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Study of the immune response engendered by differents combined measles, mumps and rubella (MMR) vaccines in an area of Andalusia (Spain)

Concepción Cruz Rojo [a,d,*], Manuel Rodríguez Iglesias [b], Juana Olvera [c], Manuela Álvarez Girón [d]

[a] *Distrito Sanitario de Atención Primaria Bahía de Cádiz-La Janda, Avda. Ramón de Carranza no. 19, 11006-Cádiz, Spain*
[b] *Laboratory of Microbiology, Puerto Real's University Hospital, Carretera Nacional IV Km, 665, 11510 Puerto Real, Cádiz, Spain*
[c] *Centro de Salud "San Fernando Este", Carretera La Carraca s/n, 11100 San Fernando, Cádiz, Spain*
[d] *Departamento de Ciencias Socio-Sanitarias, Facultad de Medicina, Universidad de Sevilla, Avda. Sánchez Pizjúan s/n 41009 Sevilla, Spain*

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Abstract

The objective of the study is to evaluate and compare the degree of serological protection conferred by the three components of two MMR vaccines: "Vac triple MSD®" (Aventis Pasteur MSD) and "Triviraten®" (Berna), and to study the effects of a second dose of "Priorix®" (Glaxo SmithKline), in an unprotected population. In March 2001, this study was conducted in a sample of 86 children aged 3 and 4 years, in two Basic Health Zones of Cádiz (Spain). Mumps, measles and rubella antibody titers were evaluated by IgG enzyme linked immunosorbent assay (ELISA). All the children showing lack of response were revaccinated with the vaccine "Priorix®" of GSK; in 12 of these children (all vaccinated previously with "Triviraten®"), studies confirmed the existence of seroconversion utilizing the same methodology. The most outstanding finding has been the low percentage of children vaccinated with "Triviraten®" possessing protective titers (>1:500) against mumps (14.3%) compared with those vaccinated with "Vac triple MSD®" (81.1%, $P < 0.000001$); geometric mean values (GMT) of 164 and 1631, respectively, were obtained. Significant differences, and in the same direction, were also found in respect of measles (83.7 and 100%, and GMT of 889 and 5076), although not so striking. However, all the children studied did have protective titres (>16 UI/ml) of antibodies against rubella. Of the 12 children studied who had not responded with protective titers of anti-mumps antibodies, eight children (66.7%) showed seroconversion with "Priorix®", and only one child (25%) presented seroconversion in the response to measles. We have thus proved that the "Rubini" strain provides insufficient protection against mumps in our child population. We have also found that the "Edmonston–Zagreb" strain confers less protection against measles than the "Enders" strain, although the "Schwarz" strain, after revaccination of the children who had failed with the "Edmonston–Zagreb" strain, did not achieve a satisfactory seroconversion, either.

Keywords: Measles; Mumps and rubella vaccines; Immune response; Antibodies

1. Introduction

The control of mumps begins with the development of the attenuated vaccines against this disease. Although monovalent vaccines and vaccines with two components (mumps with rubella or measles) have been utilized, in the main, trivalent vaccines (mumps, measles and rubella, or MMR) have been employed. In Spain, in 1999, the Inter-Territorial Council of the National Health System (Spanish Ministry of Health and Consumer Affairs) proposed a new vaccination schedule, different from that of 1998 [1], which stipulates the administration of the trivalent vaccine in a first dose at 12–15 months and a second between 3 and 6 years.

Several vaccines against mumps employing various attenuated strains have been utilized (Table 1). The Jeryl Lynn strain of MSD incorporated in the new vaccines of Aventis Pasteur MSD, is obtained from fibroblasts of chicken embryo, and is utilized in most of the industrialized countries. In various European countries, including Spain, the "Triviraten®" vaccine has been widely utilized; this is prepared in cultures of human diploid cells, and in all these countries sporadic outbreaks of mumps have been described [2–11], which have been associated with a failure of the

* Corresponding author. Tel.: +34-956-004706; fax: +34-956-004-703.
E-mail address: ccruz@acadiz.sas.junta-andalucia.es (C.C. Rojo).

