Epidemiol. Infect. (2004), **132**, 1121–1127. © 2004 Cambridge University Press
DOI: 10.1017/S0950268804002754 Printed in the United Kingdom

Seroprevalence and force of infection of varicella-zoster virus in Luxembourg

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 9 June 2004*)

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 including varicella-zoster virus (VZV). ELISA tests performed on 2679 sera indicated that 96 (3·6%) of the study population were seronegative. Age-specific seroprevalence rose rapidly from approximately 70% at age 4 years to 90% at age 6 years to 95% at age 12 years. Significant heterogeneity of seroprevalence was observed between the six different primary schools. For age groups 0–5, 6–11 and 12+ years, we estimated an annual force of infection of 0·361 [95% confidence interval (CI) 0·31–0·415)], 0·204 (95% CI 0·12–0·29) and 0·05 (95% CI 0·024–0·082) respectively. Our results indicate that transmission of VZV is highest in children below the age of 6 years and that much lower levels of VZV transmission occur in secondary schools and throughout adulthood.

INTRODUCTION

Primary infection with varicella-zoster virus (VZV) causes chickenpox and is almost always a self-limited 'benign' disease if occurring in children. Following primary infection, the virus becomes latent and may reactivate decades later to cause zoster or shingles. Reasons for reactivation are not clearly understood, although decreased cell-mediated immunity might be involved [1]. A live attenuated VZV (Oka) vaccine was developed in Japan in the 1970s which was initially licensed for immunocompromised patients [2]. The introduction of a varicella vaccine into the routine vaccination programme in the United States has promoted interest in Europe to follow the American example. However, several concerns regarding routine varicella vaccinations have been voiced which are either directly related to the vaccine or indirectly related to epidemiological knock-on effects of universal vaccination. A unique feature of the VZV vaccine in comparison to other childhood vaccines is that the vaccine strain can become latent and reactivate later in life to provoke zoster [3, 4]. There have also been reports of low efficacy in protecting against infection [5]. From an epidemiological point of view, universal vaccination will lead to an increase in the average age of varicella cases, possibly increasing the likelihood of complications and hospitalizations in unvaccinated individuals infected with varicella [4, 6]. Finally, indirect evidence suggests that continued exposure in life to circulating varicella virus decreases the risk for developing shingles [7–9]. It is feared that the benefits of reducing VZV morbidity in children through vaccination are offset by the costs of increasing zoster-related or varicella-related morbidity in older individuals, who have not received the VZV vaccine.

* Author for correspondence: Dr J. Mossong, Laboratoire National de Santé, PO Box 1102, L-1011 Luxembourg.
(Email: joel.mossong@lns.etat.lu)

Although a varicella vaccine has been licensed in Luxembourg since 1984, it has not been included in the official vaccination schedule by the Ministry of Health and is not reimbursed by national sickness funds. Vaccine uptake is thus likely to be very low, because vaccination is officially only recommended for special at-risk groups.

The National Laboratory of Health and the Public Research Centre of Health conducted a seroprevalence survey during 2000–2001 within the framework of the European Sero-Epidemiological Network (ESEN) 2 to assess the level of immunity in the Luxembourg population against eight vaccine-preventable infections. This study presents the seroprevalence of VZV antibodies and other characteristics of VZV transmission in Luxembourg of public health relevance. Results of seroprevalence of MMR virus antibodies investigated in the same survey have been published elsewhere [10]. The aims of the present study were three-fold: first to provide age-specific VZV seroprevalence estimates, secondly to identify risk factors for being VZV antibody-seropositive, and thirdly to estimate the force of infection of VZV.

METHODS

Survey design

A multi-tiered prospective sample design was followed which allowed achievement of sample sizes according to the specifications of the ESEN 2 project [11], i.e. 100 samples in each age year band in those aged 0–19 years and 200 samples for each of the age groups 20–24, 25–29, 30–34, 35–39, 40–49, 50–59 and 60+ years. Children and adolescent samples were collected in randomly selected primary schools (6 schools out of 109 in total) and secondary schools (3 schools out of 23 in total) from different geographical regions in Luxembourg. All pupils and their respective parents were given a leaflet explaining the aims the study including a short description of the diseases involved. Written consent of study participants, or their parents if participants were less than 18 years old, was obtained. The participation rate in primary and secondary schools was 47·2%, but depended on age with highest rates among 12- to 15-year-olds (>60%) and lowest for the youngest and oldest school members. Given that in Luxembourg children start kindergarten at 4 years of age, no serum samples were collected from children aged 3 years or less.

