Prevalence of Nasopharyngeal Meningococcal Carriers among Newly Enlisted Military Recruits in Taiwan

Ding-Ping Liu[1], Yi-Chien Chr[2], Chia-Chi Chang[2], Shu-Man Yau[3], Zhe-Jie Yan[2]

[1]Vaccine Developing Center, Center for Disease Control, Department of Health

[2]Fourth Group, Center for Disease Control, Department of Health

[3]Laboratory Center, Center for Disease Control, Department of Health

Abstract

In this study, systemic sampling was done on newly enlisted military recruits in Taiwan between November and December of 2001. Specimens and questionnaires of 1,988 recruits were collected to comprehend the prevalence of meningococcus among males between 20 and 24 in the community. Results showed that the prevalence of meningococcal carriers was 2.3%. Most of the carriers had type B bacteria (53.3%), followed by type Y (17.4%), W135 (15.2%), non-typable (10.9%), and Z (2.2%). The pattern was similar to that of confirmed meningococcal cases in 2001, but the carrier rate and type distribution were significantly different from those in 1974.

In this study, ethnic group, career (transportation), and smoking were found to be the risk factors for meningococcal carriage. Among ethnic groups, the aborigines had the highest prevalence. This may be caused by their crowded living environments and smoking related to socioeconomic status. However, this may also related to a high prevalence of hereditary complement deficiency among aborigines.

Repeated examinations were performed on the original subjects and their contacts in camps having positive subjects. The results showed that a positive rate

was not significantly different from the first examination. Three weeks after recruitment, contacts had a significantly higher carrier rate than those of non-contacts. While the study was progressing, a confirmed case of meningococcal meningitis was found in Jing-Liou-Jie Camp, Yi-Lan. The case and contacts were then subjected to testing, and a positive rate of 22.7% was found, significantly higher than the positive rates from random sampling or of contacts of carriers, suggesting that the carrier rate would significantly rise around positive cases.

Introduction

Meningococcal diseases were first described by Vieusseux in 1805 (1). The pathogen, *Neisseria meningitidis*, was then isolated in 1887 (2). Humans are the only natural reservoir of *Neisseria meningitidis*. In foreign countries, 5-10% of healthy adults are asymptomatic carriers (3). The bacteria are distributed through nasopharyngeal secretions. Most people are only transient carriers, and protective antibodies are formed after the transient periods. However, in a few individuals *Neisseria meningitidis* can penetrate into the blood stream through the mucosa, leading to disease.

The clinical presentation of meningococcal diseases varies, including meningitis, bacteremia, sepsis, respiratory tract infection such as pneumonia, focal infection such as conjunctivitis, purulent pericarditis, etc., and hence meningococcal diseases are difficult to diagnosis and often misdiagnosed as other more common and milder diseases. Once infected, meningitis would develop in 50% of patients (5). The symptoms of meningococcal meningitis – sudden-onset of headache, fever, neck stiffness – are similar to other bacterial meningitis. *Neisseria meningitidis* can be isolated from the blood in three out of four patients, but only 5-20% of them will develop meningococcal sepsis. Once septicemia occurs, patients will develop precipitous onset of fever, rash or pupurae, often accompanied by sudden-onset hypotension, acute adrenal hemorrhage (Waterhouse-Friderichsen syndrome), and multiple organ failure with a very high mortality rate (5, 6).

In 2001, there were significant rises in reported and confirmed cases of meningococcal meningitis, and several outbreaks in foreign countries also occurred concomitantly. In May, a passenger developed the disease while traveling on an airplane, and protocols were developed for similar situations (7). A high school student in Ohio, USA, also died of the disease, and community-wide vaccination was then administrated. In June, a case of sudden death of a soldier occurred in Cheng-Kong Mountain, leading to considerable media attention. However, related local studies and data were lacking, and there has been a significant difference in epidemiology from that between the 1960s and the 1990s. Since foreign studies may not be applicable in our country, this study of nasopharyngeal carrier status of a group of people with two risk factors (young male and newly enlisted military recruit) was the beginning of efforts to reveal the epidemiological status of meningococcus in our country.