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Table 1
Trivalent MMR vaccines (and strains utilized) currently available in Spain[a]

Name (Laboratory)	Measles	Mumps	Rubella
"Vac triple MSD®" (Aventis Pasteur MSD)	Enders hyper attenuated	Jeryl Lynn	Wistar RA 27/3
"Priorix®" (GlaxoSmithKline Beecham)	Schwarz	RIT4385, derived from the Jeryl Lynn	Wistar RA 27/3
"Triviraten®" (Berna Institute)	Edmonston–Zagreb	Rubini	Wistar RA 27/3

[a] Modified from the "Manual de Vacunas en Pediatría" [12].

vaccine, in its mumps (Rubini strain) component, to confer protection. This evidence justified the Spanish Ministry of Health and Consumer Affairs in 1999 in recommending that the use of this preparation should be avoided in the systematic vaccination of the population. However, since it had been widely utilized up to that time as the first dose, it would be reasonable to suppose that there was a group of the child population with deficient protection against the mumps virus, and this has actually been demonstrated by surveys of seroprevalence [12–15].

Our specific reason for undertaking this study was the outbreak, in January 2001, of a mumps epidemic in the town of Chiclana de la Frontera (Cádiz, Spain) that affected more than 200 children, preferentially those of preschool age. When the first cases appeared, it was advised that the infection should be confirmed (by determination of the serum IgM of mumps) and that the titres of antibodies against the three diseases of the triple virus vaccine, mumps, measles and rubella, should be determined.

The objectives of the present study are: (1) to evaluate and compare the degree of serological protection given by the three components of two MMR vaccines: "Vac triple MSD®" of Aventis Pasteur MSD, and "Triviraten®" of Berna, utilized in our area; (2) to confirm by laboratory techniques that the vaccine "Triviraten®" is not protecting against mumps, nor against measles and/or rubella in some cases; (3) to study the effects of a second dose of vaccine in the population left unprotected.

2. Materials and methods

The study was conducted in March 2001 in two Basic Health Zones (BHZ) of the Cádiz conurbation (Province of Cádiz, Andalusia): "San Fernando-East" of the town of San Fernando and "Pinillo Chico" of the town of El Puerto de Santa María. No cases of mumps or measles had been declared in either of the BHZs for more than 5 years prior to the study (according to the records of the Epidemiological Monitoring System of the Healthcare District), and the MMR vaccination coverage in those years (the 1997–2000 cohorts) was more than 98.5%. The BHZ is the smallest of the geographic areas into which Andalusia is divided for purposes of health administration.

The selection of the age range of the children to be included in the sample took into account that our objective was to measure the serological response to the first dose of MMR vaccine, and this is administered at 15 months of life, in accordance with our vaccination program. Therefore we selected children aged between 2 years (thus leaving a margin to ensure that the first dose of trivalent vaccine should already have been administered) and 5 years, when the subjects would not yet have received the second dose, which is administered at 6 years of age. Of these cohorts, it was confirmed that the younger children (of 2 years of age) had almost all been vaccinated with the "Priorix®" vaccine, and the children of 5 years of age had almost all been vaccinated with "Triviraten®", only the children between 3 and 4 years of age had been vaccinated with the two preparations that we wanted to evaluate, "Triviraten®" and "Vac triple MSD®", although a higher proportion with the first than with the second. For this reason, the target population obtained was all children born in the two BHZ between April 1997 and March 1998 (aged between 3 and 4 years) and vaccinated with the first dose with "Triviraten®" (260 children) and "Vac triple MSD®" (123 children).

Next we calculated what size of sample it was necessary to select for a α of 0.05, an accuracy of 5% and with a seroprevalence of antibodies for the three diseases of at least 95%. The result obtained utilizing the exact method [16] was 58 children for "Triviraten®" and 46 for "Vac triple MSD®". Of these, 15 could not be located or did not respond (14%), therefore 89 blood samples were obtained under standard conditions, of which three could not be processed for various reasons and were eliminated from the study.

