

## Seroepidemiology of Varicella and the Reliability of a Self-reported History of Varicella Infection in Singapore Military Recruits

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### Abstract

**Introduction:** Varicella is an acute disease with significant morbidity. However, there is little knowledge on the seroepidemiology of the disease in Singapore. The objective of this study was to assess the seroprevalence of varicella zoster virus (VZV) antibodies in military recruits in Singapore and to ascertain the predictive value of a self-reported history of varicella. The latter is a possible proxy for seroprevalence, and may be used to provide efficient identification of candidates for vaccination. **Materials and Methods:** From September 2000 to October 2005, 2189 servicemen were selected during their pre-enlistment medical check-up. Blood samples were obtained to determine the varicella IgG levels via enzyme-linked immunosorbent assay (ELISA). Information about the participant's race, history of varicella and vaccination, and other clinical variables were obtained through a questionnaire. **Results:** The overall prevalence of VZV seropositivity in military recruits was 76.0% (75.8% in the 16 years to 20 years age group). For the reported history, 73.7% of Chinese participants, 73.0% of Malays, and 63.6% of Indians reported having had varicella infection and/or vaccination. Overall, the sensitivity, specificity, positive and negative predictive values of a self-reported history of varicella for serologically confirmed immunity were 87.2%, 83.2%, 94.3% and 67.1% respectively. **Conclusions:** The prevalence of VZV antibodies in pre-enlistees to the Singapore Armed Forces (SAF) is high. Incidence of varicella in the SAF is on the wane, indicating an increase in herd immunity against VZV. A recalled history of varicella infection was also a good predictor of serological immunity and may be used for selection for vaccination.

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**Key words:** Antibody, Chickenpox, Cost-effective, Military, Seroprevalence, Varicella zoster virus

### Introduction

Varicella is an acute, self-limiting exanthematous disease characterised by a prolonged period of morbidity lasting up to 2 weeks.<sup>1</sup> Despite being mostly benign in healthy individuals, varicella infection can result in substantial loss of productivity. A study by Lee et al<sup>2</sup> estimated that the disease costs Singapore US\$11.8 million per annum, with the largest proportion attributable to days lost from work. Despite an overall decline in the national incidence of varicella since 1996, the average annual incidence of the disease in Singapore remains substantive, with 506 cases per 100,000 individuals<sup>3</sup> with occasional outbreaks. In 2004, reported cases of varicella increased by 32% compared with 2003, with 206 institutional outbreaks island-wide. Within the Singapore Armed Forces (SAF), varicella rates

have decreased over the years but outbreaks continue to contribute substantially to workdays lost. Knowledge of the seroprevalence of varicella within the SAF is important in developing vaccination strategies and other public health measures to minimise the healthcare and economic impact.

There have been many international surveys documenting the seroepidemiology of varicella in both temperate and tropical regions worldwide.<sup>1,4-11</sup> Studies have also shown that self-reported history of varicella may provide a reliable estimate for seroprevalence of infection.<sup>4,9,10</sup> However, there are few studies about the recent epidemiology of varicella in Singapore, including inter-ethnic differences among the major ethnic groups. In addition, no local studies have identified the reliability of self-reported history of infection. This study aims to determine the seroprevalence

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of varicella zoster virus (VZV) antibodies in the SAF sub-population, and the local reliability of self-reported history of infection. The latter is important as a possible proxy for seroprevalence to provide effective identification of candidates for vaccination.

## Materials and Methods

A cross-sectional study was conducted on pre-enlistees at the SAF Central Manpower Base Medical Classification Centre from September 2000 to October 2005. A systematic sample was obtained every 6 months during the main intake of recruits, where the first consecutive series of 200 pre-enlistees were selected during their pre-enlistment medical check-up.

