Epidemiol. Infect. (2003), **132**, 11–18. © 2004 Cambridge University Press
DOI: 10.1017/S0950268803001584 Printed in the United Kingdom

Seroprevalence of measles, mumps and rubella antibodies in Luxembourg: results from a national cross-sectional study

J. MOSSONG[1,2]*, L. PUTZ[1,2] AND F. SCHNEIDER[1]

[1] *Laboratoire National de Santé, PO Box 1102, L-1011 Luxembourg*
[2] *Centre de Recherche Public–Santé, 18 rue Dicks, L-1417 Luxembourg*

(*Accepted 2 October 2003*)

SUMMARY

A serological prevalence survey was carried out in Luxembourg during 2000–2001 to determine the antibody status of the Luxembourg population against vaccine-preventable infections. Blood samples of children and adolescents were collected prospectively in randomly selected schools. Samples of adults were obtained through volunteer patients of the national health laboratory or of the mandatory pre-nuptial test. Measles, mumps and rubella (MMR) virus antibody concentrations were measured using commercial ELISA tests. Age-standardized prevalence of measles, mumps and rubella virus antibodies was found to be 96·58, 75·40 and 95·69% respectively. Significant age-dependence of serology was observed for all three infections, with study participants born after the introduction of the MMR vaccine experiencing a gradual decline of antibodies following vaccination in childhood. Older study participants who were more likely to have antibodies from natural infection had consistently higher titres than younger individuals. Present vaccination coverage with MMR appears to be sufficient to prevent large local outbreaks of measles and rubella, but probably not mumps.

INTRODUCTION

During 2000–2001, the National Laboratory of Health and the Public Research Centre of Health carried out a seroprevalence survey to assess the level of immunity in the Luxembourg population against eight vaccine-preventable infections. Such a population-based serosurvey has never previously been carried out in Luxembourg. The survey was conducted within the framework of the European Sero-Epidemiological Network (ESEN) 2, the continuation of the ESEN programme [1]. The principal aim was to monitor and evaluate the serological impact of the national vaccination programme at the population level and to assess the need for policy changes in view of vaccination targets set by the WHO Region Europe (e.g. to eliminate measles by the year 2007) [2].

This study presents the results of the seroepidemiology of measles, mumps and rubella (MMR), although other infections (pertussis, diphtheria, varicella, hepatitis A and B) were also investigated in the same survey.

The combination MMR vaccine obtained its licence and came into routine use in Luxembourg in 1986, but monovalent measles, mumps and rubella vaccines were available before 1986. However, vaccination rates prior to 1986 for measles, mumps and rubella are known to be low because infant immunization with the monovalent vaccines were not recommended by the Ministry of Health and parents were not reimbursed by the national sickness funds. Rubella vaccination was, however, recommended to women who were rubella virus antibody-negative in compulsory pre-marital testing.

* Author for correspondence: Dr J. Mossong, Laboratoire National de Santé, PO Box 1102, L-1011 Luxembourg.

A recent survey in 1996 has shown that coverage rates for the first dose of MMR vaccine were high (91·1%), whereas in 1990 coverage was estimated to be approximately 80% based on return vaccination certificates [3]. Currently, the official vaccine schedule recommended by the Ministry of Health consists of two doses, a first dose to be administered at 15–18 months and a second dose at 5–6 years, prior to entering primary school. Immunizations within Luxembourg's official vaccination programme (which follows the WHO Expanded Programme on Immunization) are offered free of charge. The Ministry of Health covers the cost of the vaccines and the doctor's fee is fully reimbursed by the national sickness funds. Immunization is not compulsory as such, but parents are strongly encouraged by paediatricians and family doctors to follow the recommended vaccination schedule [4]. The survey carried out in 1996 has shown that paediatricians rather than general practitioners administered more than 90% of the MMR vaccines [3].