Serum samples of adults were obtained from adult volunteer blood donors at the national Red Cross Centre, from adult volunteers attending routine blood tests at the National Health Laboratory and from adults undergoing compulsory premarital testing (prior to marriage, all couples must undergo the following compulsory laboratory tests: syphilis, TB skin test and blood group for all, and additional testing for toxoplasmosis and rubella for females). HIV testing is offered on a voluntary basis. All study participants were offered test results via a doctor of their choice that could give advice on additional vaccinations if deemed necessary.

Serology

Serum samples were tested with an automated BEP® 2000 analyser (Dade Behring, Marburg, Germany) using a commercial Enzygnost® Anti-VZV/IgG kit (Dade Behring, Marburg, Germany) with reported sensitivity and specificity of 99·3 and 100% respectively, according to the manufacturer. Positive and negative status of sera was determined as follows: sera with corrected absorbances strictly less than 0·1 units (equivalent of 50 mIU/ml) were defined as negative, those $\geqslant 0{\cdot}1$ units as positive.

Hospital admission data

Anonymous hospital admission data based on medical billing data was obtained from the Contrôle Médicale, Luxembourg. Their database contains all hospitalizations in Luxembourg which involve reimbursement by the mandatory national sickness funds. A 3-year period, 2000–2002, was chosen due to the small numbers involved and an average was calculated to get an annual number of varicella hospital admissions. Data included age, dates of admission and discharge and up to four ICD-10 discharge diagnostic codes. Patients with a length of stay of 0 days were excluded from analysis.

Statistical analysis

Seroprevalence of VZV antibodies was calculated for both gender and age groups allowing standardization according to the population of Luxembourg in 2000 [12]. Associations between serological status and independent variables – site of sample collection, age (categorized in Fig.), sex and nationality [Luxembourg, Portuguese, other European Union (EU) and non-EU] – were tested using the χ^2 statistic. Risk

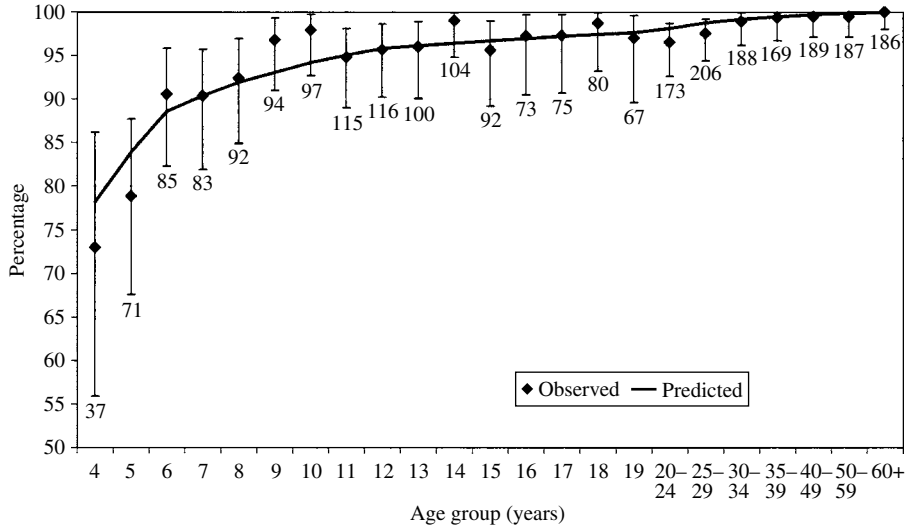

Fig. Observed *vs.* predicted seroprevalence of antibodies against varicella zoster virus in Luxembourg, 2000. Observed point-estimates of age-specific prevalence in the serosurvey is shown by diamonds, together with exact binomial 95% confidence intervals. Numbers shown below the confidence interval represent the total number of samples in that age group. Model predictions are indicated by the solid line. The model consists of estimating the force of infection for three age groups, 0–5, 6–11 and 12+ years as indicated in the text.

factors for being VZV antibody-negative compared to VZV antibody-positive were determined using multivariate logistic regression. Age was modelled using fractional polynomials, to assess potential nonlinear functional relationship between serostatus and age [13]. Ninety-five per cent confidence intervals (CIs) of prevalence estimates were calculated using the exact binomial method. Associations between serostatus and independent variables, 95% CIs of point-estimates and logistic regression were calculated with Stata 8.0 (StataCorp, Texas, USA).

