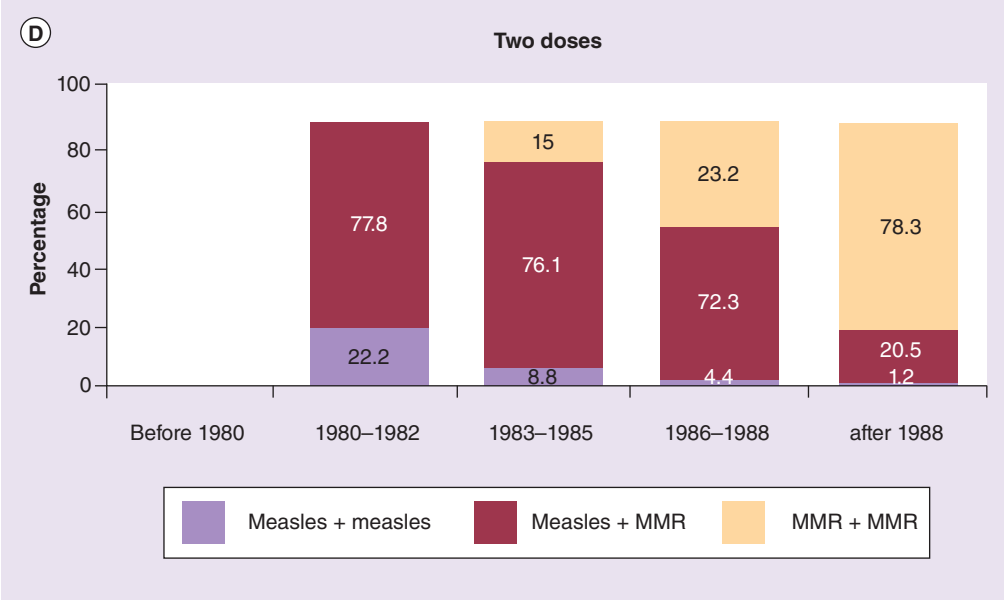


Figure 2. Vaccinated students (see facing page). (A) Percent of vaccinated students regardless of vaccination schedule according to date of birth class; **(B)** percent of vaccinated students depending to vaccination schedule (one or two doses), according to date of birth class; **(C)** percent of vaccinated students depending on vaccination with one dose of measles vaccine only or MMR vaccine, according to date of birth class; **(D)** percent of vaccinated students depending on vaccination with two doses of measles vaccine only or two MMR vaccine doses, according to date of birth class.
MMR: Measles, mumps and rubella.

or during or since 1980 (3651 students), corresponding to the date when the vaccination recommendation began [1]. The students born before 1980 showed a significantly higher prevalence of positive antibodies (93.0%, $\chi^2 = 31.755$, $p < 0.0001$) than those born after 1980 (83.6%) **(Figure 3A)**. As illustrated below, vaccination significantly increased ($\chi^2 = 34.994$, $p < 0.0001$) the rate of equivocal results in the latter group (8.0%) compared with the former (0.9%). If equivocal results were processed as positive, no statistical difference was observed.

The second goal of this round of analysis was to correlate the seropositivity and vaccine schedule. Therefore, the vaccination history of the subpopulation of the 1744 students with a booklet of vaccination and without a personal history of measles was analyzed. These students were subdivided into three subgroups as follows: unvaccinated (185 students), vaccinated once (927 students) and vaccinated twice (632 students).

The prevalence of measles antibodies in all subjects is shown in **Figure 3B**. The student with a booklet attesting no vaccination showed a significantly lower prevalence (61.1%) than those vaccinated once (84.8%, $\chi^2 = 54.459$, $p < 0.0001$) and those vaccinated twice (86.9%, $\chi^2 = 60.234$, $p < 0.0001$). Again, an increase of equivocal results was observed in both the once- (9.4%, $p = 0.0353$) and twice-vaccinated subjects (9.0%, $p = 0.0547$) compared with the unvaccinated subjects (4.3%). This difference (data not shown) was significantly more evident in males (the 4.8% rate of equivocal results in the unvaccinated students increased to 12.0% after one dose and to 12.8% after two doses) than in females (the 4.1% rate of equivocal results in the unvaccinated students increased to 8.4% after one dose and to 7.8% after two doses). In contrast the females showed significantly higher ($p = 0.0286$) prevalence of positivity (88.7%) than the males (81.4%) after two doses, but no significant difference was measured in seropositivity according to the number of vaccine doses.