METHODS

Study population and survey design

A multi-tiered approach was chosen to collect serum samples prospectively to obtain a sufficient number of serum samples as defined by ESEN [5]. Samples from children and adolescents were collected from randomly selected primary and secondary schools. Seven primary schools were chosen at random from different geographical regions. The number of schools chosen in each region was proportional to the population size of the region; one primary school was selected at random in each of the northern and eastern political regions, two in the central region and three in the southern region. Furthermore, three secondary schools were chosen at random; one from each of the northern, central and southern regions. In the selected schools, all pupils and students (respectively their parents) were given a leaflet explaining the aims the study including a short description of the disease involved.

Serum samples of adults were obtained from two separate sources: adult volunteers donating blood at the national Red Cross Centre and adults attending compulsory pre-marital testing at the National Health Laboratory. All study participants were offered the test results via a doctor of their choice who could give advice on additional vaccinations if deemed necessary.

Serology

Serum samples were stored frozen until ready for testing. Three commercial enzyme immunoassay kits were used with an automated BEP® 2000 analyser (Dade Behring, Marburg GmbH, Marburg, Germany): Enzygnost® Anti-Measles Virus/IgG, Anti-Parotitis Virus/IgG and Anti-Rubella Virus/IgG with reported sensitivities of 99·6, 95·4 and 100% respectively and specificities of 100, 93·7 and 98·5% respectively according to the manufacturer. Positive and negative status of sera were determined using the cut-offs specified by the manu facturer; sera with corrected absorbances strictly less than 0·1 were defined to be negative, those strictly greater than 0·2 were defined to be positive and those in between these cut-off values were defined to be equivocal. Quantitative antibody titres were obtained using the α-method as specified by the manufacturer.

Statistical analysis

Prevalence of virus antibodies for both gender and age groups were calculated. Overall prevalence was obtained using direct standardization, with the 2000 population of Luxembourg [6] as the standard population. χ^2 tests for homogeneity were applied to study the association between serological status and site of sample collection, age (categorized as shown in Fig. 1), sex and nationality [Luxembourg, Portuguese, other European Union (EU) and non-EU]. Multivariate logistic regression was run to determine risk factors for being virus antibody-negative compared to virus antibody-positive (i.e. equivocal samples were ignored). Age was modelled using fractional polynomials, a flexible method when the relationship between independent and continuous response variable is curved rather than linear [7]. Log antibody levels were regressed against age also using this method. Antibody titres below the lower detection threshold were assumed to be half of the threshold value. All calculations were done with Stata 8.0 (Statacorp, TX, USA).

RESULTS

Sample collection and response rates for school surveys

Overall, 2673 serum samples were obtained. The Table shows the number of sera obtained in each sampling site. All but one school in the eastern region

Table. *Frequency of samples obtained by site*

Sampling site	Frequency	%
Primary schools	725	24·65
Secondary schools	659	27·12
National laboratory	729	27·27
Pre-nuptial test	559	20·91
Hospital*	1	0·04
All sites	2673	100·00

* A pupil who was absent at school on the day of sampling had blood taken at a hospital at a later date to participate in the study.

agreed to participate in the study. Of the 2920 pupils approached at the six participating primary and three secondary schools, 1379 (47·2%) blood samples were collected. Actual participation rates were slightly higher, but in a small fraction of volunteer pupils, no sufficient blood sample could be drawn. The participation rate was highest among the 12–15 years age group (>60%) and lowest for young children and the older students in secondary schools; the association between age and participation was highly significant ($P<0{\cdot}0001$). There was no statistically significant association between participation and nationality ($P=0{\cdot}075$). Sample collection among adults in the two centres continued from July 2000 until April 2001, but no data were available on participation rates.