Antibodies to measles and mumps were determined using a commercial EIA (Enzygnost, Behring). This test yielded quantitative results without titration of the sample prediluted 1:231 (single-point quantification) [17]. Antibodies to rubella were also determined by ELISA (Cobas Core, Roche); the results from this are expressed in IU/ml. In order to determine the degree of protection provided by each component of the MMR vaccine, we have classified the titers of antibodies obtained into three groups, negative, low positive and protective level. The low-level range for antibodies against measles was defined as $1:150 < 1:375$, mumps 1:230–1:500 and rubella 7–16 IU/ml.

All children showing not response were revaccinated with the "Priorix®" trivalent vaccine of GSK. Later, from the 12 children registered with the San Fernando-East Health Centre who had previously been vaccinated with "Triviraten®", a new serum sample was taken to confirm the existence of

seroconversion, utilizing the same methodology. All these children had shown a lack of protection against mumps, and in four of them also against measles.

The statistical significance of the differences observed between the two vaccines has been calculated for the proportions (*z*-test) and for the geometric mean of titers of antibodies (Student's *t*-test, conducted on logged titres), the magnitude of these differences being assessed at 95% confidence interval. Also the Pearson's coefficients of correlation between the titers of anti-mumps and anti-measles antibodies of those children vaccinated with "Triviraten®" and those with "Vac triple MSD®" have been calculated.

3. Results

We have studied 86 children (34 girls and 52 boys), of whom 60 were registered with the "San Fernando-East" Health Centre, and 26 with the "Pinillo Chico" Health Centre of El Puerto de Santa María; the age of the youngest child was 3 years and 1 month, and that of the oldest was 4 years and 1 month. Also, according to the type of vaccine administered, 49 children had been vaccinated with "Triviraten®" and 37 with "Vac triple MSD®".

The most outstanding finding has been the low proportion of children vaccinated with "Triviraten®" that possessed protective titers against mumps, only 14.3% (7/49 children), against 81.1% (30/37 children) of those vaccinated with "Vac triple MSD®" ($P < 0.000001$, z of 7.7). Differences have also been shown in the percentages of children with protective levels of antibodies against measles, between those vaccinated with "Triviraten®" and those with "Vac triple MSD®" (83.7 and 100%, respectively), differences that are also significant ($P < 0.001$, z of 3.3), although not so striking as in the case of mumps. In contrast, however, it has been demonstrated that all the children studied did have protective titres of antibodies against rubella (Table 2).

When we present the data quantitatively, that is, with the geometric mean (GMT) of the titers of antibodies (expressed in dilution factor or IU/ml) obtained against each of the diseases, it is again notable that there exist clear differences between the children who were vaccinated with "Triviraten®" and those with "Vac triple MSD®", both for mumps component (GMTs of 164 and 1631, respectively) and for measles component (GMTs of 889 and 5076, respectively), with the differences in both cases being very significant ($t = 9.7$; $P < 0.000001$, and $t = 8.5$; $P < 0.000001$, respectively) (see Table 3). And, in agreement with the results of the qualitative analysis, it has been shown that the geometric mean of the titers of antibodies against rubella are practically equal in both types of vaccines (GMT = 62 and GMT = 59 for "Triviraten®" and "Vac triple MSD®", respectively, $t = 0.28$; $P = 0.39$).

If the quantitative results obtained in each serum sample (according to the type of vaccine received) are associated with the level of anti-mumps and anti-measles antibodies, a linear and positive correlation is observed in both cases, although the correlation of GMT antibodies titers with the "Vac triple MSD®" vaccine is higher ($r = 0.47$ for "Vac triple MSD®" and $r = 0.36$ for "Triviraten®") (see Fig. 1).

The results evident from the serological analysis after revaccination with the "Priorix®" trivalent MMR vaccine indicate that, of the 12 children studied who had not responded with protective titres of anti-mumps antibodies after administration of the "Triviraten®" vaccine, in eight of these seroconversion was demonstrated (66.7%). Of those 12 children, four had not responded to the measles component, and following the revaccination only one (25%) presented seroconversion in the response to measles. Lastly, one child retained a low combined response to mumps and to measles after the revaccination dose.