To document the epidemiology of varicella, participants were provided with a questionnaire which included questions on demographics and clinical history, including the past history of chickenpox and age at infection. These results were compared with the recorded incidence of varicella in the SAF from 1991 to 2005. The incidence of varicella in the SAF was also compared with that of the national population.<sup>3</sup>

To determine the seroprevalence of infection, a 4-mL sample of blood was drawn from each subject for varicella IgG antibody level detection via enzyme-linked immunosorbent assay (ELISA). The ELISA test was performed at Quest Laboratories (Singapore), with the test sensitivity ranging from 86% to 97%, and the specificity from 82% to 99% in detecting antibodies after natural infection. Positive antibody serology titres (Low Positive: 1.10 to 2.00 EU/mL; Mid Positive: 2.01 to 2.92 EU/mL and High Positive: >2.93 EU/mL) were assumed to confer the subject with immunity to varicella, whilst negative (<0.90 EU/mL) and equivocal (0.91 to 1.09 EU/mL) serology titres were assumed to be reflective of non-immunity.

### Statistical Methodology

Sample size calculation was based on the level of agreement between self-reported history and seroprevalence of infection for the 3 major ethnic groups. Based on an estimated range of kappa statistic values of 0.2 to 0.8, and a standard error range of 0.007 to 0.011, a sample size of at least 100 per ethnic group was needed to achieve a level of significance of 0.05. The prevalence of Indians (the smallest of the 3 major ethnic groups) in Singapore is about 8.7%,<sup>12</sup> necessitating an overall sample of 1149 to achieve the ideal sample size. Our study assumed a sample size of 2000 to provide for greater accuracy.

Data from the questionnaire and the serological survey were analysed according to the history of varicella and seropositivity among the 3 major ethnic groups. Analyses were performed to determine differences among the 3

ethnic groups using chi-squared tests, analysis of variance (ANOVA), and the Scheffe test for post-hoc analysis of variance. To determine if the trends of varicella infection within the SAF and the nation are similar, the Chow test was used to ascertain if the slopes and intercepts of the respective linear regression equations are similar.

For the level of agreement between self-reported history and seroprevalence for infection, the self-reported history of varicella was compared to the serological results and the measure of agreement was determined by the kappa statistic. For this analysis, we excluded all who had prior varicella vaccination (through medical records or history), as vaccination would confound the serological results. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the self-reported history were also calculated.<sup>13</sup>

All statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) for Windows, version 13.0 (Chicago, IL, USA). Means are presented, with *P* values and/or 95% confidence intervals (CI) where appropriate.

To determine the cost-effectiveness of using self-reported history to identify candidates for vaccination, costs were calculated for 3 different strategies for an estimated 20,000 recruits – serology for all and vaccination for those with negative serology; vaccination of all with a negative history; and serology with those with a negative history and vaccination for those with negative serology. The 2006 cost to the SAF for a serological immunoglobulin test was S\$40, and the cost of varicella vaccination was S\$54.

## Results

A total of 2189 servicemen were identified for this study, with a participation rate of 100%. The demographics of the participants are shown in Table 1. All participants were male, with a median age of 18 years (range, 16 to 36). Of the study participants, 63.6% (n = 1392) were Chinese, 30.6% (671) were Malays, 5.03% (110) were Indians, and 0.73% (16) were of other ethnic groups.

The mean age of reported varicella infection was 9.8 years (range, 1 to 22), and was statistically similar across the 3 ethnic groups. From the study, 73.7% of Chinese participants, 73.0% of Malays, and 63.6% of Indians reported having had a history of either varicella or vaccination or both, but there was no statistical difference across the 3 ethnic groups (*P* = 0.09). Malays and Chinese participants displayed a higher incidence of positive varicella history at 71.7% and 70.8% respectively compared to Indians who had the lowest incidence at 58.2%. As shown in Table 2, statistically significant differences were found between Indians and Malays (*P* = 0.005), and Indians and Chinese (*P* = 0.007).