Measles

Overall, 88 (3·28%), 89 (3·32%) and 2502 (93·39%) serum samples were negative, equivocal and positive respectively for anti-measles virus antibodies. This corresponds to a standardized prevalence of 1·79% of negatives, 1·63% of equivocals and 96·58% of positives in the Luxembourg population above 4 years of age. No significant association was found between seroprevalence and gender ($P=0{\cdot}19$). Seroprevalence was found to be homogenous within the six primary schools ($P=0{\cdot}65$), but some heterogeneity was observed for the three secondary schools ($P=0{\cdot}049$), the secondary school in the north having the higher seroprevalence. Seroprevalence in adult samples collected from the two centres were similar ($P=0{\cdot}083$). However, seroprevalence was significantly associated with age ($P<0{\cdot}001$). Whereas virtually all adults born prior to 1970 are measles virus antibody-positive (99·5%), this level reduces to 91·3% for adults born between 1970 and 1979, and to 89·9% for individuals born after 1980. Figure 1*a* shows how serological status varies as a function of age.

Analysis of actual titres rather than status reveals that individuals who were born after the MMR vaccine was introduced (those aged <15 years) have experienced waning antibody titres (Fig. 2*a*). Older individuals who acquired infection naturally tend to have higher titres than younger individuals who are most likely to have acquired their immunity from vaccination. Furthermore, schoolchildren aged 10–16 years living in the north of the country (who had a greater chance of being exposed to a circulating virus in a measles epidemic in 1996 which was due to low vaccination coverage [8]) had significantly higher titres than children of a similar age living in other regions of the country where no epidemic was observed (Fig. 3).

Seroprevalence differed between nationalities, which was mainly due to non-EU nationals having lower seroprevalence (79%) compared to EU nationals (93%), which could not be explained by age difference alone. In the multivariate logistic model, age and being of non-EU origin [odds ratio 3·6 compared to Luxembourg nationals; 95% confidence interval (CI) 1·9–7·0] remained significant predictors of being virus antibody-negative.

Mumps

A total of 354 (13·21%) sera from the study population were negative for anti-mumps virus antibodies, 399 (14·89%) were equivocal and 1926 (71·89%) were positive. This yields population estimates of 10·52% mumps virus antibody-negatives, 14·08% equivocals and 75·40% virus antibody-seropositives. A slight but significant difference of serological status was observed with respect to gender ($P=0{\cdot}042$), a higher proportion of males being equivocal. Nationality was associated with serology ($P=0{\cdot}001$), with more non-EU nationals being virus antibody-negative (22%). No heterogeneity was observed within secondary schools ($P=0{\cdot}135$) and between the two adult collection centres ($P=0{\cdot}058$). Differences were seen within primary schools ($P<0{\cdot}001$); one primary school in the northern region having lower virus antibody-positive rates (49·6%) compared to the other primary schools (average of 68·5%). The serological status of mumps was also significantly associated with age ($P<0{\cdot}001$), although the pattern seen in Figure 1*b* is not as clear as for measles. In the multivariate logistic

