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## Seroprevalence of antibody to varicella zoster virus in England and Wales in children and young adults

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### SUMMARY

This is the first large-scale study to investigate the seroprevalence of varicella zoster (VZV) in the general population of England and Wales. The study focused on those aged 1–20 years, that age group in whom most infections occur. Prevalence rose rapidly with age, with 53% of children showing evidence of prior infection by the age of 5 years and most young adults having experienced infection. In addition to using a fixed cut-off recommended by the manufacturer, a mixture modelling technique was also used to define the proportion of the population seropositive in each age group. This was shown to be a more accurate approach to categorizing data from an epidemiological perspective.

### INTRODUCTION

Varicella zoster virus (VZV) is an alpha herpes virus. In common with other members of the herpesviridae family, it is able to establish latent infection of the host following primary infection. This occurs in the dorsal root and trigeminal ganglia. VZV therefore persists for the life of the host and may reactivate at intervals. The two diseases caused by VZV are varicella (chickenpox) and herpes zoster (shingles). Varicella is the manifestation of primary VZV infection and is often acquired as a child. Its symptoms are generally clinically mild and easily identifiable by the

characteristic vesicular rash and fever. It is considered highly infectious since airborne transmission occurs via respiratory droplets or from shedding of infectious virions from vesicles on the skin, and the lifetime risk of acquiring varicella is over 95%. Herpes zoster is the disease caused by reactivation of the virus from its latent state and usually occurs in adults, the incidence rising steeply in the elderly [1–3].

Various licensed vaccines for VZV have recently become available that have been shown to be safe, protective and immunogenic [1–4]. However, none is currently used routinely in the United Kingdom and vaccine is only recommended for ‘at risk’ health workers [5]. This is because a number of issues relating to the cost-effectiveness and impact of vaccination on the incidence of herpes zoster need to be more fully understood before widespread use can be recommended [6–8].

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Previous epidemiological studies using data from a variety of surveillance perspectives suggest the majority of people have acquired infection by adulthood, and the epidemiology of VZV in the United Kingdom may be changing [1–3, 9, 10]. This is characterized by a decrease in the average age of infection, with an increasing proportion of those aged <5 years acquiring infection. A likely cause suggested is the increasing frequency with which young children are now attending pre-school establishments, facilitating exposure and transmission amongst this age group [1, 3]. There is also some suggestion of a small decline in prevalence amongst adults [3]. A recent cross-sectional serological study investigating VZV IgG prevalence using sera collected over a 25-year period from persons aged 1–39 years supported these observations [11]. However, the number of specimens available for testing was limited for this study, a feature also common to the only other published report of VZV seroprevalence in the United Kingdom [12].

The aim of this study was, therefore, to carry out a comprehensive serosurvey of VZV infection in England and Wales to determine the prevalence of VZV-specific IgG in persons aged up to 20 years, the age group in which most infection occurs. A validated commercial ELISA kit was therefore used in conjunction with substantial numbers of samples selected from a convenience collection derived from residual blood samples obtained for diagnostic and screening tests which is considered the closest approximation to the general population available [13]. Results were analysed by age, sex and region. In addition to the fixed cut-off recommended by the manufacturer for categorizing results qualitatively, a mixture modelling technique was applied to the serological data to try and obtain the most accurate estimate of the proportion seropositive in the population investigated [14, 15]. Mixture modelling was included because prevalence of antibody is expected to increase with age and it is the proportion positive at each age (rather than individual results) that is of interest, so use of a fixed cut-off as recommended by most commercial ELISA assays could result in a lack of sensitivity. This is because such fixed cut-offs are often deliberately chosen to err on the side of specificity as the assay is primarily intended for use in situations where results may be used for individual patient management, and such an approach allows accurate identification of true antibody-negative individuals.

## METHODS

### Samples

A total of 2091 serum samples (collected in 1996) from persons aged 1–20 years were used. Of these, 1006 (48%) were from females and 1085 (52%) from males. For those aged 1–4 years 117, 136, 145 and 182 sera respectively were tested in each one-year age group. For those aged 5–20 years approximately 100 sera in each one-year age group were screened. All were anonymized residues of specimens submitted for microbiological or biochemical testing to 16 Public Health Laboratories and two NHS Laboratories in England and Wales, collected as part of the PHLS serological surveillance programme [13].