Table 2
Levels of protective, low positive, and negative antibodies against mumps, measles and rubella

		Trivalent MMR vaccines					
		"Triviraten®" ($n = 49$)			"VAC TRIPLE MSD®" ($n = 37$)		
		Protective	Low	Negative	Protective	Low	Negative
Mumps	N	7	12	29	30	6	1
	%	14.3	24.5	59.2	81.1	16.2	2.7
	CI	8.3–20.3*	18.5–30.5	53.3–65.2	75.1–87.1*	10.2–22.2	0–8.7
Measles	N	41	4	4	37	0	0
	%	83.7	8.2	8.2	100	0	0
	CI	77.7–89.7**	0–14.2	0–14.2	94–100**	0–6	0–6
Rubella	N	49	0	0	37	0	0
	%	100	0	0	100	0	0
	CI	94–100	0–6	0–6	94–100	0–6	0–6

By type of vaccine, number of children, percentage and 95% confidence intervals. *N*: number of children; %: percentage; CI: 95% confidence interval.
* $P < 0.000001$.
** $P < 0.001$.

Table 3
Geometric mean of the titers of antibodies, and 95% confidence intervals, against mumps, measles and rubella, by type of MMR vaccine

Type of MMR vaccine		Mumps (titration)	Measles (titration)	Rubella (IU/ml)
"Triviraten®"	GMT	164*	889*	62
	CI95	106–201	590–1098	48–71
"Vac Triple MSD®"	GMT	1631*	5076*	59
	CI95	1071–2113	3716–6352	44–70

GMT: geometric mean of the titers of antibodies; CI95: 95% confidence interval.
* $P < 0.000001$.

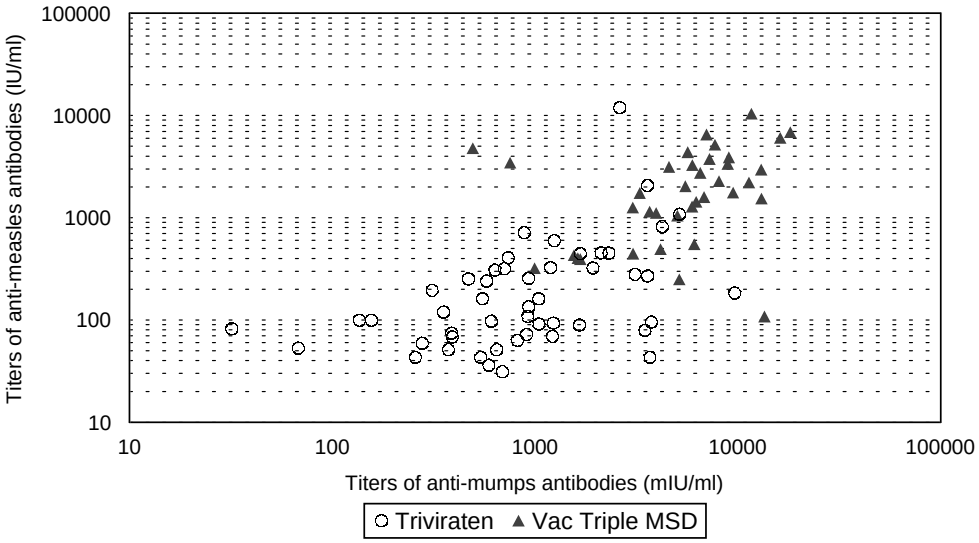

Fig. 1. Relationship of the serological response against mumps and measles in children vaccinated with two different trivalent MMR vaccines.