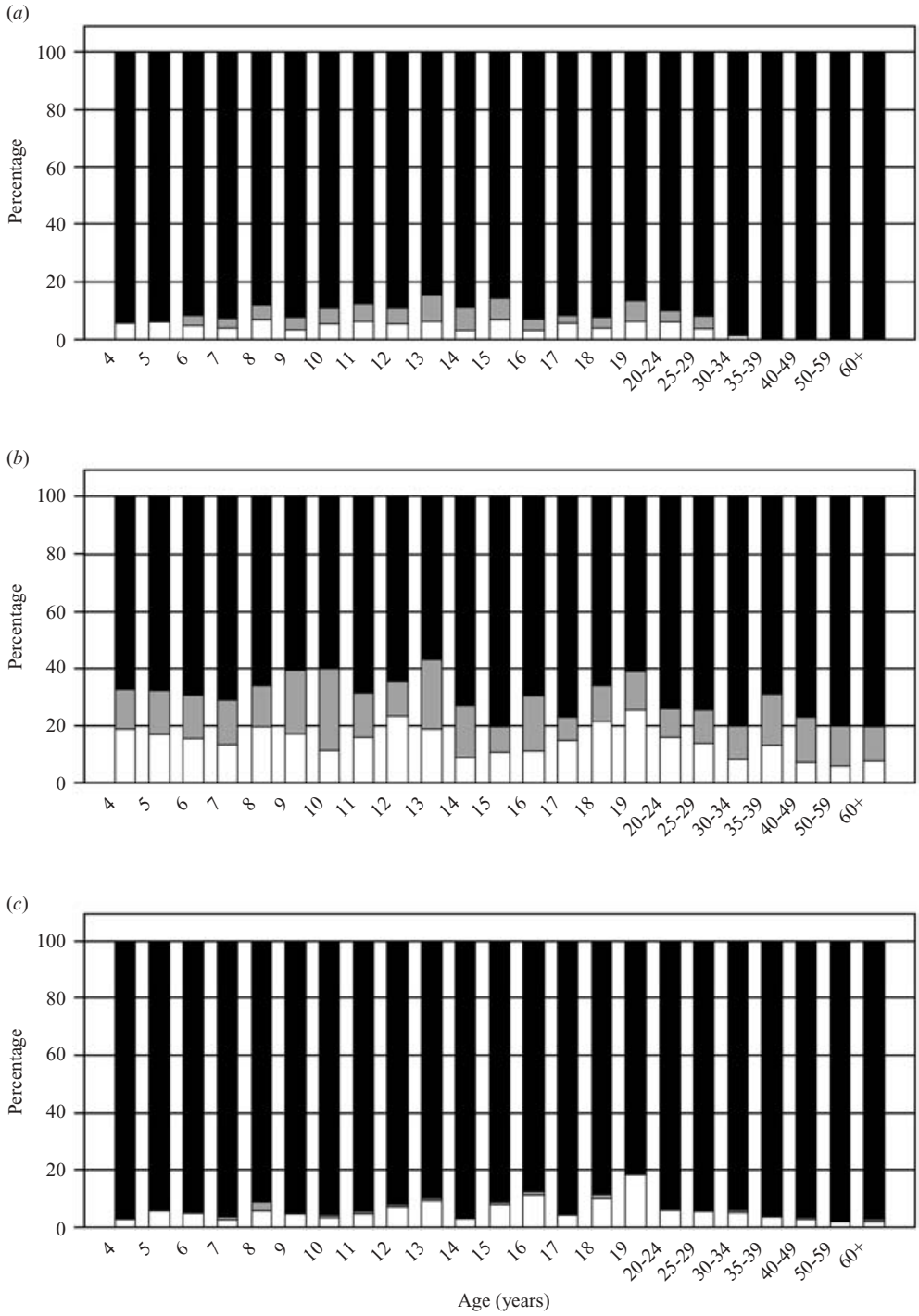

Fig. 1. Seroprevalence by age category of antibodies against (*a*) measles, (*b*) mumps and (*c*) rubella. □, Negative; ▒, equivocal; ■, positive.

model significant predictors of serological status were: age, being a non-EU national, and pupils in the primary school in the north. Figure 2*b* shows the results of regressing titres against age, the decline of titres in school-aged children being less important than for measles.

Rubella

A total of 133 (4·96%) samples of the study population were negative for anti-rubella virus antibodies, 17 (0·63%) were equivocal and 2529 (94·4%) were positive. This equates to a prevalence of 3·7% of

