Are measles, mumps and rubella a public health issue in young adults? Results from a seroprevalence survey in university students in Italy

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A survey was conducted amongst university students to assess their level of susceptibility and knowledge of measles, mumps and rubella (MMR) diseases and their prevention, and find factors associated to the seropositivity for MMR viruses.
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The prevalence of IgG antibodies was 93.2 % for measles, 91.4 % for mumps and 81.3% for rubella. The susceptibility for measles was higher in the 21-25 and over 31 age groups . The seroprevalence profile of mumps was similar to that of measles while the level of immunity to rubella was very low in students aged under 20 years (76.7%) and 21-25 years(81.2%). Only 111 students declared to be vaccinated against measles, 46 against mumps and 103 against rubella. Most students demonstrated poor knowledge concerning MMR and were not practicing preventive behaviours.
Conclusions:
The susceptibility was particularly high for rubella. Concerted efforts are needed to educate young adults about the benefits of vaccination and to raise their level of consciousness so as to motivate them to request vaccination.

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Author's name

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Keywords

University students, Italy, immunization, measles, mumps, rubella.

Introduction

In Europe, the incidence rate of measles varies among countries. In some nations it is a big Public Health concern whilst in others, it is almost overcome (WHO 2003). Italy has been facing an upsurge of measles cases after an incidence rate of 1/100 000 inhabitants in 2006. Between September 2007 and May 2008, 2 079 cases were notified and the incidence rate increased to 3.4 cases/100 000 inhabitants. Using national age-specific population figures as denominators, adolescents aged 15-19 years had the highest incidence rate, namely 15.8/100 000 (EUVAV/NET 2007; Filia et al. 2008).

Regarding rubella, from January 2005 to May 2008, four Italian Regions (Lazio, Campania, Tuscany and Piedmont) reported 27 notifications of suspected congenital rubella syndrome (CRS), and in 4 cases, the diagnosis was confirmed by laboratory tests. In the same period, ten Regions reported 75 suspected cases of rubella during pregnancy (in 30 cases the diagnosis was confirmed by laboratory tests). The mean age of cases was 29 years (range 17-46 years) (Giambi et al. 2008).

Concerning mumps in Italy, an endemo-epidemic course can be observed with outbreaks every 2-4 years and its incidence had remained almost unvaryied until 2001, with a range from 25.9 to 125.1 cases per 100 00 inhabitants being noted. Since 2002 cases of mumps have decreased with an incidence of 4.5 per 100 00 inhabitants in 2004 (Gabutti et al. 2008).

In Italy vaccinations for measles, mumps and rubella (MMR) are not mandatory. They were introduced separately, during the 1970s for rubella and measles, and in 1982 for mumps. No specific target for voluntary vaccinations had been set by central health authorities, and no organic policy offering this type of vaccinations existed until 1995 when the Ministry of Health (no. 13, 6 June 1995) established an active offer of MMR

combined vaccination, free of charge, targeting 15 month old children and started specific informative and educational programmes (Bonanni et al. 2002).

The National Health Plan 1998–2000 set a 95% coverage goal for both compulsory and recommended vaccinations. The immunisation schedule recommended that the first dose of MMR vaccine should be given between age 12 to15 months, and a second dose from age 5 to 6 or from age 11 to 12 years of age (Ministerial Decree 1999). Rubella vaccination, recommended since 1972 for pre-adolescent girls, continues to be recommended until high levels of coverage in the second year of life are reached (Ministry of Health 1999).

All described programmes have already reduced the MMR incidence in Italy but outbreaks are still recurrent in non immune subjects. In addition, no region achieved the target coverage of 95% with the first dose in 2006 (Filia et al. 2008; Giambi et al. 2008; Ministry of Health 2006). Furthermore, homogeneous results have not yet been achieved in Italy (Bonanni et al. 2007; Ciofi degli Atti et al. 2006; Langiano et al. 2005; Langiano et al. 2007) and there are great variations in the coverage value not only among the Regions but also among Local Health Units within the same Region. Although this situation has led to a slowdown in the circulation of viruses, values capable of preventing their spread have not yet been reached (Ministerial Decree 1999). This explains the shift in the average age of cases, thus exposing adolescents and young adults to a greater susceptibility to infection (Trevisan et al. 2005).