The seroprevalence data were re-analysed to estimate the force of infection of VZV. The force of infection is the rate at which susceptible individuals acquire infection and is a parameter reflecting the contagiousness of an infectious agent. We assumed that a constant infection rate has acted upon all members of the population and changes with age of the proportion susceptible are assumed to be the result of VZV transmission, i.e. individuals who seroconvert remain seropositive for the rest of their lives. The force of infection was estimated using the likelihood method explained in more detail in ref. [14], by maximizing the log-likelihood function:

$$L=\sum_{j=1}^{23} M_{x_j}\log(F(x_j))+(N_{x_j}-M_{x_j})\log(1-F(x_j)),$$

where j is the index for the 23 age groups indicated in the Figure, x_j is the average age in age group j, N_j is the total number of persons sampled in age group j, M_j is the number that are VZV antibody-seropositive in age group j, and $F(x)$ is the estimated proportion of individuals at age x (in years) who are VZV antibody-seropositive. $F(x)$ was assumed to be piecewise constant for age groups 0–5, 6–11 and 12+ years, with the exception of all babies below 6 months of age who were assumed to be protected from maternal antibodies [15]:

$$F(x)=\begin{cases}1 & \text{if } x<0{\cdot}5\\ 1-\exp(-\lambda_1 a) & \text{if } 0{\cdot}5\leqslant x<6\\ 1-\exp(-\lambda_1(6-0{\cdot}5)-\lambda_2(x-6)) & \text{if } 6\leqslant x<12\\ 1-\exp(-\lambda_1(6-0{\cdot}5)-\lambda_2(12-6)-\lambda_3(x-12)) & \text{if } 12\leqslant x\end{cases}$$

Further details regarding the mathematical details of the model can be found in ref. [16]. The log-likelihood was maximized to obtain simultaneously point-estimates of the parameters (λ_j) in the model and the profile log-likelihood was used to obtain 95% CIs assuming an approximate χ^2 distribution. Goodness of fit of the model was assessed using the deviance, i.e. the difference in 2∗(log likelihood) between the saturated model and the fitted model, which has approximately a χ^2 distribution [17]. Maximization of the log likelihood was performed using the solver add-in of Excel 2000 (Microsoft, Redmond, WA, USA).

A crude age distribution of VZV cases occurring in Luxembourg in 2000 was predicted by multiplying the total population in each age group (birth cohort of 5626 in 2000) by the proportion of seronegatives predicted by the model $(1-F(x))$ and by the age-specific force of infection.

RESULTS

Overall, 96 (3·6%) of the 2679 obtained serum samples were found to be negative for anti-VZV antibodies and 2583 (96·4%) samples were found to be positive, corresponding to an age-standardized prevalence of 2 and 98% of negatives and positives in the Luxembourg population above 4 years of age. No significant association was found between seroprevalence and gender ($P=0·395$). Seroprevalence was found to be homogenous within the three secondary schools ($P=0·837$), but significant heterogeneity being observed for the six primary schools ($P=0·006$). This heterogeneity remained significant when age was controlled for using the fractional polynomial method (likelihood ratio test, $P=0·003$). Seroprevalence in adult samples collected from the two centres were similar ($P=0·304$). As expected, seroprevalence was significantly associated with age ($P<0·001$), with seronegative samples being more common among the youngest participants. The Figure shows how serological status varies as a function of age. Although in univariate analysis seroprevalence differed between nationalities ($P=0·01$), this difference disappeared after controlling for age using the fractional polynomial method (likelihood ratio test, $P=0·44$).

Force-of-infection estimates of age groups 0–6, 6–12 and 12+ years were 0·361 (95% CI 0·31–0·415), 0·204 (95% CI 0·12–0·29) and 0·05 (95% CI 0·024–0·082) respectively [deviance: 13·84 with 20 degrees of freedom (D.F.), $P=0·84$]. The annual rate of infection per susceptible is clearly age-dependent, the highest force of infection being observed in the youngest age group, a two-fold lower force of infection in primary-school children and a seven-fold lower force of infection in adolescents and adults.