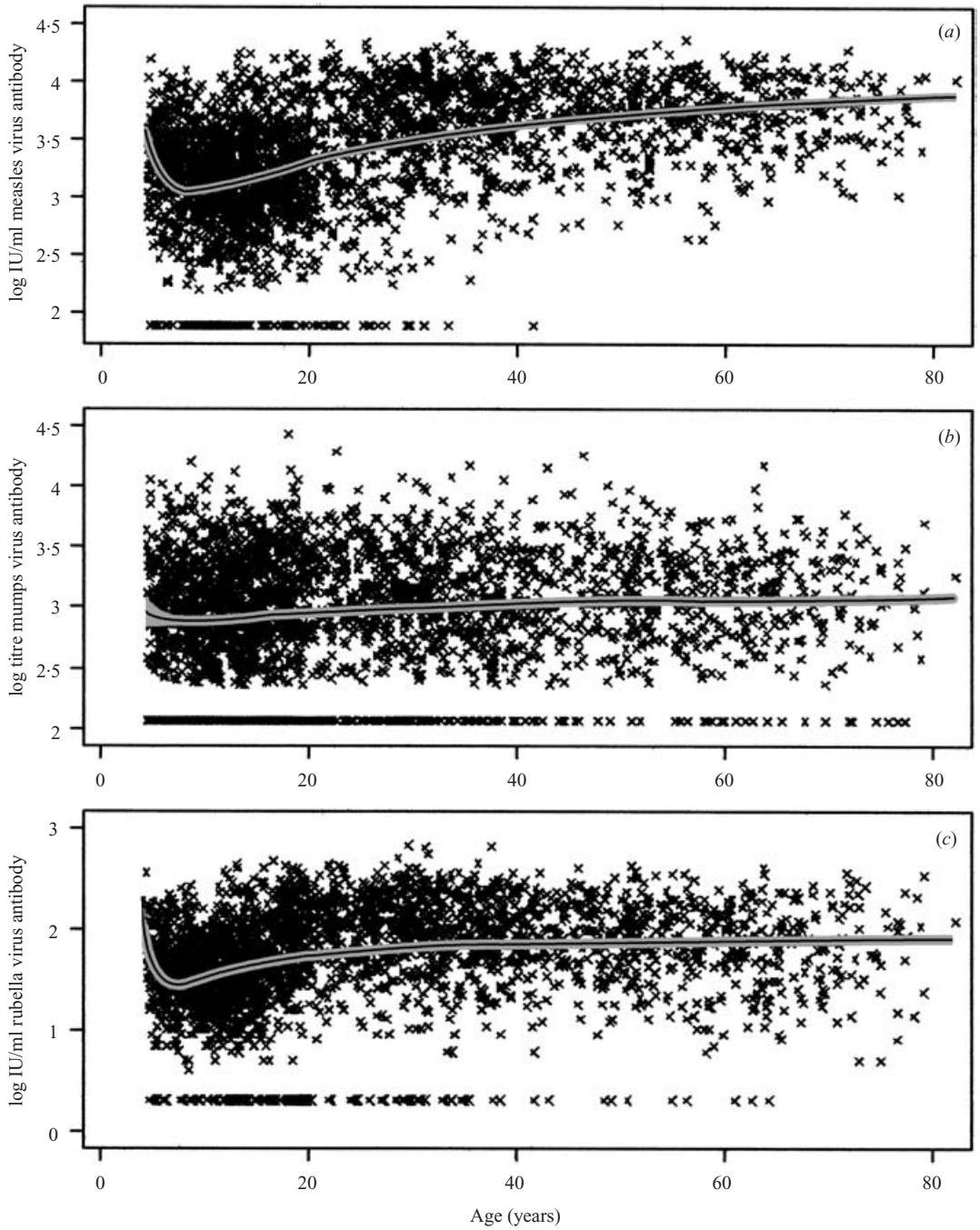

Fig. 2. Age-dependence of logged antibody titres of (*a*) measles, (*b*) mumps and (*c*) rubella virus antibodies.