In the province of Frosinone (Lazio Region – Central Italy), which has 490,000 inhabitants, MMR vaccination coverage prevalence values were 14.5% in 1995 and have risen to 62.9% in 2000. An increase in the percentage of subjects aged 10-14 years

for measles cases and 15-19 year-olds for rubella cases was observed between 1995 and 2003 (Langiano et al. 2005; Langiano et al. 2007).

Low prevalence of positive antibodies among subjects aged about 20 years to mumps, or measles, or rubella or to all diseases simultaneously was largely witnessed by Italian and international studies (Langiano et al. 2005; Trevisan et al. 2005; Hamilton-West 2006; Giambi et al. 2007; Donaghy et al 2006; WHO 2005).

This epidemic trend and the low MMR vaccination coverage rate in this geographic area has suggested we pay special attention to infections in young adults' because of more important implications for population health due to more serious complications for these individuals (Gabutti et al. 2008; Hamilton-West 2006; Presidency of the Council of Ministers 2003; Banatvala and Brown 2004; Kare 2005; Ouhilal 2000; Langiano et al. 2009).

On the basis of these considerations, the aims of our study were:

a) to assess the level of susceptibility for Measles, Mumps and Rubella in a sample of university students in order to evaluate possible windows of susceptibility accumulating within this specific sub-population;

b) to find factors associated to the seropositivity for MMR viruses.

c) to examine the knowledge of these students regarding MMR diseases and their prevention.

Subjects and Methods

Study population

A cross-sectional study was conducted involving 961 students from the University of Cassino, the only campus present in the Southern Lazio area (Central Italy). The University of Cassino has about fourteen thousand students prevalently from Southern Lazio and in small part from the neighbouring regions (especially Campania). The student population consists of 57 % females and 43% males aged 18-44 years.

Sampling and the questionnaire

All students attending the Faculties of Literature and Philosophy, Economics, Law, Engineering, Sport Science and the degree course of Nursing Sciences were invited to participate in the survey. The only criterion of inclusion was being university students. Students attending the first year were informed directly in the classroom while others were informed through an invitation form sent by the secretary's office. Informed consent for taking blood specimens and the use of personal data was obtained from all students who participated in the survey, with data being collected using a specific form. Blood samples were collected in a medical university laboratory, after which students filled in the questionnaire which was specifically designed for the study and contained three different areas of inquiry. The first section of the questionnaire concerned socio-demographic data as age, gender, marital status, family structure and parental educational level and occupation. The second section regarded information about previous history of MMR diseases and previous history of MMR immunization. The third section evaluated the students' knowledge about the following topics: definition of MMR, transmission pathway of the infection, risk during pregnancy, risk in adulthood, MMR vaccine and precautions after vaccination.

Self reported history of MMR vaccination or the Official certification of vaccination issued by paediatricians or Local Health Agency was requested to all the participants. Certification of the vaccination status was not a prerequisite for the study participation.

Serological assay

The concentration of human IgG antibodies for mumps, measles and rubella was determined using a commercial ELISA immunoenzymatic test (Radim) according to the manufacturer's instructions.

Enzyme immunoassay method was used for the qualitative determination of anti IgG antibodies for measles and mumps. The calculation of results obtained by qualitative assay, considered the optical density of each negative, positive and cut-off control.

For qualitative IgG detection, the cut off recommended by the producer was used (OD>0.110): samples with optical density lower than the cut-off control were considered non reactive (negatives), samples with optical density higher than the cut-off control were considered reactive (positives), whilst samples with absorbance values ranging within ±10% of the cut-off control were considered questionable and repeated for confirmation. The diagnostic specificity of the method was evaluated on a representative group of individuals with no immunity against Measles or Mumps IgG infection and results were respectively 94.1% for Measles and 100% for Mumps. The clinical sensitivity of the method was evaluated on a representative group of individuals having undergone Measles or Mumps IgG infection and the results were respectively 91.2% for Measles and 95.5% for Mumps.