Table 1. Demographics, Clinical History, and Varicella Serology, Stratified by Ethnicity

Variable		Ethnic group		
		Chinese (n = 1392)	Malay (n = 671)	Indian (n = 110)
<b>Demographics</b>				
Age (y)	Mean (SD)	18.7 (1.6)	18.6 (1.3)	18.3 (1.3)
<b>History</b>				
Reported history of varicella	Yes	986 (70.8%)	481 (71.7%)	64 (58.2%)
	No	406 (29.2%)	190 (28.3%)	46 (41.8%)
Age at infection (y)	Mean (SD)	9.8 (3.9)	9.8 (3.5)	10.5 (3.2)
Reported history of vaccination	Yes	123 (8.8%)	67 (10.0%)	11 (10.0%)
	No	1269 (91.2%)	604 (90.0%)	99 (90.0%)
Treatment with acyclovir	Yes	69 (7.0%)	35 (7.3%)	2 (16.7%)
	No	917 (93.0%)	446 (92.7%)	61 (95.3%)
<b>Varicella serology</b>				
Mean titre (EU/mL)*		4.46†	4.05†	5.24†

\* Mean titre for those who tested positive for varicella IgG on ELISA

† Statistically significant ( $P < 0.05$ ) between Chinese and Malay, and Indian and Malay, by Scheffe test

Table 2. Reported History of Varicella Infection between Races

	Race	
	Chinese	Indian
Yes (%)	70.8	58.2
No (%)	29.2	41.8
	$P = 0.007$	
	Indian	Malay
Yes (%)	58.2	71.7
No (%)	41.8	28.3
	$P = 0.005$	
	Chinese	Malay
Yes (%)	70.8	71.7
No (%)	29.2	28.3
	$P = 0.717$	

Comparing the use of antivirals (Table 1), a higher proportion of Malay patients provided a history of treatment with acyclovir (7.3%) compared with Chinese (7.0%) and Indians (4.7%) ( $P = 0.517$ ). Forty-three participants (2%) reported 2 episodes of chickenpox.

From the serological tests, the mean antibody titre obtained among the seropositive participants was 4.36 EU/mL, as shown in Table 1. Malays had the lowest antibody titre (4.05 EU/mL), followed by Chinese (4.46 EU/mL), and Indians (5.24 EU/mL). The differences were significant between Malays and Chinese ( $P = 0.015$ ), and Malays and Indians ( $P = 0.001$ ).

The proportion of subjects who reported previous varicella

Table 3. Contingency Table of Recalled History of Varicella versus True Seroconversion

	Recalled history of varicella		Total	
	Negative	Positive		
Antibodies to varicella	Negative	395	80	475
	Positive	194	1318	1512
Total	589	1398	1987	

infection or immunisation remained fairly constant with a mean of 74.7%, and a low of 67.7% in September 2000, and a high of 80.2% in October 2004 (Fig. 1). From the ELISA serology tests, 76.0% of the 2189 samples assayed were seropositive, with 75.8% of those in the 16 years to 20 years age group possessing antibodies to VZV. Of all seropositive participants, 87.1% gave a history of varicella infection, while 9.1% gave a history of vaccination. 83.4% of the participants were tested to be immune to VZV in serology in October 2005, a 17.1% increase in immunity compared to those tested in September 2000.

Figure 2 shows the incidence of varicella in the SAF and the national population. The incidence of varicella in the SAF has been on a steady decline over the past 15 years. The decline in SAF varicella incidence is significantly larger than the decline in incidence in the general population ( $P < 0.01$ ).

Table 3 shows the recalled history versus serology to varicella. For the overall level of agreement between self-reported history and seroprevalence for infection, the Kappa statistic was 0.65 ( $P < 0.01$ ). The overall positive predictive

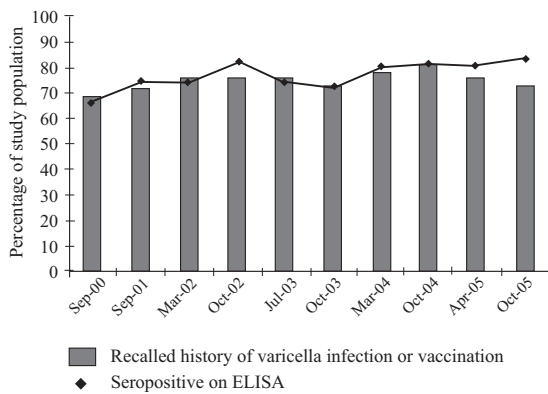


Fig. 1. Comparison of history of varicella or vaccination versus actual serologic immunity by ELISA from September 2000 to October 2005.

value (PPV) for serologic immunity given a history of chicken pox was 94.3% (Table 4). The overall negative predictive value (NPV) for the lack of serologic immunity given a negative history of chicken pox for non-immunity was 67.1%. The overall sensitivity and specificity of the recall history of varicella infection were observed to be 87.2% and 83.2% respectively.