The predicted age distribution of primary VZV infections and hospitalization rates are presented in the Table. Of the approximately 6000 VZV cases occurring each year in Luxembourg, the overwhelming majority are expected to occur in children prior to entering primary school (80%), and only a tiny fraction (1·5%) are expected to occur in adults, who are most likely to suffer from VZV-related complications and thus be hospitalized. The average age for VZV infection is estimated to be 4·25 years, the median being between 2 and 3 years. Overall, estimated hospitalization rate per case is estimated to be 0·21%, although it is found to be age-dependent, with approximately a 20-fold higher hospitalization rate in adults compared to children and adolescents.

Table. *Predicted age distribution of estimated VZV cases occurring in Luxembourg in 2000 and estimated hospitalization rates*

Age group (years)	Estimated no. of cases (%)	Hospitalizations* (%)	Hospitalization rate per case (%)
0–5	5068 (80·86%)	6·33 (47%)	0·1%
6–11	786 (12·55%)	2·66 (20%)	0·3%
12–18	317 (5·05%)	0·33 (2·5%)	0·1%
19+	97 (1·54%)	4 (30%)	4·1%
Total	6268 (100%)	13·33 (100%)	0·2%

* Average annual number in years 2000–2002.

DISCUSSION

To our knowledge, this is the first time that a serosurvey on VZV has been conducted in Luxembourg. Even if the collected serum bank is large (2673 serum samples correspond to 0·6% of the total resident population), a large sample size is no guarantee for representativeness. While selective participation might be an important source of bias when investigating seroprevalence of vaccine-preventable diseases [18], the low rate of varicella vaccination in Luxembourg and the high incidence of VZV shown in this study probably minimize this bias. As reported previously, we could not detect any significant difference in participation between nationalities ($P=0·075$) [10]. Our study clearly shows that age is associated with VZV antibody status, but due to the age-stratified study design of our sampling scheme, this factor was appropriately controlled for. With hindsight it would have been appropriate to include participants below the age of 4 years as transmission is highest in this age groups, but a 'random' sample collection in this age group is difficult to implement.

For the sampling of school-aged children and adolescents, schools were chosen at random in different

regions to ensure a certain degree of geographical and social diversity. It is interesting to note that we observed a significant amount of heterogeneity of VZV antibody seroprevalence between primary schools ranging from 85 to 96%. This might be due to the fact that seroprevalence of VZV observed in schools depends on whether a VZV epidemic had taken place recently within the primary school prior to blood sample collection. This has implications for the design of serosurveys. Future school-based studies ought to be conducted in multiple schools spread over a certain region to 'average out' the influence of recent and local VZV epidemics. On the other hand, no significant heterogeneity was observed in the secondary schools, although this was probably due to a lack of statistical power given the high seropositive rate in secondary schools and the low force-of-infection estimate in this age group. A unique feature of the Luxembourg demography is the large proportion (35%) of the resident population of foreign origin. As far as VZV is concerned, we did not observe any differences in seroprevalence after adjusting for age.

In comparison to results from other countries, the seroprevalence of VZV antibodies we observed in Luxembourg follows a remarkably similar pattern to the one seen for neighbouring Belgium in that approximately 70% of 4-year-olds are VZV antibody-positive [6]. This age-specific seroprevalence is higher than has been reported for Switzerland [19], Germany [20], and Italy [21] and indicates that kindergarten and pre-school-mixing patterns play a major role in VZV transmission in the two neighbouring countries. Although numbers are probably too small to allow a formal comparison, hospitalization rates per case in Luxembourg are similar to those found in the United Kingdom and Canada [4].

In this study, we estimated the force of infection of varicella using three age groups only. The force of infection is a valuable measure of the transmission potential of an infectious agent. It is defined as the rate at which susceptible individuals acquire infection. Our study suggests that in Luxembourg, the annual rate of acquiring varicella for a susceptible child below 6 years of age is approximately 36%, for a susceptible child aged 6–12 years it is approximately 20% and for older children, this rate declines to approximately 5%. Increasing the number of age groups to five (0–4, 4–6, 6–12, 12–18, 18+ years) categories did not improve the model fit significantly (likelihood ratio test against model with three age groups, $P=0{\cdot}28$) and the parsimonious model with three age groups was therefore retained. The Figure also shows that the fit of the catalytic infection model to the observed proportion of seropositives is adequate, as model predictions always lie within the 95% CI of point-estimates in each age group.