### Laboratory methods

All serological tests for VZV-specific IgG were performed at Preston Public Health Laboratory using a commercial ELISA assay (VZV IgG Enzyme Immunoassay test kit, Diamedix Corporation, Miami, FL, USA) according to the manufacturer's instructions. This assay allows results to be expressed quantitatively or qualitatively using a fixed cut-off.

### Mixture modelling

In addition to analysing results using the fixed cut-off suggested for the ELISA test, serological data were also analysed using a mixture model. The assumption underlying mixture modelling is that the samples tested are taken from a mixture of persons who have previously experienced infection (the previously infected population) and those who have not (the uninfected population).

Initially, an age-stratified distribution of quantitative results needs consideration. This yields information that is necessary to influence the choice and construction of the mixture model to most accurately represent the serological prevalence data observed, related to the 'previously infected' and 'uninfected' population groups. Twenty-four parameters were then estimated from 500 data-points that represented the distribution of results in the 20 one-year age groups. Twenty parameters describe the proportion in each component (i.e. whether uninfected or previously infected) for each one-year age group. Four parameters describe the shape of the two distributions seen: a Normal distribution has a mean and standard deviation, and a Gamma distribution is described by

shape and scale parameters. A detailed description showing how the model was constructed is provided in the Appendix.

## RESULTS

Using the fixed cut-off suggested by the assay manufacturer, seroprevalence of IgG to VZV increased with age. Of those aged 5 years, 53% showed evidence of infection. This rose to ~85% in those aged 15–20 years (Fig. 1). There was no significant difference in prevalence between sexes or regions.

An age-stratified distribution of reactivity of samples in the assay used in this study is shown in Figure 2. This shows data falling into two distinct distributions, assumed to reflect those uninfected and those previously infected with VZV within the population used. The distribution of reactivity in the uninfected component was best fitted by a Gamma distribution whilst the previously infected component was best fitted by a Normal distribution (Fig. 2). The resulting mixture model that was applied to the data is illustrated in Figure 3.

The position of the fixed cut-off suggested by the manufacturer relative to the age-stratified distribution of results is also highlighted by Figure 2. This illustrates that the recommended fixed cut-off actually intersects the distribution considered to represent those who have been previously infected, rather than lying between the two distributions.

The mixture model used provided a good fit to the serological data, which is reflected by the deviance ( $D=560.87$  using 476 D.F.). The model estimates for antibody prevalence were very similar to results using the recommended fixed cut-off for children aged 1–10 years (Fig. 1). However, the model suggested prevalence was significantly higher ( $P<0.05$ ) in those aged 11–20 years than that obtained using the suggested fixed cut-off, estimating prevalence to rise to >90% in those aged >15 years (Fig. 1).

## DISCUSSION

This study was designed to investigate the prevalence of VZV infection in the population of England and Wales in the age group in which most primary varicella infections occur. The subjects used were not a random sample of the population, but were persons whose serum was submitted to microbiology or biochemistry laboratories for routine diagnostic

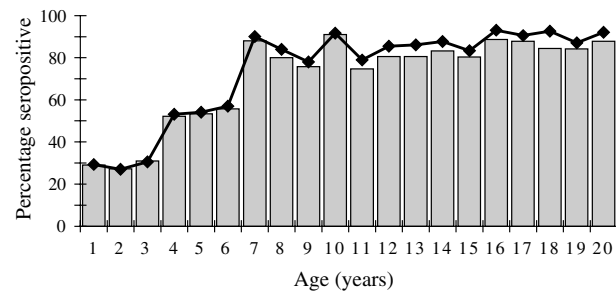
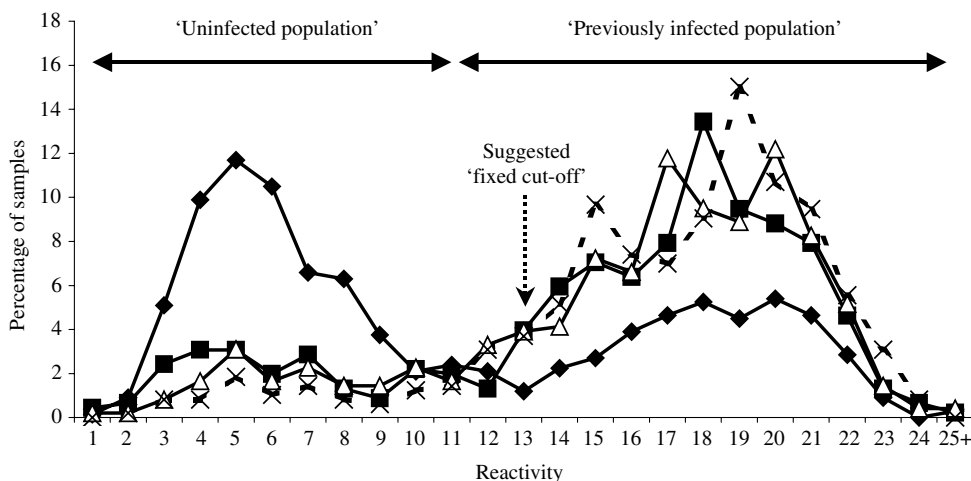