Enzyme immunoassay method was used for quantitative detection of IgG antibodies for rubella virus. Samples with IgG values lower than 15 IU/mL were considered non reactive for antirubella IgG antibodies (negative), samples with IgG values higher than

30 IU/mL were considered reactive for antirubella IgG antibodies (positive) and samples with IgG values between 15 and 30 IU/mL were considered questionable and repeated for confirmation in accordance with the producer's instructions indicated in the Rubella IgG RADIM kit. In this quantitative assay, the calibrators were calibrated against the 2nd IS WHO 67/182, 1986. The 2nd British Standard for Anti-Rubella Serum, Human (67/182) was established by the National Biological Standards Board (NBSB) in 1986. The diagnostic specificity of the method was evaluated on a group of more than 100 samples with no immunity against Rubella infection andthe result was 97.2%. The diagnostic sensitivity of the method was evaluated on a group of more than 200 samples having undergone past Rubella infection and the result was 100%.

Statistical analysis

Differences in qualitative variables among goups were assessed using the χ^2 test and a P-value <0.05 was considered statistically significant. The logistic regression was used to identify significant predictors of seropositivity. Results of the logistic analysis were expressed as Odds Ratio (OR) and 95% Confidence Intervals (95% CI). Finally, three different multivariate models, one for each outcome variable (seropositivity), were built using the stepwise approach (backward elimination procedure).

Statistical analysis was carried out using the program SPSS 12.0.

Results

Study population

961 Students participated in the survey (7.4% of Cassino University population), with a median age of 22.0 years (range 18-44), 86.8% female, 13.2% male, living mainly in

the nearby area (province of Frosinone 78.2%). The distribution according to Faculty was as follows: Literature and Philosophy (28.5%), Nursing Sciences (12,1%), Sport Science (8.7%), Law (6.9%), Engineering (6.0%), Economics (4.7%); non responders were 33.2%.

Seroprevalence

As described in Table 1, the highest prevalence of specific IgG antibodies was detected for measles (93.2%), followed by mumps (91.4%) and rubella (81.3%). Lower seropositivity levels for measles and mumps were found in the $\geq$ 31 years age group and in the 21-25 years age group. Seroprevalence for IgG antibodies against rubella was always lower than 85% up to the age of 30 years, resulting particularly low in students under 20 (76.7%) whilst the highest prevalence (93.6%) was seen in the $\geq$ 31 years group. Questionable results were observed for mumps (1.1%) and for rubella (1.1%), without age or gender differences

Only 0.3% of students had no IgG antibodies for all the three viruses under study and 70.4% were positive for all three diseases. No statistically significant differences in the seroprevalence of positive antibodies were identified for gender for all three diseases.

Personal history of previous MMR infections and MMR vaccination

Most students interviewed declared they had had measles (63.1%), mumps (28.5%) and rubella (28.6%). Thisfact was supported by serological assays: 96.9% for measles, 97.8% for mumps and 88.3% for rubella. Table 2 shows the history of the diseases crossed with the laboratory results and is statistically significant for all three.

The reporting of *unknown* was 17.9% for measles, 33.7% for mumps and 30.5% for rubella. Having little or no memory of having contracted mumps or rubella could be

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due to the fact that both these infections are characterized by a high percentage of asymptomatic cases.

The majority of students who were asked to indicate whether they had had the combined vaccination or any of the three single vaccine in the past, said that they were not vaccinated or that they didn't remember it. Only 111 students said to be vaccinated against measles, 46 against mumps and 103 against rubella. Primarily females (94), aged 21-25 years (42) and under 20 years (28), resulted vaccinated against rubella. In any case, we did not identify statistically significant differences among males and females. Vaccination history was confirmed by serological tests in 97.3% of cases for measles, 93.5% for mumps and 90.3 % for rubella. When vaccination memory and serology tests were crossed, statistically significant results were seen only for measles. Only a negligible percentage of subjects (<1%) documented previous immunizations with the certificate issued from the Local Health Unit or the paediatrician.

The low percentage of self declared vaccinations suggests that the presence of antibodies is likely to be related to natural disease. Thus, we preferred postponing the analysis of other variables to further detailed study.

General Knowledge

As far as knowledge inferred from the questionnaire was concerned, only 2.3% of the students for measles, for mumps 19.7%, and for rubella 6.9% were aware of the complications of MMR infection contracted in adulthood by Only 1% of the students interviewed knew that the rubella virus could cause spontaneous miscarriage. Only 3.3% recognized a generic risk to the foetus but to the more specific answer regarding the transmission of the virus in pregnancy, the risk was perceived by most students (78.0%). Only 7.8% of responders knew that an infant with CRS? is highly contagious

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for a long time and 54.1% thought that their knowledge about rubella was insufficient. This result confirmed the high percentage of students who did not know the Rubeo test (43%). Regarding mumps 26.2% of students recognized a risk to pregnancy. Although vaccination was recognized as the best prevention tool available against measles (61.2%), mumps (56.8%) and rubella (59%), non responders to this question were quite high (39.8%).