The results were further stratified by race. The PPV of a history of chicken pox for serologic immunity was lower in Malays (92.4%) when compared to Indians (94.9%) and Chinese (95.2%) ( $P < 0.01$ ). The NPV of a history of chicken pox for serologic immunity was lowest in the Malay subpopulation (58.0%) and higher amongst the Indians (65.0%) and Chinese (72.1%). The sensitivity of recalled history for serologic immunity was lower amongst Indians (80.0%), compared to Malays (83.7%) and Chinese (89.4%). The specificity of recalled history for serologic immunity was lower amongst Malays (76.6%), compared to Chinese (85.9%) and Indians (89.7%).

From the cost-effectiveness analysis, serology for all and vaccination for those with negative serology will ensure 100% coverage, but cost S\$800,000 for screening and S\$259,200 for vaccination. Vaccination of all with a negative history will achieve 96% coverage at a cost of S\$319,000. Serology with those with a negative history and vaccination for those with negative serology will achieve 96% coverage at a cost of S\$236,000 for screening, and S\$105,000 for vaccination.

**Discussion**

The SAF constitutes a large pool of young adult male Singaporeans, a proportion of whom are susceptible to varicella infection. The overall seropositivity rate of 76.0% in our study is in contrast to a 1989 SAF study of 200 recruits that showed a 33% seropositivity to varicella (SAF, unpublished data). The incidence of varicella in the SAF has been on the decline over the past 15 years (Fig. 1), from

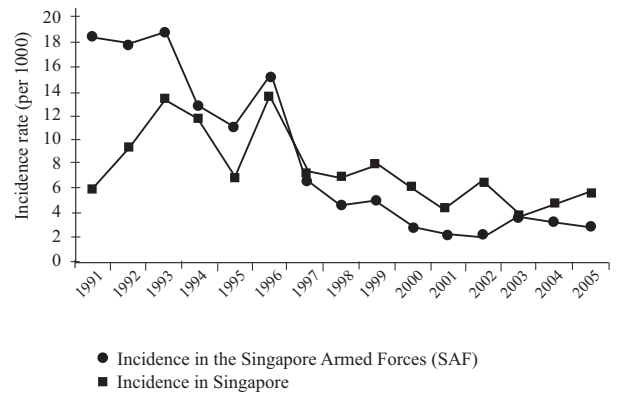


Fig. 2. Annual incidence of varicella in Singapore and the SAF from 1991 to 2005.\*

\*The mean age (in years) for varicella infection in Singapore and the SAF from 2002 to 2005 were as follows: the mean age was 16.3, 17.3, 16.7, 17.0 for Singapore in the respective years; and 21.9, 21.6, 21.8, 22.0 for the SAF in the respective years.

a high of 18.32 per 1000 persons in 1991 to a low of 2.62 per 1000 persons in 2005, an overall decline of 85.7%. This period also saw a shift in the national varicella burden towards the childhood age groups.<sup>3</sup> The increase in immunity in younger age groups could be due to an increased exposure to the virus and the greater use of varicella vaccination. SAF pre-enlistees would therefore have increasing immunity towards varicella, corresponding to the increase in seropositive rates and the declining incidence of varicella over this study’s five-year period.