Materials and Methods

Population

Males between age 20 and 24 in Taiwan.

Subjects and Sampling

I. Sampling numbers: 2,000 individuals were sampled with at least 1,800 valid samplings.

II. Echelon selection: since meningococcal infection has seasonal distribution

(5), to avoid bias caused by seasons, three echelons between November and December (echelon #1885 – 1887) were selected to complete the sampling.

III. Camp selection: since newly enlisted recruits are called up to nearby training camps, to include recruits from every county, multiple training camps around the country were selected. In this study, 12 training camps were included, including Jing-Liou-Jie in Yi-Lan, Kuan-Si in Sing-Jr, Do-Huang-Ping in Tou-Feng, Cheng-Kong Mountain in Tai-Chung, Chi-Ding and Chung-Keng in Chia-Yi, Guan-Tian, Da-Nei, and Sing-Chung in Tai-nan, Wei-Wu-Ying in Kaohsiung, Tai-Ping in Tai-Dong, and Bei-Pu in Hua-Lien.

IV. Sampling numbers in each county: Sampling numbers in each county were proportional to the ratio of its population aged between 20 and 24 to that of the whole country of the same age groups.

V. Principles of sampling of each echelon/county/camp: To avoid overloading and quality degradation, for each echelon, samples in each laboratory were limited to no more than 200, and for each camp, samples were limited to no more than 130 in each echelon.

VI. Sampling of newly enlisted recruits from the same camp in the same county: since the time of check-in and the allotment of echelon were random, we sampled those who came first.

Education and Training

Before sampling, education and training were given. The content included the goal of the study, techniques involved, processes of sampling, practice dealing with nasopharyngeal swab sampling, plating, and drills. Seventy-nine military officers and soldiers and forty-one staff from the CDC attended the training and education program.

Sampling and Laboratory Examination

I. Immediately after their check-in and before any group activity occurred, sampling was done with nasopharyngeal swabs by military officers.

II. After the head was fixed, a nasopharyngeal swab was slowly moved into one nostril until the nasopharynx was touched. Slow rotation was done for 20 to 30 seconds before rapid withdrawal of the swab.

III. The swab was directly inoculated onto a modified Thayer-Martin (MTM) medium according to standardized procedures, and the plate was kept at room temperature. Samples were sent to laboratories in the same day of collection and incubated in incubators containing 5-10% CO_2.

IV. laboratory examination was done by the Laboratory Center of the CDC and laboratories of its branches. Samples were limited to no more than 200 in a single laboratory to avoid degradation of quality.

V. The standardized examination protocol is illustrated in Fig. 1.

Questionnaire

Possible risk factors of carrier status in the literature were assessed by questionnaires. Recruits sampled must have their questionnaire completed. Specialists were required to give explanations for each question, and incomplete questionnaires were required to be completed.

Analysis of Questionnaires

Questionnaires were first transformed into EXCEL files, and then analyzed by SPSS 8.0 and Epi Info 6.04.

Secondary Sampling

After the sampling of echelon #1885 (Nov. 6) was completed. Secondary nasopharyngeal sampling was done in those camps with positive carriers three weeks later (Nov. 26 – 27) on recruits sampled in the first examination in order to compare effects of group living on the rate of nasopharyngeal carriage. Simple questionnaires were filled in to ascertain general heath status and usage of antibiotics among subjects.

Sampling of Contacts

Contacts are defined as living together with the case. According to the situation in the camps, recruits of the same company were viewed as contacts. After the sampling of echelon #1885 was completed, recruits of companies having positive subjects were sampled three weeks later(Nov. 26 - 27). Simple questionnaires were then completed to ascertain general heath status and usage of antibiotics among subjects.

Results

In this study, systemic sampling was done on fresh military recruits of echelon # 1885, 1886, and 1887 in 2001. 1,998 valid questionnaires and samples were gathered. Secondary sampling and sampling of contacts were done 3 weeks later in camps having the nine positive cases of echelon # 1885. 71 recruits were only involved in the secondary sampling, 236 were contacts and subjects of the second sampling, and 600 were only contacts.