Age group and gender were the main independent variables associated significantly with personal knowledge concerning MMR. The 21-25 years age group and gender (female) influenced responses given from the interviewed regarding basic knowledge of contagiousness of all three viruses, including transmission modality and prevention against MMR (p<0.05). Therefore only the 21-25 years age group influenced degree of awareness regarding existence MMR vaccination.

Univariate logistic analysis

The logistic regression model, exemplified in Table 3, demonstrated that measles and mumps, on the one side, and rubella on the other side, show different patterns of predictors. In fact, while for measles and mumps the only significant predictor of seropositivity was the region of residence (higher OR in Lazio region), being over 25 years of age and vaccination were independent predictors of seropositivity for rubella (OR=1.72).

Multivariable logistic analysis

Table 3 shows the multivariable analysis, thatrevealed that the following variables were predictors of seropositivity: for Measles age over 25 (OR = 2.04; 95% CI: 1.02 – 4.10), region of residence other than Lazio (OR = 0.51; 95%CI: 0.28 – 0.90) and vaccination against measles (OR = 2.96; 95%CI: 1.00 – 9.67); for Mumps region of residence other

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than Lazio (OR = 0.51; 95%CI: 0.29 − 0.89); for Rubella age over 25 (OR = 1.74; 95%CI: 1.14 − 2.65) and vaccination against rubella (OR = 2.39; 95%CI: 1.18 − 4.86).

Discussion

Several surveys investigated the MMR seroprevalence in different countries in similar settings and age specific profiles of MMR have shown a wide variation (Trevisan et al. 2005; WHO 2005; Dominguez et al. 2006). In these similar studies however, most students were vaccinated and MMR vaccination coverage was higher.

The Italian student population enrolled that was susceptible to measles (6.8%) was close to the estimated European level that must not exceed in over nine years old in order to eliminate this disease (Presidency of the Council of Ministers 2003; WHO 2005; Ramsay 1999). Positive results according national strategies were reached for mumps too (Presidency of the Council of Ministers 2003). On the other hand, the level of susceptibility to rubella is very high, in all age groups tested, and was always over 5% and particularly high in subjects under 25 (from 18.7% to 22.6%).

The measles seropositivity rates in young adults found in our survey are higher than those obtained in a similar study conducted in Italy (Trevisan et al. 2005), and lower than those obtained in others conducted in Spain (WHO 2005; Dominguez et al. 2006). The mumps seropositivity rates are higher than that obtained in Italy (Trevisan et al. 2005), and similar to others obtained in Spain (WHO 2005; Dominguez et al. 2006). On the other hand Rubella seropositivity is always lower compared to the other European studies (WHO 2005; Dominguez et al. 2006). The percentage of those reporting to have had measles and were immune is very similar to the percentage reported in a study carried out in Italian children born before 1990 (Grandolfo et al. 1998) but, as

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multivariate logistic regression suggests and other surveys confirm (Baer et al 2005; Murray and Linch 1998), historical information on measles, mumps and rubella infection cannot be a reliable predictor.

The high measles seroprevalence in these mostly unvaccinated students can hardly be a surprise. Our results are supported by a study conducted on pupils born in the late 1980s when 50% of children from 36 months in Southern Italy to 5-6 years in Northern Italy (Grandolfo et al. 1998) achieved natural infection.

Although the susceptibility to measles is not very low in the population studied, suboptimal MMR vaccination coverage in our province (Langiano et al. 2005) increases the risk that one may contract measles. Other serological studies are needed that involve younger cohorts because of the low vaccination coverage in the past. A probable unvaccinated status, and fewer opportunities to acquire natural infection increase the spread of the virus.

Our results underline the importance of catch-up activities among adolescents and young adults to reduce the rate of susceptibility in this age-group and to reduce the regional differences in vaccination coverage (Bonanni et al. 2007; Ciofi degli Atti et al. 2006; Presidency of the Council of Ministers 2003).