Given these data, for the third round of analysis, we addressed the question of whether seroprevalence was influenced by the time interval (years) since the last dose. The persistence of positive antibodies was therefore evaluated using a time interval of 10 years (≤10>) since the last dose in both the individuals vaccinated with one dose and those vaccinated with two doses. The rate of antibody positivity decreased (not

Table 2. Age of vaccination, interval between vaccination and marker measurement, and interval between first and second dose (if any) of 1559 students at the Padua University Medical School with a booklet of vaccination and declaring no history of disease.

	Mean ± SD	Median
One dose of vaccine (927 students)		
Age (years) of vaccination	4.07 ± 3.8*	2
Interval between vaccination and marker measurement	19.1 ± 4.4	20
Two doses of vaccine (632 students)		
Age (years) of first vaccination	2.5 ± 1.6*	2
Age (years) of second vaccination	12.5 ± 2.3	12
Interval between first and second dose	9.9 ± 2.3	10
Interval between vaccination and marker measurement	9.4 ± 3.3	9

*$p < 0.0001$ between age at the first vaccine dose according to vaccination schedule (one or two doses).

significantly) from 87.7% within 10 years of the vaccination to 84.6% 10 years after the vaccination in individuals who received one dose of vaccine. Moreover, no change was observed aggregating equivocal with positive data (94.7 and 94.1%, respectively). In the twice-vaccinated subjects seropositivity significantly ($p = 0.0127$) increased from 84.1% within 10 years of vaccination to 91.3% 10 years after the vaccine. On the other hand, aggregating equivocal and positive results, no statistical difference was observed, increasing seropositivity to 94.7% (one, ≤10 years), 94.1% (once, >10 years), 94.9% (twice, ≤10 years) and 97.5% (twice, >10 years).

Discussion

The results of the present research support that vaccination compliance, according to the hypothesis that the parents of children born after vaccine recommendations [1] were more inclined to vaccinate their children, has been found to have consistently and progressively increased in medicine, dentistry and health profession students born since 1980 compared with those born before 1980.

In contrast, birth before 1980 appears to be a condition for positive antibodies against measles. In 1984, 50% of children 2.9–5.5 years were recognized to be naturally immune to measles in Italy [20]. This, in the prevaccination era, is most likely related to wild-type virus circulating in Italy before vaccine recommendations. According to self-reported personal histories, 41.0% of students born before 1980 declared measles infection compared with 13.1% of those born after 1980. In addition, the geometric mean of positive antibody titres was higher in students born before 1980 (4395 IU/l) than in those born since 1980 (1595 IU/l), supporting this evidence. Accordingly, other authors [21] ascribe this seropositivity to the acquisition of natural immunity from exposure to widespread circulation of wild-type virus in the prevaccination era.

Table 3. Year of birth of students at the Padua University Medical School according to the age of their only (one-dose schedule) or first (two-dose schedule) measles vaccine†.

Birth date	Age at vaccination (1 dose schedule)		Age at the first dose (two dose schedule)		p-value‡
	No.	*Mean ± SD*	*No.*	*Mean ± SD*	
Before 1980	28	6.2 ± 4.9	§		
1980–1982	43	7.7 ± 6.8	9	2.8 ± 1.3	0.0115
1983–1985	349	3.9 ± 3.5	112	2.6 ± 2.2	<0.0001
1986–1988	263	4.2 ± 0.9	261	2.4 ± 1.2	<0.0001
After 1988	244	3.3 ± 2.9	250	2.6 ± 1.7	0.007

†Casuistry is the same as **Table 1**.
‡Mann–Whitney U test.
§No child born before 1980 was vaccinated twice.
SD: Standard deviation.