Even though the sample size of children aged 4–5 years is small, and we did not obtain data for children aged <4 years, our model suggests that prior to entering kindergarten, a substantial majority of children have already experienced infection. It is likely that this is partially due to increasing availability of pre-school child care and crèche facilities. Indeed, in 1998 the Luxembourg government introduced a voluntary pre-school programme for children aged between 3 and 4 years. During 2000–2001, 2377 (42% of the total number of children born in 1997) children attended this pre-school programme [22]. In 2002 there were an additional 2677 places available in public and private crèches for children aged <4 years [12], it is, therefore, probable that a majority of children have attended day-care or pre-school facilities prior to entering kindergarten. It is clear that the trend for earlier socialization of children will have an influence on the epidemiology of VZV by keeping the age of acquisition of VZV infection and immunity low. The proportion of seropositives has risen to approximately 90 and 95% by the time they enter primary school at age 6 years or secondary school at age 12–13 years respectively. As the low force of infection of 0·05 per year indicates, much lower levels of VZV transmission seem to occur in secondary schools and throughout adulthood.

As far as estimates of the force of infection based on the same serosurvey methodology are concerned, there is only one recent study in Australia which showed a much lower force of infection (0·139 in the 0–4 years group and 0·195 in the 5–9 years group) and higher average age of infection of 8·15 years [23]. This suggests that varicella is less easily transmitted in Australia. Reasons for this are unclear, but could include varying mixing patterns in relevant age groups thereby affecting the transmission of the virus.

Data from the United Kingdom and Canada in the early 1990s also show a much lower force of infection in pre-school children (0·18 and 0·16 respectively), but similar levels among adolescents (0·09 and 0·1 respectively) and adults (range 0·07–0·09 and 0·04–0·09 respectively) [4]. Some of the difference could be explained due to the study design: United Kingdom and Canadian estimates are based on medical billing data, which might underestimate the number of infections

in young children, due to parents not seeking medical treatment for their children. Moreover, studies in Scotland have indicated a general downward trend of the age of VZV infection between the early 1980s and 1998 attributed to an increased attendance of pre-school children at nursery or day-care centres [24, 25].

One of the main implications of our study is that, although almost all children in Luxembourg get varicella, the burden on the health system due to primary VZV infection is limited, because very few infected children need to be hospitalized. The risk for hospitalization, on the other hand, is approximately ten-fold higher for adults with primary VZV infection. It is clear that were a vaccine introduced in the routine schedule, the average age of infection in those not immunized would rise and might lead to an overall increase in hospitalizations and in-patient days due to VZV. Such a deleterious effect from routine vaccination has been observed for rubella in Greece [26]. It has been shown that this negative impact of vaccination depends critically on the achieved vaccine coverage, and that total number of hospitalizations in adults could decrease if vaccine coverage is high enough to eliminate VZV transmission. Also it is unclear how routine vaccination might impact the prevalence of zoster [27]. For future work, it is envisaged to use our data to develop and parameterize mathematical transmission models of VZV to investigate the hypothesis whether, and when, introducing varicella vaccine in the routine vaccination programme might lead to such adverse outcomes.

Our study data has been presented to the national body advising the Minister of Health on vaccination policy and has helped to further essential knowledge of VZV epidemiology for policy makers. This body took a decision in July 2003 not to include the varicella vaccine in the routine vaccination programme given the uncertainties related to vaccine efficacy and possible medium- to long-term adverse epidemiological consequences.