A significantly smaller proportion of the Indians in the study population reported a history of varicella compared to the Chinese and Malays. This may reflect real differences in the incidence of varicella. Data from the Ministry of Health, Singapore, for the year 2004, indicate that Indians had a chickenpox incidence of 327.5 per 100,000 population, which was lower than the Chinese (481.2 per 100,000), and Malays (851.0 per 100,000).<sup>3</sup>

Malays who were seropositive were found to have a lower mean antibody titre than Chinese or Indians. The statistical significance remained after correction for possible confounders such as history of vaccination or history of treatment with acyclovir. The reason for this difference is unknown and should be subject to further research.

For the reliability of the reported history of varicella, the overall PPV of reported history for serological immunity among SAF participants (94.3%) was comparable to other studies that have documented an average PPV of 97.3% amongst military personnel<sup>14-16</sup> and 95.6% amongst healthcare workers.<sup>13,17,18</sup> The NPV from this study of 67.1% was higher compared to other studies, where the NPV ranged from 6%<sup>18</sup> to 44%.<sup>16</sup> The higher NPV may have been attributed to the comprehensive explanations of the questions in our self-administrated questionnaire.

Table 4. PPV, NPV, Specificity and Sensitivity of a Reported History of Varicella, Stratified by Race

Variable	Ethnic group			
	Overall	Chinese	Malay	Indian
PPV* (%)	94.3 (93.2, 95.2)	95.2 (94.0, 96.3)	92.4 (90.2, 94.3)	94.9 (87.5, 98.6)
NPV† (%)	67.1 (64.5, 69.3)	72.1 (68.9, 74.7)	58.0 (52.8, 62.4)	65.0 (54.1, 70.5)
Specificity‡ (%)	83.2 (80.0, 85.9)	85.9 (82.2, 89.1)	76.6 (69.8, 82.5)	89.7 (74.6, 97.2)
Sensitivity§ (%)	87.2 (86.2, 88.0)	89.4 (88.2, 90.4)	83.7 (81.7, 85.4)	80.0 (73.7, 83.1)

\* Positive predictive value: the probability that a person is seropositive to varicella zoster virus (VZV), given a positive answer on historical enquiry

† Negative predictive value: the probability that the person is seronegative to VZV, given a negative answer on historical enquiry

‡ The ability of a negative answer on historical enquiry to identify all subjects susceptible to VZV

§ The ability of a positive answer on historical enquiry to identify all subjects immune to VZV

95% confidence intervals for PPV, NPV, specificity and sensitivity are shown in parentheses

Another explanation could be the younger age of our study population, as shown by studies that have observed that the PPVs and NPVs were higher in younger persons.<sup>7,19</sup>

We also found that 2% of the respondents reported having had chickenpox twice and all had tested positive for varicella IgG. True second episodes of chickenpox are highly unlikely in immunocompetent adults<sup>20,21</sup> and a study among young military recruits in the United States concluded that genuine repeat infections of varicella are rare and that the reliability of history of previous infection was a possible confounder.<sup>14</sup>

The current high rates of immunity to VZV among study participants support existing SAF protocols of not providing blanket varicella vaccination for all incoming recruits. This is further supported by the larger decrease in varicella infections within the SAF compared to the general population. The high PPV of a history of previous chickenpox for actual immunity indicate that positive history can reliably distinguish those who are immune from those who are non-immune. This provides a cost-effective method for future selection of individuals for vaccination. The strategy of using a negative history only, or in combination with serological tests, reduces costs by S\$740,000 and S\$718,000 respectively, while maintaining coverage needed to achieve herd immunity.

One limitation of this study is that the results cannot be generalised to the entire population of Singapore. This is because the study only covers SAF enlistees, who are all young adult males. The study also excluded participants from other races due to the small numbers and the representation by a variety of races. Further studies should be conducted in the general population and in specific high-risk groups to determine if this is true for these populations.

## Conclusion

This study demonstrates that the prevalence of varicella

antibodies in pre-enlistees of the SAF is high. Varicella incidence in the SAF is on the wane, which may be a result of the increase in herd immunity amongst SAF enlistees. This is consistent with the national trend, which shows a decline in the incidence of varicella in the teenage and young adult age groups. For future varicella vaccination programmes within the SAF, recalled history of previous VZV infection or vaccination is a suitable and cost-effective proxy for seropositivity.

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