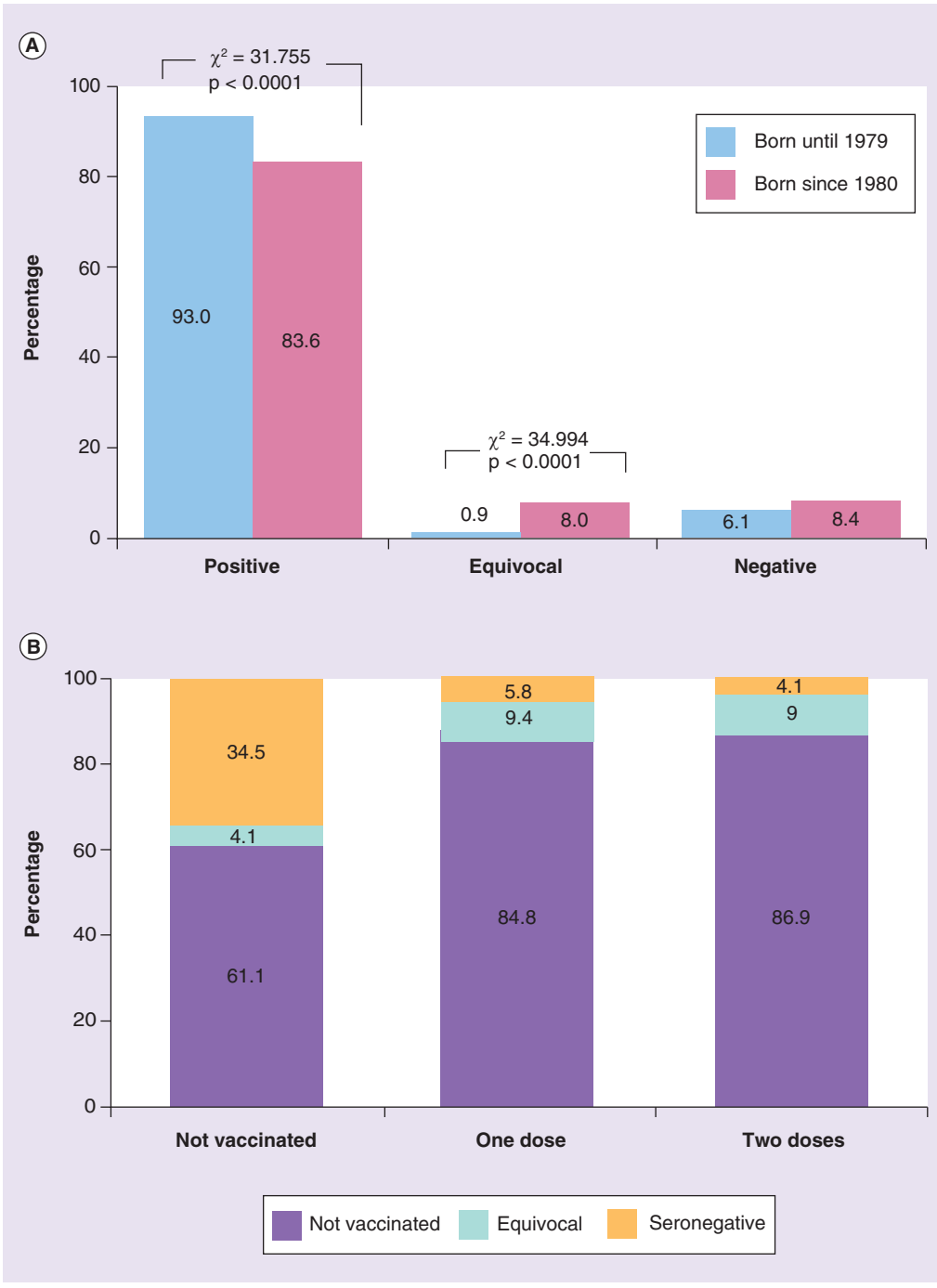


Figure 3. Seroprevalence of measles antibodies. (A) Seroprevalence of measles antibodies in 4195 students enrolled in the study according to their birth before or since 1980, **(B)** and seroprevalence of measles antibodies in students with the booklet of vaccination.