I. Carrier rate of meningococcus and serological distribution

Among the 1,988 samples, 46 showed positive meningococcal reaction, and the rate of carriage was 2.3%. As shown in table 1, among the 46 positive cases, 23 (50%) were from northern Taiwan, 12 (26.1%) from central Taiwan, 9 (19.6) from southern Taiwan, and 2 (4.3%) from eastern Taiwan. No positive case was found in recruits from Pong-Hu, King-Men, and Ma-Tsu islands. Positive rates were 2. 7% (23/861), 2.4% (12/496), 1.6% (9/565), and 3.8% (2/53) in northern, central, southern, and eastern Taiwan, respectively. Type B accounted for 53.3%, type Y 17.4%, and type W135 15.2% among positive cases. Geographically, Type B followed by type Y was dominant in northern and central Taiwan, while type B followed by type W135 was dominant in southern and eastern Taiwan.

Type B was also dominant among ethnic groups, except one case of W135 and Y in the mountain aboriginal group.

II. Risk factors of meningococcal carriers

1. Variables of socioeconomic demography

As shown in Table 2, the average age of negative cases was significantly higher than that of positive cases. There was no significant difference among geographic areas. Ethnic group was another significant risk factor ($p < 0.0001$). Positive rates were lower in recruits of descent of Ming-Nan (1.7%) or other provinces (1.8%). Recruits of Hakka descent had a relatively high positive rate (5.6%). Plains and mountain aboriginals had the highest positive rate (12.5%). Significant Kai-square test result ($p = 0.002$) was obtained when ethnic groups were divided into aboriginal and non-aboriginal groups. The risk of recruits of aboriginal descent was 6.63 times higher than that of recruits of the non-aboriginal group (statistically significant, CI = 2.56 ~ 17.26).

Taking their careers three month before recruitment into consideration, those who had had a career in transportation had the highest positive rate (7.5%). Positive rate was 2.1% among previous workers, and 0.7% among previous students.

2. Other risk factors

As shown in table 3, the risk of positive results in smokers was 2.92 times higher than that in non-smokers (statistically significant, 95% CI = 1.56 ~ 5.51).

Logistic regression analyses were also done, and age, ethnic group, previous career, smoking, symptoms of common cold/cough, and medications within two weeks were taken into consideration. The risk among aborigine descents was 4.39 times higher than other groups (95% CI = 1.42 ~ 13.56). The risk among recruits having previous career in transportation was 3.14 times higher than that among recruits having other careers (95% CI = 0.90 ~ 10.94). The risk among smokers using less than one pack per day was 1.39 times higher than that among non-smoking recruits (95% CI = 0.53 ~ 3.68). The risk among smokers using one to two packs per day was 3.22 times higher than that among non-smoking recruits (95% CI = 1.60 ~ 6.47). Ethnic group, previous career, and smoking (one to two packs per day) were significant risk factors (Table 4).

III. Results of secondary examinations in camps having positive carriers

Secondary examinations were performed done in three camps (Guan-Si, Chi-Ding, and Guan-Tien) having the nine positive carriers of echelon # 1885 (Nov. 6). 337 soldiers tested in the first sampling were re-examined three weeks later (Nov. 26 – 27). Excluding those who left the army or refused to take the test, 307 soldiers were tested. Among them, 14 (4.6%) were positive,. a two-fold increase when compared with the positive rate before (2.6%).

We then used Fisher’s exact test to analyze the variables involved in conversion from negative to positive carrier status after three weeks (12 cases were selected together with 287 control cases). The results showed that contact with positive carriers and smoking were significant variables (Table

5).

IV. Results of sampling of contacts

Nine positive cases were identified in echelon #1885 (carrier rate 1.3%). 836 contacts in the six companies having positive cases were sampled after three weeks of recruitment (Nov. 26 – 27). Among them, 60 were positive, with a carrier rate of 7.2%, 5 times higher than that of the echelon and three times higher than that of the whole population.