4. Discussion

The present study analyses the degree of serological protection provided by each of the components of two MMR vaccine, in a sample of children aged 3 and 4 years in two Basic Health Zones (BHZ) of the province of Cádiz, Spain. The decision to include all the children of these ages resident in the BHZs and not to limit the study to those whose parents initially agreed to the request for a blood sample from their child, has meant that we were able to work with completely healthy subjects. Despite not being able to locate some of the children selected, and despite not obtaining the authorisation of the parents in some other cases, there is nothing to suggest that these circumstances implied any differences in relevant characteristics in these children that might bias the results. Despite the age range of the children in the sample studied not being very wide (only 12 months), it should be noted that the children vaccinated with Triviraten® were older than those with MSD. This could represent a bias or factor of confusion, since it is known that the titres of antibodies fall in line with the length of time elapsed since vaccination. However, it has been shown that most of the children vaccinated with MMR remain seropositive for between 3 and 4 years following the inoculation [18–20]. In our study, except for one child in which the time elapsed between his vaccination and the measurement of his titres of antibodies was 36 months, in all the children studied, this length of time was less (between 18 and 34 months). Further, we do not believe that the circumstance of the children studied belonging to two different municipalities constitutes a factor of confusion. Because the two BHZs have percentages of well-vaccinated children of close to 100%, and given the absence of declared cases of these diseases (mumps and measles), the circulation of wild viruses in the two areas is very unlikely.

The findings obtained with the sample studied demonstrate in a very significant way the different serological response against mumps according to the type of vaccine administered: the protective response was 14.3% for "Triviraten®" and 81.1% for "Vac triple MSD®". The low response with the Rubini strain has been sufficiently documented in studies conducted in countries where it has been widely utilised [4,5]. Toscani et al. [21], describe an mumps outbreak in Geneva, and find a level of protection in the population studied of 12.4% with the Rubini strain and 64.7% with the Jeryl Lynn strain. The results obtained by Germann et al. [5] are similar for the Rubini strain (lack of immunization in 87% of the population studied). In a recent study of Tischer and Gerike [22], they detect a response in 38% of the subjects vaccinated with Rubini (although more than half the subjects have low titres), similar to the figure

that we would obtain in our study if we included those with a low level of antibody titers (38.6%). The results found with the Jeryl Lynn strain fall between 61 and 96.5% [22–25].

An interesting aspect on analysing the results obtained with the Rubini strain is the importance of the technique utilised. Thus, Tischer and Gerike [22] manage to detect levels of antibodies by neutralizing antibody test in VERO cells in 93.4% of the samples, and Schwarzer et al. [26] also find a detectable cellular response in all the samples of subjects vaccinated with the Rubini strain. Similarly, Khalil et al. [27] confirm that, in assessing the response of antibodies induced by the Rubini strain, the indirect immunofluorescent test is superior to ELISA. It can be deduced from all this that the response is not null but that it is insufficient in numerous cases and depends on the technique utilised.

Poltera and Herzog [28] defend the immunogenicity of the "Triviraten®" vaccine for the Rubini and Edmoston–Zagreb strains, adducing that the commercial kits, such as Enzygnost do not utilise the original viral strains of "Triviraten®", and cite a series of studies that throw up high percentages of seropositivity. Equally, ELISA as the way of correctly assessing the immunogenicity of that MMR vaccine is questioned, and others such as the plaque neutralisation test are preferred. However, they do not respond to the fact that various outbreaks of mumps have been associated with populations of children vaccinated with "Triviraten®", as we have already described. We believe that laboratory tests do serve as methods of evaluation of immunogenicity, but that epidemiological observations on the effectiveness of the vaccination are more decisive. It could also be deduced that the assessment of immunogenicity from both perspectives (laboratory tests and epidemiological assessments) would, in practice, help to indicate whether humoral or cellular-based trials are more specific and sensitive for detecting adequate or inadequate protective responses produced by the different vaccines utilised.

The response against rubella from both types of vaccine (both containing the same Wistar RA 27/3 strain) confers 100% protection in all the cases, with no appreciable or significant differences having been found in the geometric means of titres of antibodies; this finding largely coincides with other studies in which the degree of serological protection of various triple virus preparations is analysed [22,26,29,30].