The susceptibility seen in our young adult students could expose them to a risk of contracting measles and rubella with more serious implications (Bonanni et al. 2007; Hamilton-West 2006). Therefore, there is a strong need to raise their level of consciousness and to motivate them to request vaccination.

In the last outbreaks reported in European countries, including Italian adolescents, young adults were particularly affected by measles, because most of the poplation were

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unvaccinated or incompletely vaccinated (Filia et al. 2008; Andrews et al. 2008; Nardone et al. 2003).

Outbreaks of measles, mumps and rubella in older susceptible cohorts have occurred in Europe in the recent years and they are still occurring (Hamilton-West 2006; Andrews et al. 2008; Sartorius et al. 2005; Schmidt et al. 2006) The cases of Mumps notified in Italy in 2006 by the Ministery of Health confirm the susceptibility in young adults (www.ministerosalute.it).

The herd immunity for mumps is estimated at about 75-86% (Donaghy et al. 2006), and the theoretical level of antibodies required to interrupt the transmission of mumps is estimated between 85% and 90% (Nardone et al.2003). Although asymptomatic mumps infections may be more common especially in adults (Sartorius et al. 2005), the last epidemic peak in the province of Frosinone reported 137 cases with median age of 7 vs. 285 with median age of 10 reported in 1995 (Langiano et al. 2007). In this setting, mumps seroprevalence was always greater than 90% in all age groups considered and it seems that a guaranteed level of acquired immunity through natural infection has been reached although these data need to be integrated with other serological studies assessing immunitary status in other younger age groups and completed with MMR vaccination coverage of 95% with the first dose in all Italian regions.

The susceptibility rates for rubella obtained in our results are far from the value obtained in national surveys in similar age groups (8%) and even farther from the target of 5% set by the National Plan for Measles and Congenital Rubella Elimination launched in 2003 (Giambi et al.2008; Presidency of the Council of Ministers 2003).

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Suboptimal seroprevalence rates without gender difference, especially for rubella, highlight the failure of previous selective rubella vaccination policy targeted at pre-adolescent girls that had been recommended in Italy since 1972 (Osborne et al. 2000).

 As a consequence of the suboptimal vaccination coverage, a high proportion of subjects remain susceptible to rubella virus and a recent survey suggested that the late increase of MMR vaccination coverage in childhood has not had an impact on seroprevalence in childbearing age women.In fact, it is believed that over 5% of them are still susceptible to rubella infection (Rota et al. 2007). Vaccination is one of the most effective measures in the prevention of congenital rubella syndrome (CRS) (WHO 2005) but despite its documented benefits our sample show that there are still childbearing women who are not being adequately immunized. In fact, only a small proportion of females reported having received rubella vaccination, mainly those aged under 24. From a public health perspective, this leads to a very inadequate coverage (highest proportion susceptible for rubella). The susceptibility value among our students neither prevents the circulation of the virus nor the risk of CRS (WHO 2003; Presidency of the Council of Ministers 2003; WHO 2005).

Nowadays, interventions targeting childbearing age women are not yet satisfactory and, although rubella vaccine is free of charge and preconceptional screening is available, these opportunities are substantially missed reflecting that this offer is prevalently based on providing immunization through vaccination services though the active support of public health services and widespread awareness campaigns targeted towards this group are lacking.

Surveillance data in fact, , show that less than 25% of women with suspected rubella during pregnancy had undergone rubeo-test before pregnancy, even if it is offered free

charge by the Italian National Health Service before and during pregnancy (Giambi et al.2008).

The present study has some interesting strengths and limitations. Concerning the strengths, the results from the regression analysis revealed that factors such as geographical residence (for MMR), age and vaccination (for measles and mumps) are significantly associated to seroprevalence of these three infections. Moreover, our study demonstrated that students have low levels of knowledge concerning measles, mumps and rubella and were not practicing preventive behaviours that reduce their level of risk (e.g. MMR vaccination). These findings have important implications for school health and suggest that education programmes should be considered and implemented.