Examination results of contacts and non-contacts three weeks after recruitment were compared. Among the 307 newly enlisted recruits, 71 non-contacts were all negative in the second test, but 14 of the 236 contacts were positive (5.9%,). The difference is statistically significant by Fisher's exact test ($p = 0.046$).

Discussion

I. Comparison with previous studies

The carrier rate in this study was 2.3%, representing nasopharyngeal meningococcal carrier status of males aged from 20 to 24 in Taiwan. Foreign studies have showed carrier rates around 5 – 10% (3, 9). When compared to similar foreign studies, newly enlisted recruits have been shown to have a carrier rate around 40% (10, 11), significantly higher than our results. As to the incidence of meningococcal diseases, considering a non-endemic area such as the United States for example, the incidence is around 0.9 to 1.5 cases per one hundred thousand people and has been stable since 1960s (12). Although cases increased significantly in 2001 in Taiwan, the incidence was still around 0.15 cases pee one hundred thousand people, about one tenth that in other countries.

In the study done by Yang et al. in 1974-75 (13), newly enlisted recruits were examined in the four seasons in various counties in Taiwan. the carrier rates were 10.4% to 19.1% with an average of 13.7%. The rates in winter and spring were lower than summer and fall, and were higher than the rate obtained in our study (2.3%). Two endemic periods occurred in Taiwan between 1919 and 1926, and between 1933 and 1946. 300 cases were reported annually at that time, with a peak of more than 600 per year. Incidence decreased significantly after 1946. In 1971, less than 5 cases were reported annually. Between 1981 and 1991, only the rare case was reported (8). The incidence increased after 1991, and in 2001, confirmed cases were up to 43. Although the incidence was lower in 1974, the carrier rate was higher.

There is no absolute relationship between carrier rate and incidence. Hence, WHO does not recommend predicting epidemics by carrier rate surveillance (14). However, the carrier rate would be significantly higher among contacts of cases than non-contacts once an outbreak occurred (15). The carrier rates in endemic countries are also higher than those in non-endemic countries. Exposure to meningococcus and immunity against it might be the causes of low carrier rate as compared with foreign countries or with the past.

II. Comparison of types of carriage

46 strains of meningococcus were isolated in our study. Among them, type B was the most often found (53.3%), followed by type Y (17.4%), W135 (15.2%), non-typable (10.9%), and type Z (2.2%). In the study done by Yang et al. in 1974 to 1975, the most common strain isolated was type A (43.3%), followed by type X (38.1%), type C (12.2%), and type B (10.2%). Hence in the past 25 years, type of carriage has changed significantly. Type A and X

have disappeared, and type B has become the dominant type. Type Y, W135, and Z were not isolated in the study in 1974. Among the six typable strains out of the seven cases of meningococcal meningitis which occurred in the army in 1991, 3 (50%) were type B, two (33.3%) were W135, and one (16.7%) was type Y, similar to the result of our study. It is obvious that different types of strains have been introduced and produce significant changes in ecology between meningococcal strains.

Antibiotics sensitivity tests have also been performed on the 19 strains isolated. 89.5% was sensitive to penicillin, and 10.5% had intermediate resistance. With the exception of one strain which was resistant to rifampicin, all strains were sensitive to common antibiotics (16).

Carrier rate, types, and case number of current and previous studies having been taken into consideration, increased immunity induced in the two high incidence periods may account for the low incidence and carrier rate thereafter. Besides the balance between human immunity and bacteria, effects of antibiotics usage on the ecology of bacteria must be taken into consideration. The health environment in Taiwan has improved gradually since the 1960s, and antibiotics are used more prevalently. This has not only improved the treatment for bacterial infection, but also decreased the carrier rate of meningococcus. After a long period of low carrier rate and low incidence, community immunity has started to decrease gradually. International travel and intercommunication also introduced new strains of bacteria, leading to increased incidence. However, studies are lacking and further investigations are required to validate this point of view.