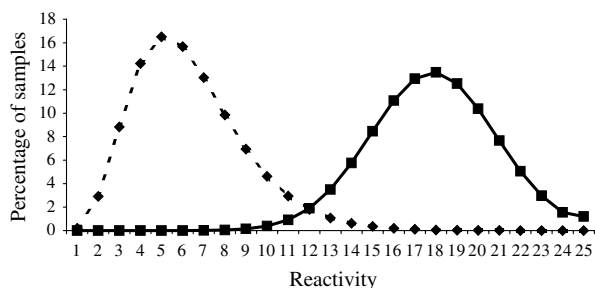
Fig. 1. Age-specific VZV antibody prevalence determined using a fixed cut-off (□) and the mixture model (—◆—).

examination. Given the comprehensive diagnostic service that each offers, substantial differences between laboratories regarding the reasons for which sera were submitted are unlikely. The collection may be considered to be representative of the general population in terms of its exposure to varicella [13]. Additional evidence also suggests that such convenience sampling strategies are comparable to random cluster surveys [16].

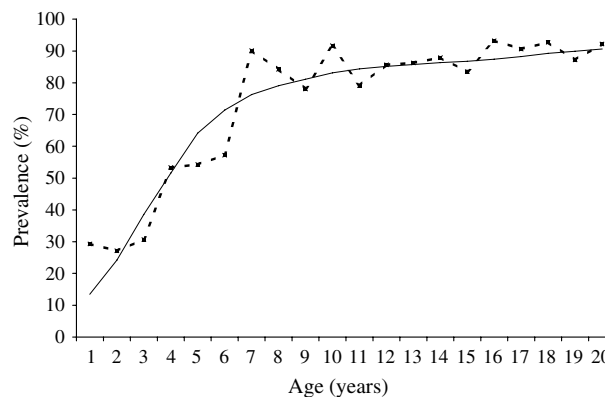
Two methods of estimating prevalence were used; a fixed cut-off recommended by the assay manufacturer and a mixture modelling technique. Using both methods, antibody prevalence to VZV was observed to rise sharply with age and demonstrated that most children showed evidence of VZV infection by the age of 10 years using samples collected in 1996, with a high rate of acquisition of antibody amongst those aged <5 years. However, whilst prevalence estimates using the mixture model closely reflected those using the fixed cut-off for children aged 1–10 years, estimates were up to 5% higher for those aged 11–20 years using the mixture model (Fig. 1). The recommended fixed cut-off clearly intersects that distribution of results representing persons previously infected with VZV (Fig. 2) suggesting it has been deliberately chosen specifically for diagnostic purposes. Rather than attempting to identify any samples with evidence of previous infection with VZV, its purpose is to distinguish only those that contain clinically significant levels of antibody to ensure a high specificity and a high positive predictive value. In contrast, for epidemiological analysis the primary consideration is not the clinical significance of individual results, but the proportion seropositive at each age. Therefore, from an epidemiological perspective, the mixture model estimate is the more appropriate and sensitive method. Whilst such discrepancies are small, they should not be ignored as they will affect an understanding of the epidemiology of VZV in those aged



**Fig. 2.** Distribution of VZV EIA results by age group. —◆—, 1–5 years; —■—, 6–10 years; -△-, 11–15 years; --×--, 16–20 years.



**Fig. 3.** The modelled distribution of reactivity in the VZV EIA for IgG-positive (previously infected, —■—) and IgG-negative (uninfected, --◆--) samples.



**Fig. 4.** VZV prevalence estimates (—) (derived from MSGP4 surveillance data adjusted for under reporting) compared to mixture modelled seroprevalence (---) data.

> 10 years [3]. This illustrates the value of analysing data quantitatively using an appropriate method.