However, the results of this study need to be interpreted with caution, since several limitations are recognised. First of all, it only involved students participating on voluntary basis, therefore results might not be generalized to other student populations. Replication studies need to be conducted at universities of different sizes and in different geographical areas. A possible selection bias could have occurred concerning gender, since the prevalence of participants were women. Likewise, higher responses were obtained in the Faculties where female students were the majority. However, we have to consider that the over represented number of females in our sample could bias the results. Given that infections occur with equal frequency in men and women, as well as the immunological response, and that gender appeared to have an influence only for measles, it remains unclear whether females have a better serological response to the measles vaccine than men (Dominguez et al 2006; Gdalevich et al. 2002). As such, the selection bias in this study is likely to be limited. Moreover our situation presents a low percentage of subjects vaccinated for measles. On the other hand, the preponderance of

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women allowed us to better investigate rubella susceptibility, which is of great importance for the women considering the teratogenic effects which may occur from infection during pregnancy.

Concerning the study design, we must consider that vaccination status is self-reported, and a possible recall bias could have occurred since almost all students involved did not supply vaccination certificate and thus results could have been distorted. However, this possibility may not influence the study in a systematic way since the clinical signs of these infectious diseases are the most useful indicator of the presence of the diseases themselves. The likelihood of recognising the diseases, even by non health personnel, and subsequent related recall is very high.

This study shows that sampled young adults can be exposed to an increasing risk of MMR infection related to the history of vaccination campaigns and outbreaks in the area where they lived their childhood. Concerted efforts are necessary to educate young adults about the benefits of MMR vaccination particularly for rubella. Co-operation is required not only between national and regional levels but especially at a Local Health Unit level and should be enhanced in order to increase immunity rates for rubella in childbearing women.

Conclusions

Public Health Local authorities should promote, in their action plan against MMR, much more incisive educational programmes in order to encourage young adults to have MMR vaccinations and achieve a MMR vaccination recovery.This method is the only way to increase the seroprevalence rate needed to interrupt transmission of MMR and could be easily and quickly reached. Special attention should be paid to the recovery of

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rubella susceptible females in these age cohorts in order to reduce the risk of CRS in

non- immune pregnant women. In this context, an added value could be represented by

the process of formal Health Technology Assessment of these vaccinations, with the

aim of considering not only the epidemiological, clinical and economic impact of the

vaccinations, but also the legal, social and bioethical aspects related to the vaccinations

themselves (La Torre et al. 2007). Finally, the need to establish collaborations is urgent

in order to train and create of an operational network among professional staff in the

health care area (e.g. general practitioners, gynaecologists and obstetricians) that could

help women to increase their knowledge of their immune status and encourage the use

of rubeo-test and rubella vaccination.

Ethical approval

All procedures were approved by the appropriate academic committees.

Conflict of interest

The authors declared that they have no conflict of interests.

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Authors' Response to Reviewers' Comments

Reviewer #1: Manuscript Number: JOPH-D-09-00054

Dear Author,

the submitted article ("Are measles,mumps and rubella a Public Health issue in young adults? Results from a seroprevalence survey in university students in Italy") needs some major changes before it can be resubmitted.

Proof-reading of the article by a native English speaker is strongly recommended. It'll enhance the understanding of several parts made by the authors.

Materials Methods

The study design is not precisely described, for example:
-    The description of the study population is not precise: Exclusion / Inclusion criteria are missing. Is it a representative sample? Are the students included in the survey from one or different study programmes?

Answer: **We did not used criterions of exclusion, since the matter object of the search, the criterion of inclusion was being university students, the population target of our study.**

-    The questionnaire should be described in more detail

Answer: **following the suggestion of the reviewer we added more details.**

(The first section of the questionnaire concerned socio-demographic data as age, gender, marital status, family structure and parental educational level and occupation. The second section regarded information about previous history of MMR diseases and previous history of MMR immunization. The third section evaluated the students' knowledge about the following topics: definition of MMR, transmission pathway of the infection, risk during pregnancy, risk in adulthood, MMR vaccine and precautions after vaccination).

-    The information on the vaccination status through self-reporting is rather invalid. It is not described by the authors if the official certification of the vaccination status was a prerequisite for the study participation.

Answer: **We added "Certification of the vaccination status was not a prerequisite for the study participation" at the end of the paragraph *Sampling and the questionnaire.***

-    It is unclear whether all students of the university were asked to participate. For this study design it is a prerequisite to sign an informed consent. It remains unclear in the manuscript.