III. Geographic distribution of carriers

In the current study, recruits from eastern Taiwan have the highest carrier rate, and recruits from southern Taiwan have the lowest. This might be related to the higher proportion of aborigines in the population in eastern Taiwan. However, Chi-square test does not reveal a significant relationship between geographic regions and carrier rates. Taking confirmed cases of meningococcus in 1991 into consideration, 21 cases occurred in northern Taiwan (incidence: 2.2 cases per million people), 13 in central Taiwan (2.3 cases per million people), 8 in southern Taiwan (1.3 cases per million people), 1 in eastern Taiwan (1.7 cases per million people). No case was reported on off-shore islands. The trend was similar to the positive rate in each geographic region. The incidence of confirmed cases of meningococcus in persons between 20 to 24 years of age in 1991, was 7.1, 3.8, and 6.8 per million population in northern, central, and southern Taiwan, respectively. No cases were reported in eastern Taiwan. This trend did not correspond with the results of the current study. Hence, carrier rate is unrelated to disease incidence, and WHO does not recommend making predictions based on carrier status in the community (14).

VI. Risk factors of meningococcus carriage

Cross analyses were done on the test results of nasopharyngeal swabs and the questionnaires. Significant risk factors include: ethnic group (aborigine), career (transportation), smoking (one to two packs per day). Our results are similar to those obtained in other studies. Ethnic group might be closely related to socioeconomic status. However, hereditary complement deficiency is found in certain aborigines, especially the Tai-Ya tribe(17). The complement deficiency has been known to be an important factor of meningococcal diseases. Whether the deficiency is related to carrier status requires further investigation.

V. Effects of military life on carrier rate

The results of secondary examination three weeks after recruitment showed that the carrier rate increased two fold after living in the army for three weeks, this, however, is not statistically significant. The results of Anderson and Yang et al. (10, 13) showed that the carrier rate started to increase one week after recruitment, and peaked in two months. Because fresh recruits were only kept in training camps for four weeks and dispersed thereafter, third and forth examinations are inapplicable. However, results of the second examination were similar to other studies. From another viewpoint, the one-month long training system might be better for meningococcal prevention than the previous ten-week long training.

The results of secondary examination also showed that 71 non-contacts were still negative in the second examination. However, 14 of the 236 contacts were positive, with a positive rate of 5.9%. This results were statistically significant by Chi-square test, suggesting that recruits belonging to the same companies as positive carriers, that is, close contacts, have significantly higher carrier rates than non-contacts. This result once again explains why meningococcal outbreaks may easily occurr in training camps. Since certain recruits are carriers already, carrier status is easily spread by crowded living condition, and hence increases the opportunity and morbidity of sensitive subjects exposed to pathogens.

While the current study was progressing, one confirmed case of meningococcal disease was reported in the Jing-Lieu-Jie Camp. 110 contacts were sampled, and a carrier rate of 22.7% was found, significantly higher than the 4.6% found in the secondary examination three weeks after recruitment and the 7.2% found in contacts of positive carriers. A case-induced significant increase in carrier rate has also been found in other studies (15).

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Fig. 1 Protocol of meningococcal laboratory examination