Brisson et al. [1] described the epidemiology of VZV in the United Kingdom using consultations to general practitioners (GPs) for varicella recorded in the fourth National Survey of Morbidity in General Practice (MSGP4). This survey of general practices covered a 1% sample (over 500 000 patients) of England and Wales in 1991–1992. Brisson et al. included an age-specific adjustment for under-reporting, since not all children who contract varicella consult a GP, from which age-specific prevalence estimates can be made. The mixture model estimate of VZV seroprevalence made by this study compares very favourably to, and therefore supports, the age-stratified varicella prevalence estimate that can be derived from the incidence data of Brisson et al. [17], particularly in those aged > 10 years (Fig. 4).

The observed prevalence of VZV-specific IgG in those aged 1 year was surprisingly high (29%)

compared to the findings of other studies [1, 12]. This is unlikely to be due to the persistence of maternal antibody since all persons providing specimens involved with this study were aged at least 12 months. A more probable explanation may be that this could reflect a specificity problem with the assay when testing samples from the very young. For example, there may be some cross reactivity with antibody to other herpes viruses with a high prevalence in this age group (e.g. HHV-6 and HHV-7) [12].

This is a cross-sectional study presenting prevalence data obtained from a single point in time, namely 1996. Caution should, therefore, be exercised when using it to comment on suggested changes in VZV epidemiology. However, the high rate of acquisition of antibody seen amongst those aged < 5 years is consistent with those reports describing an increasing

proportion of pre-school children contracting the disease due to an increasing proportion attending pre-school establishments. It is more difficult to use these data to support an upward shift in age of onset towards older groups, especially since the study was limited to those aged  $\leq 20$  years. The high antibody prevalence observed amongst young adults suggests there was limited susceptibility in this group in 1996, but inferences cannot be made as to how this may be changing with time.

This is, therefore, the first large-scale seroprevalence study to describe evidence of antibody to VZV in England and Wales using samples thought to reflect the general population. It also illustrates the benefits of using mixture models to interpret prevalence data, a technique that facilitates the categorization of data qualitatively from an epidemiological perspective. To confirm any changes to the epidemiology of VZV requires additional seroprevalence studies, ideally using sera collected at varying points in time. This would be particularly useful should more widespread use of vaccine be introduced. It would also be valuable to extend such studies to incorporate the whole age range. This would enable an assessment to be made of any tendency of antibody levels to wane over time, which may be an important consideration regarding susceptibility to zoster that could occur later in life.

**APPENDIX**

**Description of the mixture model**

The reactivity  $x$  in the assay was defined as the logarithm of the optical density (OD) of the test sample compared to the relevant mean negative control OD. Individual results were aggregated into 500 data points comprising 20 one-year age groups (from ages 1–20 years) by 25 reactivity categories:  $n_{jk}$  denotes the number of results from person in age group  $j$  ( $j=1, \dots, 20$ ) falling in the  $k$ th reactivity category ( $x_{k-1} < x \leq x_k$ :  $x_0 = -\infty$ ,  $x_{25} = \infty$ ,  $x_k = -0.5 + 0.1k$  for  $k = 1, \dots, 24$ ).

**Mixture model**

Let  $f^-(x)$  and  $f^+(x)$  denote the distributions for the negative and positive components respectively. Let  $p_j^-$  and  $p_j^+$  denote the proportion of samples from the negative and positive components respectively in age group  $j$  ( $p_j^- + p_j^+ = 1$ ). Then the overall density of

results at age  $j$ ,  $f_j$ , is a mixture of the two component densities,

$$f_j(x) = p_j^- f^-(x) + p_j^+ f^+(x).$$

**Parameter estimation**

Let  $N_j$  denote the number of individuals of age  $j$ , so that  $N_j = \sum_k n_{jk}$ . Then  $(n_{j1}, \dots, n_{j25})$  is multinomial with index  $N_j$  and probabilities  $\pi_{jk}$ , where

$$\pi_{jk} = \int_{x_{k-1}}^{x_k} f_j(x) dx.$$

Maximum-likelihood estimates for the parameters were obtained by minimizing the deviance,  $D$

$$D = 2 \sum_{j=1}^{20} \sum_{k=1}^{25} n_{jk} \log \left( \frac{n_{jk}}{\pi_{jk} N_j} \right).$$

Likelihood-based 95% confidence intervals for the age-specific prevalence were obtained by finding the maximum and minimum values for which the deviance was within 3.84 of the minimum.

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