With respect to the induction of anti-measles antibodies with the "Triviraten®" vaccine, four cases of lack of response (8.2%), and another four (8.2%) with a level of antibodies giving only low serological protection, have been detected. These results indicate that the Edmonston–Zagreb strain has less immunogenic capacity than the Enders strain utilised in the Aventis Pasteur vaccine, a fact very evident when the geometric means of the titers of antibodies obtained with each vaccine are analysed; this latter datum coincides with previous publications [22,26]. Other studies are not conclusive when the Edmonston–Zagreb strain is compared with the Schwarz strain (of the "Priorix®" vaccine of GSK): serological responses are similar or not, but it has always been observed that the Schwarz strain presents a higher geometric mean of anti-measles antibodies [30–33]. Bruno et al. [34] and Hussey et al. [35] find a serological response three times more powerful with the Schwarz strain. The reasons for this presumed low response are not clear; they could be associated with the attenuation in human diploid cells in the case of the Edmonston–Zagreb strain, although it has not been possible to find differences in respect of biological characteristics, reactivity, immunogenicity and degree of attenuation between the genomes of the viral strains utilised [36,37].

Moreover, in our study a relationship, albeit weak, is confirmed in the serological response of the vaccine against measles and against mumps, independently of the type of trivalent MMR vaccine administered, this response being higher for those vaccinated with "Vac triple MSD®". St. Sauver et al. [38] also find, among schoolchildren vaccinated with MMR vaccine, a modest although significant correlation between the components anti-measles and anti-mumps. This finding only serves to show another quite natural conclusion, that the biological factor also influences the higher or lower serological response, and in the same direction, in the face of different viral antigens of a vaccine, independently of the commercial preparation.

The serum samples taken after revaccination with "Priorix®" could not be obtained sooner than 8 months later, and although the ideal time for checking the immunogenicity of the vaccine preparation is 2–4 weeks after the inoculation, various studies have demonstrated that, after the administration of the triple virus vaccine, most of the vaccinated subjects remain seropositive against the three diseases in the following 3–4 years [18–20]. The results obtained have demonstrated that a new dose seroconverts most of the children who did not respond adequately to the anti-mumps vaccine and removes any doubt about the need to make the second dose obligatory [39]. With respect to measles, the response was lower (three children out of four were left without protection against measles) and was combined with mumps in one case. This phenomenon could be associated with mechanisms of recognition of the virus and homozygocity of the HLA antigens [40], therefore we have initiated a study in this group of children.

With this study we believe we have proven very clearly, and in our own area (Andalusia, Spain), the message that the mumps outbreaks in different parts of our country and in other countries have been signalling as a warning: the "Rubini" strain provides insufficient protection against mumps. The appearance of these outbreaks led the Spanish Ministry of Health and Consumer Affairs in 1999 to recommend that the vaccine "Triviraten®" should not be utilised in a systematic way. Similarly, the WHO in November 2001 recommended that vaccines with the Rubini strain should not be utilised in programs of immunisation against mumps due to its demonstrated low effectiveness [41]. We have also confirmed that the "Edmonston–Zagreb" strain confers less

protection against measles than the "Enders" strain; although the "Schwarz" strain, after revaccination of the children who had failed to respond with the "Edmonston–Zagreb" strain, did not achieve a satisfactory seroconversion, either.

In conclusion, we consider it advisable to continue assessing the effectiveness of the vaccines against mumps that we are currently utilising. Considering a 95% immunity response and 95% vaccination coverage as standard for preventing the transmission of the virus in our community, we only obtain a serological protection of 81.1% in the case of the "Vac triple MSD®", and the study of revaccination with "Priorix®" has made us aware that the effectiveness of the latest vaccines not is conclusive. Epidemiological vigilance of these diseases for which vaccines are available, together with studies of the levels of protection provided by new vaccines, is essential in order to guarantee the correct immunisation of our children.