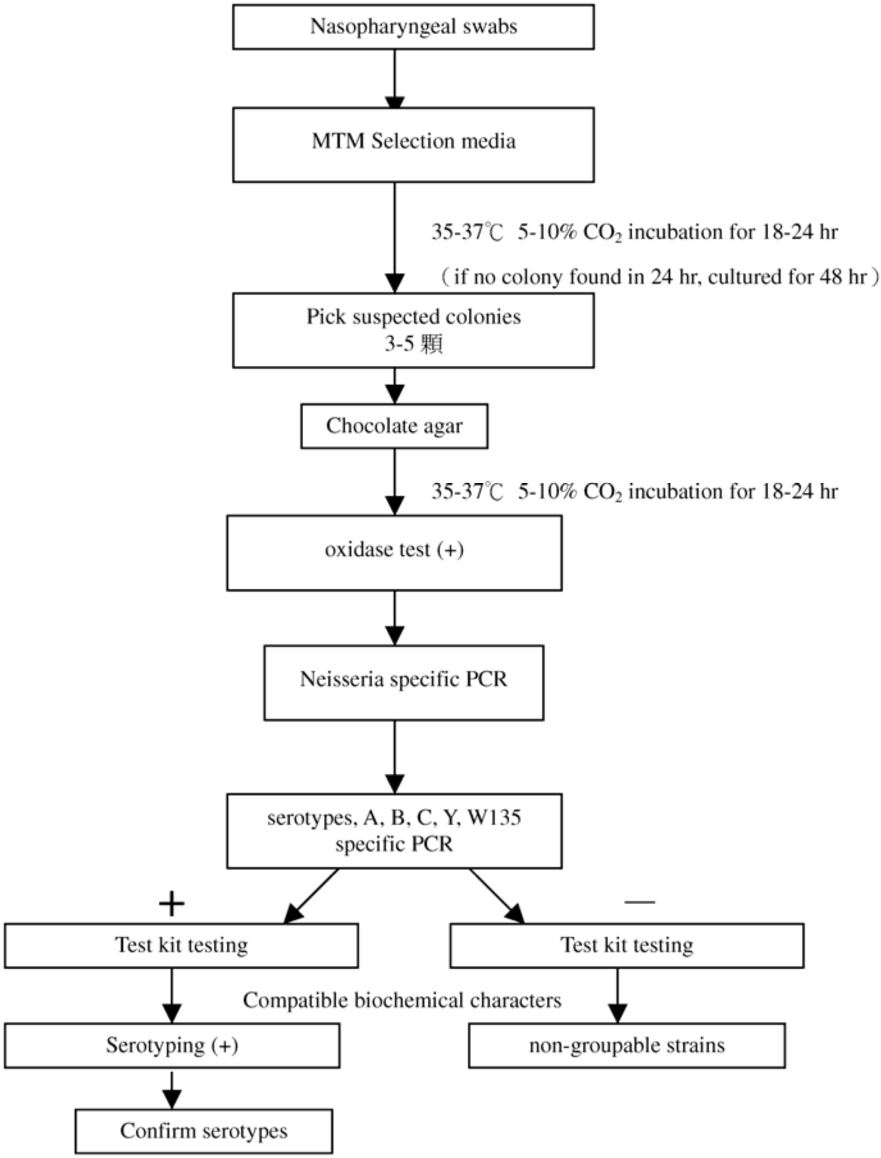

Table 1 Distribution of serological types in ethnic groups and geographic areas

		B		W135		Y		Z		Non-typable	
		Number	(%)	Number	(%)	Number	(%)	Number	(%)	Number	(%)
Area	Northern	14	60.90%	2	8.70%	4	17.40%	1	4.30%	Number	(%)
	Central	6	50.00%	1	8.30%	3	25.00%	0	0.00%	2	8.70%
	Southern	4	44.40%	3	33.30%	1	11.10%	0	0.00%	2	16.70%
	Eastern	1	50.00%	1	50.00%	0	0.00%	0	0.00%	1	11.10%
	Total	25	54.30%	7	15.20%	8	17.40%	1	2.20%	0	0.00%
										5	10.90%
Ethnic groups	Ming-Nan	16	61.50%	3	11.50%	4	15.40%	0	0.00%		
	Others	2	66.70%	0	0.00%	1	33.30%	0	0.00%	3	11.50%
	Hakka	5	41.70%	2	16.70%	2	16.70%	1	8.30%	0	0.00%
	Plains aboriginal	2	66.70%	1	33.30%	0	0.00%	0	0.00%	2	16.70%
	Mountain aboriginal	0	0.00%	1	50.00%	1	50.00%	0	0.00%	0	0.00%
	Total	25	54.30%	7	15.20%	8	17.40%	1	2.20%	0	0.00%

Table 2 Socioeconomic Variables

		Number of subjects		Positive cases	(%)	P values
Age		21.5	(1.3)*	21.0	(1.1)*	0.005
Region	Northern	861		23	2.7%	0.641
	Central	496		12	2.4%	
	Southern	565		9	1.6%	
	Eastern	53		2	3.8%	
	Off-shore islands	9		0	0.0%	
Ethnic group I	Ming-Nan	1550		26	1.7%	< 0.0001
	Other provinces	165		3	1.8%	
	Hakka	214		12	5.6%	
	Plains aboriginal	24		3	12.5%	
	Mountain aboriginal	16		2	12.5%	
	Others	15		0	0.0%	
Ethnic group II	Non-aboriginal	1944		41	2.1%	0.002
	Aboriginal	40		5	12.5%	
Previous career	Student	569		4	0.7%	0.005
	Transportation	40		3	7.5%	
	Workers	143		3	2.1%	
	Others	1201		33	2.7%	