the Luxembourg population being rubella virus antibody-negative, 0·61% being equivocal and 95·69% being positive. No difference of rubella antibody status could be detected with respect to gender ($P=0{\cdot}973$), nationality ($P=0{\cdot}766$), within secondary schools ($P=0{\cdot}305$) and between adult collection centres ($P=0{\cdot}175$). However, seroprevalence was heterogeneous within primary schools significantly ($P=0{\cdot}010$); the primary school sampled in the northern region had a higher proportion of rubella virus antibody-negatives (10·43%) compared to the other primary schools (3·3%). Age was significantly associated with serological status ($P=0{\cdot}002$). In the multivariate logistic model, only age and the two schools in the north (one primary and one secondary) were significant risk factors for being rubella virus antibody-negative. Figure 1*c* shows that the proportion of rubella virus antibody-negative is highest

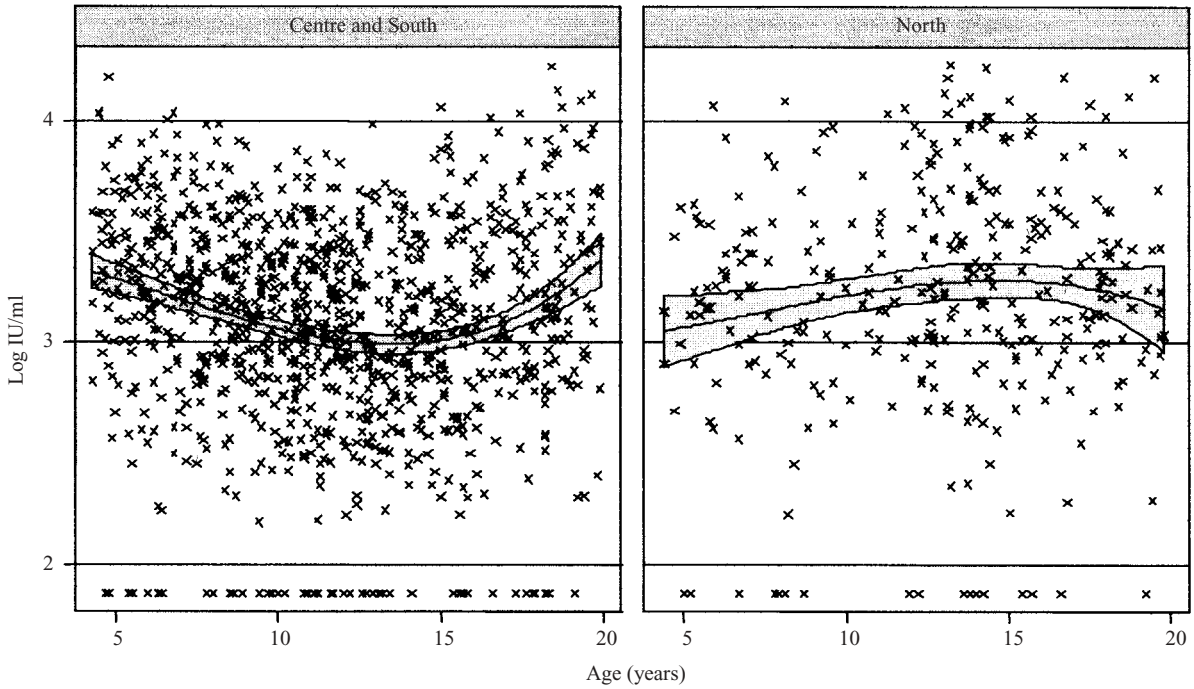

Fig. 3. Log antibody titres against measles comparing the school-aged population in the north to other regions in Luxembourg.

among the older secondary-school population. The individual titres regressed against age show a very similar pattern as the one observed for measles (Fig. 2*c*).