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References

[1] Centro Nacional de Epidemiología. Calendario unificado de vacunaciones infantiles. España 1998. Bol Epidemiol Semanal 1997;5(24):236.
[2] Matter HC, Cloetta J, Zimmermann H, Sentinella A. Measles, mumps, and rubella: monitoring in Switzerland through a sentinel network, 1986–1994. J Epidemiol Community Health 1995;49 (Suppl 1):4–8.
[3] Paccaud MF, Hazeghi P, Bourquin M, et al. Ruckblick auf zwei mumpsausbruche. Soz Präventivmed 1995;40:72–9.
[4] Dias JA, Cordeiro M, Afzal MA, Freitas MG, Morgado MR, Silva JL, et al. Mumps epidemic in Portugal despite high vaccine coverage—preliminary report. Eurosurveillance 1996;1:25–8.
[5] Germann D, Ströhle A, Eggenberger K, Steiner CA, Matter L. An outbreak of mumps in a population partially vaccinated with the Rubini strain. Scand J Infect Dis 1996;28:235–8.
[6] The Benevento and Campobasso Pediatricians Network for the control of vaccine-preventable diseases. Field evaluation of the clinical effectiveness of vaccines against pertussis, measles, rubella and mumps. Vaccine 1998,16:818–22.
[7] Gonçalvez G, de Araujo A, Montero Cardoso ML. Outbreak of mumps associated with poor vaccine efficacy, Oporto, Portugal, 1996. Eurosurveillance 1998;3:115–21.
[8] Flores R, Cremades A. Brote de parotiditis en el área sanitaria 17. Eficacia de la vacuna triple vírica (II) y comparación de la incidencia de la enfermedad según el año de vacunación. Rev Esp Salud Pública 1998;72(Suppl):123–4.
[9] Pons C, Carmona T, Castellanos T, Manrique JA, Martín-Sierra M, Vanaclocha H. Qué factores están implicados en la última onda epidémica de parotiditis ocurrida en la Comunidad Valenciana? Rev Esp Salud Pública 1998;72(Suppl):124.
[10] Peñuelas JA, Peyró R, Diestro A, Pastor MC. Efectividad vacunal, según las cepas administradas en un brote epidémico de parotiditis. Rev Esp Salud Pública 1998;72(Suppl):130.
[11] Chamot E, Toscani L, Egger P, Germann D, Borquin C. Estimation de l'efficacité de tríos souches vaccinales ourliennes au cours d'une épidémie d'oreillons dans le canton de Genève. Rev Epidemiol Santé Publique 1998;46:100–7.
[12] Comité Asesor de Vacunas. Manual de Vacunas en Pediatría. Madrid: Asociación Española de Pediatría; 2001.
[13] Pachon I, Amela C, De Ory F, León P, Alonso M. Encuesta nacional de Seroprevalencia de enfermedades inmunoprevenibles. Año 1996. Bol Epidemiol Semanal 1998;6:93–100.
[14] Gallardo V, Camino F, Garcia J, et al. Encuesta Seropidemiológica de Andalucía. Junta de Andalucía. Consejería de Salud. 1999.
[15] Vega AT, Ruíz C, et al. 1ª Encuesta Seroepidemiológica de Castilla y León. Junta de Castilla y León. Consejería de Sanidad y Bienestar Social; 1996.
[16] Cochran WC. Sampling techniques. New York: Wiley; 1963.
[17] Dopatka HD, Giesendorf B. Single point quantification of antibody by ELISA without need of a reference curve. J Clin Lab Analysis 1992;6:417–22.
[18] Watson J, Pearson J, Markowitz L, et al. An evaluation of measles revaccination among school-entry-aged children. Pediatrics 1996;97:613–8.
[19] Johnson C, Kumar M, Whitwell J, et al. Antibody persistence after primary measles–mumps–rubella vaccine and response to a second dose given at four to six versus 11 to 13 years. Pediatr Infect Dis J 1996;15:687–92.
[20] King J, Lichenstein R, Feigelman S, Luna C, Permutt T, Patel J. Measles, mumps, and rubella antibodies in vaccinated Baltimore children. Am J Dis Child 1993;147:558–60.
[21] Toscani L, Batou M, Bouvier P, Schlaepfer A. Comparación de l'efficacité de diferentes souces de vaccin ourlien: une enquète en milieu scolaire. Soz Präventivmed 1996;41:341–7.
[22] Tischer A, Gerike A. Immune response after primary and re-vaccination with different combined vaccines against measles, mumps, rubella. Vaccine 2000;18:1382–92.
[23] Briss PA, Fehrs LJ, Parker RA, et al. Sustained transmission of mumps in a highly vaccinated population: assesment of primary vaccine failure and waning vaccine-induced immunity. J Infect Dis 1994;169:77–82.
[24] Cheek JE, Baron R, Atlas H, Wilson D, Crider R. Mumps outbreak in a highly vaccinated school population. Arch Pediatr Adolesc Med 1995;149:774–8.
[25] Gay N, Miller E, Hesketh L, et al. Mumps surveillance in England and Wales supports introduction of two dose vaccination schedule. Commun Dis Rep 1997;7:21–5.
[26] Schwarzer S, Reibel S, Lang AB, Struck MM, Finkel B, Gerike E, et al. Safety and characterization of the immune response engendered by two combined measles, mumps and rubella vaccines. Vaccine 1998;16:298–304.
[27] Khalil M, Poltera AA, al Howasi M, et al. Response to measles revaccination among toddlers in Saudi Arabia by the use of two different trivalent measles–mumps–rubella vaccines. Trans R Soc Trop Med Hyg 2002;93:214–9.
[28] Poltera AA, Herzog C. Vaccine-induced antibodies assessed by comercial test kits, the case of the Rubini mumps and the Edmonston–Zagreb measles vaccine strains (Letter to the Editor). Vaccine 2001;12:398–.
[29] Miller E, Hill A, Morgan-Capner P, Forsey T, Rush M. Antibodies to measles, mumps and rubella in UK children 4 years after vaccination with different MMR vaccines. Vaccine 1995;13(9):799–802.
[30] Crovari P, Gabutti G, Giammanco G, et al. Reactogenicity and inmunogenicity of e new combined measles–mumps–rubella vaccine: results of a multicentre trial. Vaccine 1998;16(2–3):298–304.
[31] de Souza VA, Pannuti CS, Sumita LM, de Andrade Jr HF. Enzyme-linked immunosorbent assay-IgG antibody avidity test for