ACKNOWLEDGEMENTS

The authors thank the Direction de la Santé and the CRP-Santé in Luxembourg for their financial assistance; Dr Paul Koch and Thierry Ries from the Contrôle Médical in Luxembourg for providing the hospital admission data, 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. Hope-Simpson RE. The nature of herpes zoster: a long-term study and a new hypothesis. Proc R Soc Med 1965; **58**: 9–20.
2. Gershon AA, Takahashi M, White CJ. Varicella vaccine. In: Plotkin SA, Orenstein WA, eds. Vaccines. Philadelphia: Saunders, 1999: 475–507.
3. Wise RP, Salive ME, Braun MM, et al. Postlicensure safety surveillance for varicella vaccine. J Am Med Assoc 2000; **284**: 1271–1279.
4. Brisson M, Edmunds WJ, Law B, et al. Epidemiology of varicella zoster virus infection in Canada and the United Kingdom. Epidemiol Infect 2001; **127**: 305–314.
5. Galil K, Lee B, Strine T, et al. Outbreak of varicella at a day-care center despite vaccination. N Engl J Med 2002; **347**: 1909–1915.
6. Thiry N, Beutels P, Shkedy Z, et al. The sero-epidemiology of primary varicella-zoster virus infection in Flanders (Belgium). Eur J Pediatr 2002; **161**: 588–593.
7. Brisson M, Gay NJ, Edmunds WJ, Andrews NJ. Exposure to varicella boosts immunity to herpes-zoster: implications for mass vaccination against chickenpox. Vaccine 2002; **20**: 2500–2507.
8. Thomas SL, Wheeler JG, Hall AJ. Contacts with varicella or with children and protection against herpes zoster in adults: a case-control study. Lancet 2002; **360**: 678–682.
9. Edmunds WJ, Brisson M, Gay NJ, Miller E. Varicella vaccination: a double-edged sword? Commun Dis Public Health 2002; **5**: 185–186.
10. Mossong J, Putz L, Schneider F. Seroprevalence of measles, mumps and rubella antibodies in Luxembourg: results from a national cross-sectional study. Epidemiol Infect 2004; **132**: 11–18.
11. 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.
12. Annuaire statistique [Statistical Yearbook]. Service central de la statistique et des études économiques. Luxembourg, 2003.
13. Royston P, Altman D. Approximating statistical functions by using fractional polynomial regression. The Statistician 1997; **46**: 411–422.
14. Remme J, Mandara MP, Leeuwenburg J. The force of measles infection in East Africa. Int J Epidemiol 1984; **13**: 332–339.
15. Gershon AA, Raker R, Steinberg S, Topf-Olstein B, Drusin LM. Antibody to Varicella-Zoster virus in parturient women and their offspring during the first year of life. Pediatrics 1976; **58**: 692–696.
16. Griffiths DA. A catalytic model of infection for measles. Appl Statist 1974; **23**: 330–339.
17. McCullagh P, Nelder JA. Generalized linear models, 2nd edn. Monograph on statistics and applied probability, 1989. New York: Chapman & Hall.
18. 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.
19. Aebi C, Fischer K, Gorgievski M, Matter L, Muhlemann K. Age-specific seroprevalence to varicella-zoster virus: study in Swiss children and analysis of European data. Vaccine 2001; **19**: 3097–3103.
20. Wutzler P, Farber I, Wagenpfeil S, Bisanz H, Tischer A. Seroprevalence of varicella-zoster virus in the German population. Vaccine 2001; **20**: 121–124.
21. Gabutti G, Penna C, Rossi M, et al. The seroepidemiology of varicella in Italy. Epidemiol Infect 2001; **126**: 433–440.
22. Les chiffres clés de l'Education Nationale. Statistiques et indicateurs 2001–2002 [Key figures of national education. Statistics and indicators 2001–2002]. SCRIPT. Ministère de l'Education Nationale, de la Formation Professionnelle et des Sports. Luxembourg, 2003.
23. Gidding HF, MacIntyre CR, Burgess MA, Gilbert GL. The seroepidemiology and transmission dynamics of varicella in Australia. Epidemiol Infect 2003; **131**: 1085–1089.
24. Bramley JC, Jones IG. Epidemiology of chickenpox in Scotland: 1981 to 1998. Commun Dis Public Health 2000; **3**: 282–287.
25. Ross AM, Fleming DM. Chickenpox increasingly affects preschool children. Commun Dis Public Health 2000; **3**: 213–215.
26. Panagiotopoulos T, Antoniadou I, Valassi-Adam E. Increase in congenital rubella occurrence after immunisation in Greece: retrospective survey and systematic review. Br Med J 1999; **319**: 1462–1467.
27. Edmunds WJ, Brisson M. The effect of vaccination on the epidemiology of varicella zoster virus. J Infect 2002; **44**: 211–219.