Answer: **thanks to the reviewer suggestions, we added more details.**

(All students attending the university were invited to participate in the survey. Informed consent for taking blood specimens and the use of personal data was obtained from all students who participated in the survey, with data being collected using a specific form.)

-    A description of the serological test used for this survey should include more information (e.g. validated test system or not, manufacturer, detailed cut-off values etc.)

Answer: It was using a commercial ELISA immunoenzymatic test (Radim) according to the manufacturer's instructions. we added more details in the paragraph *serological assay.*

(The concentration of human IgG antibodies for mumps, measles and rubella was determined using a commercial ELISA immunoenzymatic test (Radim) according to the manufacturer's instructions.Enzyme immunoassay method was used for the qualitative determination of anti IgG antibodies for measles and mumps. The calculation of results obtained by qualitative assay, considered the optical density of each negative, positive and cut-off control. For qualitative IgG detection, the cut off recommended by the producer was used (OD>0.110): samples with optical density lower than the cut-off control were considered non reactive (negatives), samples with optical density higher than the cut-off control were considered reactive (positives), whilst samples with absorbance values ranging within ±10% of the cut-off control were considered questionable and repeated for confirmation.)

Results

The results should be described more precisely.
-    The authors should describe why the study population consists mainly out of women (86,8%)?

Answer: **we introduced in the discussion section a clearer statement about that**

-    The tables are not self-explanatory, description of p-values is not clear enough.

Answer: **we introduced in the tables p values in a more detailed way. Moreover, in table 3 p values were not reported, since we wanted to avoid replications, after presenting the 95%CI of the OR.**

-    The description of seroprevalence data is of limited value without information on the official and certified vaccination status of the subjects. It remains unclear how many positive tests are a result of vaccination or natural infection.

Answer: **The main aim of our study was to assess the level of susceptibility for Measles, Mumps and Rubella in a sample of university students. As described in "results" only 111 students said to be vaccinated against measles, 46 against mumps and 103 against rubella, and since only a negligible percentage of them (<1%) documented previous immunizations with the certificate issued from the Local Health Unit or the paediatrician, we preferred postponing the analysis of other variables to further detailed study. However, vaccination history resulted confirmed by serologic test in 97.3% of cases for measles, 93.5% for mumps and 90.3 % for rubella and disease history among positive and negative test MMR was reported in tab.2**

Discussion

-	Should be more structured and also shortened

Answer: the discussion was hopefully better structured and also shortened according to the comments of the reviewer.

-	Methodological limits of the study design, like recall- and selection bias, should be described in more detail. Are there any strengths of the study?

Answer: strengths and limitations of the study are presented in more detailed way

-	The consequences of the study concluded by the authors are not comprehensible, as it is not obvious whether the seroprevalence rate needed to interrupt transmission of MMR is due to wild type infections or MMR vaccination.

Answer: a statement on this suggestion made by the reviewer was added in the Conclusion paragraph.

Reviewer #2: The aim of the study was to assess seroprevalence against measles, mumps and rubella among Italian students and to find out whether seropositivity had been obtained from natural infection or vaccination and to assess the students' knowledge on these the infections in and outside pregnancy.
The survey yields a number of interesting findings:
1.   Almost all students had antibodies against mumps and measles whereas some 20 % of the students were still susceptible for rubella.
2.   Most of the students have converted to seropositive from infection rather from vaccination.
3.   The students' knowledge about the risks of measles, mumps and rubella with respect to pregnancy and else wise was appallingly poor.

I have some suggestions to approve the paper:

1.   Please describe the recruitment procedure in more detail: The participation rate of 7.4 % was extremely low. Were really all students invited to participate in this investigation or only those in the first year? Potential sources of ´bias need to be discussed!

Answer: **All students attending the Faculties of Literature and Philosophy, Economics, Law, Engineering, Sport Science and the degree course of Nursing Sciences were invited to participate in the survey. Students attending the first year were informed directly in the classroom while others were informed through an invitation form sent by the secretary's office. Informed consent for taking blood specimens and the use of personal data was obtained from all students who participated in the survey, with data being collected using a specific form. A more detailed discussion on possible source of bias has been added.**

2.   The table two is very difficult to understand. "Do not" probably means do not know; please correct the rows for diseases history among positive or negative test for MMR for measles, mumps and rubella. These do not evidently add up of to 100 % but should for each headline "disease history among positive for MMR" as well as among "disease history" among negative for MMR.