A significant increase in equivocal results was observed in students born after vaccine recommendation and implementation, but according to the recent position paper by the WHO [18], equivocal results should be protective, stating that "*although vaccine-induced antibody concentrations decline over time, immunological memory persists.*" Furthermore, the ELISA method to evaluate measles virus-specific antibodies underestimates seroprevalence by approximately 4% [22].

Among students with a booklet of vaccination, vaccine compliance appears to result in a large immune coverage, but it is not clear because the students who were vaccinated twice were

correctly dosed the first time in the second year of life, whereas those who were vaccinated once were dosed after the fourth year of life. Therefore, a clear age watershed was observed in the choice between one and two dose vaccination schedules corresponding to birth before or since 1985.

The two-dose schedule of measles vaccination was introduced in Italy in 1999 [3] to achieve the objective of measles elimination. The choice of a two-dose schedule is based on the evidence that the transmission of measles is possible in children who receive only one dose of vaccine [23] because primary vaccination failure occurs at rates of 4–8% [24,25].

Our results show a clear choice of a one-dose schedule for individuals born before 1985 and a two-dose schedule for those born since 1985. In addition, measles vaccine alone was administered to the former group, and the MMR vaccine was administered to the latter group.

On the other hand, Kremer *et al.* [26] suggest that a second dose of vaccine is not required because it does not further increase measles seroprevalence. Accordingly, the seroprevalence analysis performed on the entire group of vaccinated students appears to confirm this conclusion: no significant increase of seropositivity was observed in individuals vaccinated twice. The analysis performed on two subgroups of people according to the time-interval elapsing from the first or second vaccination (considering 10 years as the reference value), showed no significant increase in seroprevalence after more than 10 years after the second vaccination.

Thus, immunological response is not only persistent as previously described [17,27–29] but also potentiates over time. The explanation of these results could be related to the age of double-vaccinated individuals. Ten years after the second vaccination, subjects become adults and thus have increased measles contact due to different social and occupational habits. In this time frame, the amplified positivity corresponds to an immunological reaction to measles, which is favored by the immunological sensitization mediated by the second dose. Taken together, these findings unequivocally support the conclusion that the vaccination schedule based on two doses is recommended, although the actual coverage is far from what is expected after 15 years from the second vaccine dose [17].

Conclusion

To our knowledge, this is the first study on the effectiveness of different vaccination schedules against measles in Italy. The results raise relevant questions on immune coverage of young adults and on the effectiveness of the two-dose vaccine schedule in eliminating measles. The study clearly notes that subjects born in the prevaccination era have a higher prevalence of positive antibodies than those born in the vaccination era, most likely because they had greater exposure to wild-type virus and no significant variation of seroprevalence was detectable in time, with positivity persisting for several years (independently of the vaccination schedule). A two-dose vaccination schedule is therefore strongly recommended. Finally, although the increase in vaccine compliance after 1979 is easily explained, we have no clear explanation for the increase in the choice of the two-dose schedule since 1985 or for the increase in the use of the MMR vaccine since 1988, considering that the MMR (offered free of charge) and two-dose schedule have been recommended in Italy only since 1995 [30] and 1999 [3], respectively.

Future perspective

Measles is a diffuse infectious disease with possible serious complications, and natural or acquired immunity is decisive in preventing measles outbreaks. Apparently, the effectiveness of two doses of vaccine is greater than 99% [16], enough to achieve herd immunity, defined as 95% effectiveness in the population [31]. In contrast, our study evidenced that the compliance to vaccination is high (~82%) but insufficient and that the percentage of positive antibodies is far from the level required for herd immunity.