single sample serologic evaluation of measles vaccines. J Med Virol 1997;52(3):275–9.
[32] Garly ML, Bale C, Martins CL, et al. Measles antibody responses after early two dose trials in Guinea–Bissau with Edmonston–Zagreb and Schwarz standard-titre measles vaccine: better antibody increase from booster dose of the Edmonston–Zagreb vaccine. Vaccine 2001;19(15–16):1951–9.
[33] Grandolfo ME, Medda E, Novello F, Ridolfi B. Seroepidemiological evaluation of 1989–1991 mass vaccination campaigns against measles, in Italy. Epidemiol Infect 1998;121(3):645–52.
[34] Bruno G, Grandolfo M, Lucenti P, Novello F, Ridolfi B, Businco L. Measles vaccine in egg allergic children: poor immunogenicity of the Edmoston–Zagreb strain. Pediatr Allergy Immunol 1997;8: 17–20.
[35] Hussey G, Goddard EA, Hughes J, et al. The effect of Edmonston–Zagreb and Schwarz measles vaccines on immune responses in infants. J Infect Dis 1996;173:1320–6.
[36] Rota JS, Wang ZD, Rota PA, Bellini WJ. Comparison of sequences of the H, F, and N coding genes of measles virus vaccine strains. Virus Res 1994;31:317–30.
[37] Heider A, Santibañez S, Tischler A, et al. Comparative investigation of the long non-coding M-F genome region of wild-type and vaccine measles viruses. Arch Virol 1997;142:2521–8.
[38] St. Sauver JL, Jacobson RM, Vierkant RA, et al. Correlations between measles, mumps, and rubella serum antibody levels in Olmsted County school children. Vaccine 2001;19:1363–8.
[39] Pebody RG, Gay NJ, Hesketh LM, et al. Immunogenicity of second doce measles–mumps–rubella (MMR) vaccine and implications for serosurveillence. Vaccine 2002;20:1134–40.
[40] St. Sauver JL, Ovsyannikova IG, Jacobson RM, et al. Association between human leukocyte antigen homozygosity and antibody levels to measles vaccine. J Infect Dis 2002;185:1545–9.
[41] World Health Organization. Mumps virus vaccines. WHO position papers. Wkly Epidem. Rec. 2001;76:346–355.