Answer: **we corrected table 2 according to reviewer's suggestions**

3.   I did not find table three with the modelling very helpful. I would rather suggest to leave out this table and to present the information on the students' knowledge about measles, mumps and rubella complications in table three instead.

Answer: **we did not remove table 3, since we are convinced that the results demonstrated the influence of possible determinants of seropositivity for MMR, taking into account the control of potential confounding factors. Moreover, as suggested by the reviewer, we add information concerning students' knowledge about MMR.**

Table

Table 1- Positive seroprevalence IgG antibodies of Measles, Mumps and Rubella (MMR) stratified by gender, age group and residence

Personal date	Number tested	Measles		*p*- value	Mumps		*p*- value	Rubella		*p*- value
		n	**%**		**n**	**%**		**n**	**%**	
Age group										
18-20	271	254	93.7	0.02	251	92.6	0.7	208	76.7	0.04
21-25	432	395	91.4		389	90.0		351	81.2	
26-30	208	202	97.1		193	92.7		176	84.6	
≥31	47	42	89.4		43	91.4		44	93.6	
total	**958**	**893**	**93.2**		**876**	**91.4**		**779**	**81.3**	
Sex										
Male	127	117	92.1	0.90	111	87.4	0.1	107	84.2	0.30
Female	834	778	93.2		768	92.0		675	81.0	
total	**961**	**895**	**93.1**		**879**	**91.4**		**782**	**81.3**	
Residence										
Frosinone	752	707	94.0	0.06	695	92.4	0.04	609	80.1	0.20
Caserta	107	95	88.8		91	85		81	75.7	
Latina	45	42	93.3		43	95.5		43	95.5	
total	**904**	**844**	**93.4**		**829**	**91.7**		**733**	**81.0**	

Table 2- Disease history among the positive and negative test MMR

	Measles	Mumps	Rubella
Diseases History	*N (%)*	*N (%)*	*N (%)*
Yes	607 (63.2)	273 (28.5)	275 (28.6)
No	152 (15.8)	259 (27)	232 (24.1)
Do not know	202 (21.0)	426 (44.5)	454 (47.3)
Diseases History among positive test MMR	*N (%)*	*N (%)*	*N (%)*
Yes	586 (65.4)	267 (30.3)	242 (31)
No	121 (13.5)	221 (25.1)	176 (22.6)
Do not know	189 (21.1)	392 (44.6)	362 (46.4)
Diseases History among negative test MMR	*N (%)*	*N (%)*	*N (%)*
Yes	19 (30.2)	6 (8.8)	30 (18.1)
No	30 (47.6)	36 (52.9)	51 (30.7)
Do not know	14 (22.2)	26 (38.3)	85 (51.2)
p- value	< 0.001	< 0.001	0.01

Table 3- Predictors and associated odds ratio of MMR seroprevalence rate.

Characteristics	Measles		Mumps		Rubella	
	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
Gender						
Male (reference)	1.0		1.0		1.0	
Female	1.11 (0.53-2.31)		1.80 (0.97-3.35)		0.83 (0.49-1.40)	
Age group						
< 26 (reference)	1.0	1.0	1.0		1.0	1.0
≥ 26	1.95 (0.98-3.90)	2.04 (1.02-4.10)	1.19 (0.67-2.12)		1.72 (1.13-2.62)	1.74 (1.14-2.65)
Marital status						
Not married (reference)	1.0		1.0		1.0	
Married	3.57 (0.86-14.83)		0.71 (0.34-1.48)		1.53 (0.82-2.88)	
Having given birth						
Yes	na		0.73 (0.28-1.91)		1.75 (0.73-4.15)	
No (reference)			1.0		1.0	
Vaccination						
Yes	2.74 (0.84-8.89)	2.96 (1.00- 9.67)	1.10 (0.33-3.64)		2.34 (1.16-4.74)	2.39(1.18 – 4.86)
No (reference)	1.0	1.0	1.0		1.0	1.0
Region						
Lazio	1.0	1.0	1.0	1.0	1.0	
Other region	0.49 (0.28-0.87)	0.51 (0.28-0.90)	0.51 (0.29-0.88)	0.51 (0.29-0.89)	0.84 (0.53-1.23)	

na= not applicable