Another question is whether the negative subjects and the equivocal subjects were nonresponders or had 'lost' their circulating antibodies. The revaccination and measurement of antibodies in a small (39%) group of negative subjects and equivocal subjects shows that 86.4% became positive again (data not shown). Is it reasonable to assume that approximately 10% of the population are nonresponders? It is uncertain whether vaccination coverage equaled immune coverage even when circulating antibodies vanished. Alternatively, two vaccine doses should prevent disease outbreaks. In the near future, every effort will need to be made to ensure that good vaccine coverage is maintained.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a

financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

EXECUTIVE SUMMARY

Background

- The implementation of vaccination against measles dramatically decreased the incidence of the disease and the effectiveness of vaccination is stated greater than 95% after the first dose and greater than 99% after the second dose.

Aims

- The study was to establish the efficacy of the measles vaccination in Italy and the validity of the vaccination schedule adopted.

Results

- The results show an increased compliance to vaccination according to vaccination implementation in Italy. Aggregating positive and equivocal results 94.2% after the first dose and 95.9% after two doses of our vaccinated population showed immune coverage.

Conclusion

- The young adults are commonly favorable to vaccination against measles and vaccine seems to give a good immunization coverage. However, the question whether negative or equivocal subjects are nonresponders or whether their circulating antibodies had vanished remains an relevant issue.

References

1 Ministero della Sanità. *Circolare n° 41. Profilassi del Morbillo (Circular 41. Measles Prophylaxis)*. Rome, Ministry of Health, Rome, Italy (1979).

2 Bonanni P, Bergamini M. Factors influencing vaccine uptake in Italy. *Vaccine* 20(Suppl. 1), S8–S12 (2002).

3 Ministero della Salute. *Circolare n° 12. Controllo ed Eliminazione di Morbillo, Parotite e rosolia per Mezzo della Vaccinazione (Circular 12. Control and Elimination of Measles, Mumps and Rubella by Means of Vaccination)*. Ministry of Health, Rome, Italy (1999).

4 Ministerial Decree 29 November 2001 Essential levels of health care. *Gazzetta Ufficiale*33(Suppl. 26), 8 (2002).

5 WHO. Regional Office for Europe. *Health 21. The health for all policy framework for the WHO European Region. European Health for All Series*. 6, 49 (WHO Regional Office for Europe Copenhagen, Denmark (1999).

6 Italian Ministry of Health. La situazione sanitaria del paese 2007–2008. www.salute.gov.it

7 European Centre for Disease Prevention and Control. *Measles and Rubella Monitoring, February 2014 – Reporting on January–December 2013 Surveillance Data and Epidemic Intelligence Data to the end of February 2014*. ECDC, Stockholm, Sweden (2014).

8 Filia A, Barale A, Malaspina S *et al.* A cluster of measles cases in northern Italy: a preliminary report. *Euro Surveill.* 12, e071129.1 (2007).

9 Filia A, De Crescenzo M, Seyler T *et al.* Measles resurges in Italy: preliminary data from September 2007 to May 2008. *Euro Surveill.* 13, pii:18928 (2008).

10 Caputi G, Tafuri S, Chironna M *et al.* An outbreak of measles including nosocomial transmission in Apulia, South-East Italy, January-March 2008 – a preliminary report. *Euro Surveill.* 13, pii:18839 (2008).

11 Calza L, Rossetti N, Piergentili B *et al.* Measles outbreak in the city of Bologna, December 2007 to May 2008. *Infez. Med.* 17, 28–32 (2009).

12 Curtale F, Perrelli F, Mantovani J *et al.* Description of two measles outbreaks in the Lazio region, Italy (2006–2007). Importance of pockets of low vaccine coverage in sustaining the infection. *BMC Infect. Dis.* 10, 62 (2010).