* average (SD)

Table 3 Risk factors of Positive meningococcal Examination

		Number of subjects	Positive cases	(%)	Odds Ratio	(95% C.I.)*
Smoking	No	1079	14	1.3%	2.92	(1.56~5.51)
	Yes	865	32	3.7%		
	Cessation	39	0	0.0%		
Degree of smoking	0	1079	14	1.3%		
	Less than one pack per day	294	7	2.4%	1.86	(0.76~4.51)
	One to two packs Per day	446	23	5.2%	4.14	(2.13~8.02)
	More than two Packs per day	125	2	1.6%	1.24	(0~4.94)
Cold/cough symptoms within the past two weeks	No	971	22	2.3%	1.05	(0.59~1.87)
	Yes	1010	24	2.4%		
Medications within the past two weeks	No	1215	30	2.5%	0.84	(0.46~1.53)
	Yes	770	16	2.1%		
History of familial hereditary diseases	No/don't know	1963	46	2.3%	-	
	Yes	12	0	0.0%		
Travel abroad (self or family members) within the past three months	No	1764	37	2.1%	1.96	(0.95~4.06)
	Yes	223	9	4.0%		
Chronic diseases	No	1059	23	2.2%	1.12	(0.62~2.00)
	Yes	908	22	2.4%		

*95%confidence interval

Table 4 Logistic regression analysis of risk factors

Variables	β	S. E.	Odds ratio	95% C.I.*	
Age	-0.36	0.15	0.70	0.52	0.94
Ethnic group[1]	1.48	0.58	4.39	1.42	13.56
Previous carrer[2]	1.14	0.64	3.14	0.90	10.94
Smoking, no more than one pack/d[3]	0.33	0.50	1.39	0.53	3.68
Smoking, one to two packs/d[4]	1.17	0.36	3.22	1.60	6.47
Smoking, more than two packs/d[5]	0.09	0.77	1.10	0.24	4.92
Symptoms of cold/cough[6]	0.02	0.34	1.02	0.53	1.98
Medication within two weeks[7]	-0.16	0.35	0.85	0.42	1.70

*95%confidence interval

1 Comparison between aboriginal and non-aboriginal

2 comparison between transportation and other careers

3 Comparison with non-smokers

4 Comparison with non-smokers

5 Comparison with non-smokers

6 Comparison between those with and without symptoms

7 Comparison between those with and without medications in past two weeks

Table 5 Factors affecting test results three weeks after recruitment

		Total subjects	Positive cases	(%)	p Value
Contacts	Yes	228	12	5.3%	0.036
	No	71	0	0.0%	
Ethnic group	Aborigine	3	0	0.0%	0.884
	Non-aborigine	296	12	4.1%	
Smoking	No	117	8	6.8%	0.044
	Yes	173	3	1.7%	
	Quitted	8	1	12.5%	
Symptoms of cold/cough within past two weeks (first time)	Yes	144	7	4.9%	0.679
	No	154	5	3.2%	
Symptoms of cold/cough within past two weeks (second time)	Yes	280	12	4.3%	0.449
	No	19	0	0.0%	
Medications within past two weeks (first time)	Yes	112	3	2.7%	0.279
	No	187	9	4.8%	
Medications within past two weeks (second time)	Yes	224	7	3.1%	0.155
	No	75	5	6.7%	
Familial congenital diseases	Yes	5	0	0.0%	0.811
	No/don’t know	289	12	4.2%	
Traveling abroad (self or family members) within past three months	Yes	257	10	3.9%	0.522
	No	42	2	4.8%	
Chronic diseases	Yes	147	4	2.7%	0.390
	No	149	8	5.4%	