DISCUSSION

We have presented results of a seroprevalence study carried out on a representative sample of the Luxembourg population. To our knowledge, it is the first time that such a study has been done in Luxembourg. Although the collected serum bank is large, particularly so with respect to the small population size of Luxembourg (2673 serum samples correspond to 0·6% of the total resident population), size does not necessarily guarantee that the sample is representative. Selective participation could be an important source of bias when investigating seroprevalence of vaccine-preventable diseases [9]. Age and gender are often associated with antibody status, but due to the age-stratified study design of our sampling scheme, the bias for these variables was controlled for. Moreover for the sampling of children and adolescents, schools were chosen at random in different regions to ensure a certain degree of geographical and social diversity. A unique feature of the Luxembourg demography is that a large proportion (35%) of the resident population is of foreign origin. However, in the school-aged population at least, no difference in participation rates according to nationality was observed, thus excluding nationality as an important source of bias. Other biases could of course still be present (e.g. that parents opposing vaccination would be more likely to oppose participation in the study) but the extent of this bias is difficult to assess, although it would mean that our estimates of population immunity are possibly too high.

The seroprevalence results for measles and rubella show that the high coverage with MMR observed in the vaccine coverage survey [3] translates into a high proportion of virus antibody-positive individuals at the population level in school-aged children. It has been estimated that more than 95% of the population needs to be immune for elimination [2] and currently Luxembourg appears to be meeting this target. However, the analysis of actual titres rather than categorical antibody status shows quite clearly that a shift is occurring from a population whose immunity was derived from natural infection to a population protected by vaccine-derived immunity. Our data indicate that for all three infections, vaccinated individuals have lower titres than older individuals who had natural infection prior to the vaccination era. For measles, this has also been observed in other European

countries with a high routine coverage [10]. Whether this shift has or will have any epidemiological consequence and whether any future dose of MMR later in life may be necessary is as yet unknown. Mathematical modelling studies have attempted to address this important issue [11, 12].

Seroprevalence of anti-mumps virus antibodies appears to be lower than of anti-measles and anti-rubella virus antibodies. Several explanations are possible. First, there is some evidence the mumps virus component of the MMR vaccine elicits a less effective immune response than the measles or rubella virus components. Studies in developed countries have shown that seroconversion with a single dose of Jeryl Lynn strain mumps vaccine can vary from 80 to 100% [13]. Studies in Scandinavia document persistence of antibodies in children derived from the Jeryl Lynn mumps strain; rates of 73% of mumps virus seroconversion were reported in Sweden after a single dose [14] and 86% in Finland after two doses [15]. This level of seroprevalence concurs with those observed in our study. Secondly the lower seroprevalence could be due to the characteristics of the commercial assay. According to the manufacturer, the immunoassay we used to measure anti-mumps virus antibodies has a lower sensitivity and specificity, so some false negative results are to be expected. It is possible that the cut-off recommended by the manufacturer might be too conservative, erring on the side of caution because it is intended for diagnosis in the individual rather than for screening populations for seroepidemiological purposes. Finally, as far as persistence of mumps virus antibodies in unvaccinated individuals is concerned, age-stratified studies in most developed countries in the pre-vaccine era have shown that seroprevalence rarely exceeded 90% in adulthood [16], whereas seroprevalence was close to 100% among adults for measles and rubella. This could indicate that components used in mumps virus antibody assays may not be as good in general as those used in measles and rubella virus antibody assays. Unfortunately there is no mumps outbreak data available for Luxembourg to support the relatively high proportion of the population susceptible to mumps.

Seroprevalence in our sample for rubella virus is similar to that observed for measles virus. It is unlikely that major rubella outbreaks will occur with the currently high vaccination coverage achieved in Luxembourg. Thus the risk of susceptible women becoming infected during pregnancy is probably low. However, we observed a slightly higher proportion of rubella virus antibody-negatives in the older secondary-school students, notably the generation born between 1980 and 1985. This is not unexpected to some extent, because this was the generation that missed out on the opportunity to receive the MMR vaccine and, in consequence, remained susceptible because they had less exposure to circulating wild viruses following the implementation of routine vaccination. It is likely that a proportion of this 'window' generation might have been vaccinated outside of the recommended vaccination schedule. Nevertheless the overall antibody positivity rate of individuals born between 1980 and 1985 was 89·7%, which is 4% lower than the proportion of antibody positives of the individuals born in the vaccine era of 1986–1990, which is 93·95%.