13 Muscat M, Bang H, Wohlfahrt S, Mølbak K, EUVAC.NET group. Measles in Europe: an epidemiological assessment. *Lancet* 373(9661), 383–389 (2009).

14 Centers for Disease Control and Prevention. Measles-United States, 1990. *Morb. Mortal. Wkly Rep.* 40(22), 369–372 (1991).

15 Kaplan LJ, Daum RS, Smaron M, McCarthy CA. Severe measles in immunocompromised patients. *J. Am. Med. Assoc.* 267, 1237–1241 (1992).

16 Watson JC, Pearson JA, Markowitz LE *et al.* Evaluation of measles revaccination among schoolentry-aged children. *Pediatrics* 97, 613–618 (1996).

17 Davidkin I, Jokinen S, Broman M, Leinikki P, Peltola H. Persistence of measles, mumps, and rubella antibodies in an MMR-vaccinated cohort: a 20-years follow-up. *J. Infect. Dis.* 197, 950–956 (2008).

18 Measles vaccines: WHO position paper. *Wkly Epidemiol. Rec.* 84, 349–360 (2009).

19 Trevisan A, Morandin M, Frasson C *et al.* Prevalence of childhood exanthematic disease antibodies in paramedical students: need of vaccination. *Vaccine* 24, 171–176 (2006).

20 Santoro R, Ruggeri FM, Battaglia M, Rapicetta M, Grandolfo ME, Annesi I. Measles epidemiology in Italy. *Int. J. Epidemiol.* 13, 210–219 (1984).

21 Williamson D, Freeman J, Austin P, Allum C, Roberts S. Seroprevalence of measles antibodies among high-risk healthcare workers in Auckland, New Zealand: additional justification for assumption of age-determined immunity based on local data. *Infect. Control Hosp. Epidemiol.* 31, 1082–1084 (2010).

22 van den Hof S, van Gageldonk-Lafeber AB, van Binnendijk RS, van Gageldonk PG, Berbers GA. Comparison of measles virus-specific antibody titres as measured by enzyme-linked immunosorbent assay and virus neutralisation assay. *Vaccine* 21, 4210–4214 (2003).

23 Bloch AB, Orenstein WA, Stetler HC *et al.* Health impact of measles vaccination in the United States. *Pediatrics* 76, 524–532 (1985).

24 Markowitz LE, Orenstein WA. Measles vaccines. *Pediatr. Clin. North Am.* 37, 603–625 (1990).

25 WHO. Expanded Programme on Immunization. Global Advisory Group. *Wkly Epidemiol. Rec.* 65, 5–12 (1990).

26 Kremer JR, Schneider F, Muller CP. Waning antibodies in measles and rubella vaccinees-a longitudinal study. *Vaccine* 24, 2594–2601 (2006).

27 Güris D, McReady J, Watson JC *et al.* Measles vaccine effectiveness ad duration of vaccine-induced immunity in the absence of boosting from exposure to measles virus. *Ped. Infect. Dis. J.* 15, 1082–1086 (1996).

28 Dine MS, Hutchins SS, Thomas A, Williams I, Bellini WJ, Redd SC. Persistence of vaccine-induced antibody to measles 26–33 years after vaccination. *J. Infect. Dis.* 189(Suppl. 1), S123–S130 (2004).

29 LeBaron CW, Beeler J, Sullivan BJ *et al.* Persistence of measles antibodies after 2 doses of measles vaccine in a postelimination environment. *Arch. Pediatr. Adolesc. Med.* 161, 294–301 (2007).

30 Ministero della Sanità. *Circolare n° 13. Vaccinazioni Obbligatorie e Facoltative. Attuazione del Piano Sanitario Nazionale 1994–96 (Circular 13. Mandatory and Optional Vaccinations. Implementation of National Health Plan 1994–96).* Ministry of Health, Rome, Italy (1995).

31 Nokes DJ, Anderson RM. Measles, mumps, and rubella vaccine: what coverage to block transmission? *Lancet* 2(8624), 1374 (1988).