A lower seroprevalence in the school-aged population living in the north of the country was detected for rubella and mumps viruses. This observation in conjunction with the fact that a major measles epidemic occurred in this region in 1996 is indicative of a lower coverage in the north of the MMR vaccine and warrants further investigation.

To conclude, immunity levels in the Luxembourg population against measles and rubella are probably sufficient to prevent large outbreaks. Current vaccination coverage appears to be sufficiently high to eliminate measles and rubella nationally and to control mumps. Future cooperation of paediatricians and doctors recommending all vaccinations of the Ministry of Health to their patients is crucial if control targets set by the WHO are to be met.

ACKNOWLEDGEMENTS

The authors thank the Direction de la Santé and the CRP–Santé in Luxembourg for their financial and technical assistance, the staff of the Laboratoire National de Santé who helped with the blood sample collection and testing, as well as all local staff involved with the sample collection in schools.

REFERENCES

1. Osborne K, Miller E, Weinberg J. The European Sero-Epidemiology Network. Euro Surveill 1997; **2**: 29–31.
2. Measles. Mortality reduction and regional elimination plan 2001–2005. World Health Organization, Geneva.
3. Enquête de la couverture vaccinale au Grand-Duché de Luxembourg [Survey of vaccination coverage

in the Grand Duchy of Luxembourg]. Septembre–Octobre 1996. Direction de la Santé. Ministère de la Santé.
4. Health care systems in transition. Luxembourg. European observatory on health care systems. WHO Regional Office for Europe, Copenhagen.
5. Edmunds WJ, Pebody RG, Aggerback H, et al. The sero-epidemiology of diphtheria in Western Europe. ESEN Project. European Sero-Epidemiology Network. Epidemiol Infect 2000; **125**: 113–125.
6. Annuaire statistique. Service central de la statistique et des études économiques [Annual statistics. Central service of statistics and economic studies]. Luxembourg.
7. Royston P, Altman D. Approximating statistical functions by using fractional polynomial regression. The Statistician 1997; **46**: 411–422.
8. Mossong J, Muller CP. Estimation of the basic reproduction number of measles during an outbreak in a partially vaccinated population. Epidemiol Infect 2000; **124**: 273–278.
9. de Melker HE, Nagelkerde NJ, Conyn-van Spaendonck MA. Non-participation in a population-based seroprevalence study of vaccine-preventable diseases. Epidemiol Infect 2000; **124**: 255–262.
10. van den Hof S, Berbers GA, de Melker HE, Conyn-van Spaendonck MA. Sero-epidemiology of measles antibodies in the Netherlands, a cross-sectional study in a national sample and in communities with low vaccine coverage. Vaccine 1999; **18**: 931–940.
11. Mossong J, Nokes DJ, Edmunds WJ, Cox MJ, Ratnam S, Muller CP. Modeling the impact of subclinical measles transmission in vaccinated populations with waning immunity. Am J Epidemiol 1999; **150**: 1238–1249.
12. Mossong J, Muller CP. Modelling measles re-emergence as a result of waning of immunity in vaccinated populations. Vaccine 2003; **21**: 4597–4603.
13. Fahlgren K. Two doses of MMR vaccine – sufficient to eradicate measles, mumps and rubella? Scand J Soc Med 1988; **16**: 129–135.
14. Broliden K, Abreu ER, Arneborn M, Bottiger M. Immunity to mumps before and after MMR vaccination at 12 years of age in the first generation offered the two-dose immunization programme. Vaccine 1998; **16**: 323–327.
15. Davidkin I, Valle M, Julkunen I. Persistence of anti-mumps virus antibodies after a two-dose MMR vaccination. A nine-year follow-up. Vaccine 1995; **13**: 1617–1622.
16. Galazka AM, Robertson SE, Kraigher A. Mumps and mumps vaccine: a global review. Bull World Health Organ 1999; **77**: 3–14.