

## Protection from natural infection after live influenza virus immunization in an open population\*

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### SUMMARY

Live attenuated influenza vaccine containing the recombinant of A/Victoria/3/75 with A/PR/8/34 virus was administered to healthy adults in a field trial aimed at evaluating protection provided by immunization. The study was designed to measure the effect of vaccination on absenteeism from respiratory disease during a natural influenza epidemic. A total of 2115 male employees of the public transport service of Rome volunteered to participate in the trial, 1050 and 1065 receiving vaccine and placebo respectively, in a randomized blind fashion. Vaccination procedure was completed by the end of December 1976. A small-sized outbreak of influenza, due to a viral strain antigenically homologous to the vaccine, occurred during the month of February 1977. Analysis of absenteeism data, classified according to medical certificate, indicated that morbidity from respiratory disease was reduced in vaccinees compared with controls during the epidemic month; the rate of increase of morbidity compared with that of the preceding month was then three times lower in vaccinees than in controls and the difference in absenteeism between the two groups greatly exceeded the ordinary fluctuation that was observed during non-epidemic periods.

### INTRODUCTION

Many studies proved the safety of attenuated influenza virus vaccines (McDonald *et al.* 1962; Alexandrova *et al.* 1970), and demonstrated a low incidence of local or systemic side-effects and a practically non relevant rate of transmission to susceptible contacts (Murphy *et al.* 1972; Beare *et al.* 1973; Petrilli *et al.* 1975; Ikić *et al.* 1977). The efficacy of attenuated influenza vaccines has been demonstrated by immunological assays showing high rates of serum and nasal antibody rises as a result of immunization (Prévost *et al.* 1973; Minor *et al.* 1975; Rubin *et al.* 1976; Crifò *et al.* 1978) and a high degree of protection against infection with virus challenge (Rytel *et al.* 1975; Donikian, McKee & Greene, 1977; Rytel *et al.*

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1977). Several double blind studies on healthy target populations, in which antibody titration was used to demonstrate both the immunogenicity of the vaccine and the occurrence of natural infection, also showed that a high protection rate against natural influenza can be achieved from vaccination with attenuated virus (Mackenzie *et al.* 1975; Noble *et al.* 1975).

The purpose of this paper is to present the results of a field trial performed in Rome with an attenuated strain of current serotype A/Victoria/3/75 during the winter 1976-7. The study was intended to evaluate the protective effect of the vaccine on a group of healthy workers heavily exposed to respiratory infections because of their work. The purpose of the study was to check whether vaccination could reduce absenteeism due to respiratory causes when an influenza A serotype similar to the vaccine strain would be prevalent and other respiratory agents would also be active. The serological control of immunogenicity of the vaccine was not performed, and the evaluation of the efficacy of the vaccine was based on the assumption that protection conferred by immunization would result in an appreciable reduction of absenteeism during the epidemic due to influenza. The recognition of epidemic influenza periods was based on surveillance techniques consisting mainly of virus isolation, seroepidemiology and epidemic excess mortality, according to a previously described epidemiological base-line (Ragona *et al.* 1978).

#### MATERIALS AND METHODS

##### *Study population*

A total of 2115 healthy male employees of the public transport service, between the ages of 20 and 60 years, volunteered to participate in the trial; a complete explanation of the nature and benefits of the study had been previously given to participants in order to obtain consent.

Each participant was given a single dose of either placebo or vaccine in a randomized blind fashion; 1050 subjects received vaccine and 1065 served as controls, receiving placebo. The vaccination started on 21 December and was completed by 31 December 1976.

##### *Vaccination*

RIT 4050, a recombinant of influenza A/Puerto Rico/8/34 (H0N1) with A/Victoria/3/75 (H3N2), was kindly provided by Recherche et Industrie Thérapeutiques, Belgium; 10<sup>7</sup> EID<sub>50</sub> were given to the vaccinees by dropping 0.25 ml into each nostril. The placebo consisted of the diluent used for the vaccine and was identical in appearance to the vaccine. Vaccine administration was well tolerated by vaccinees. No clinically significant response was observed during the first five days after vaccination. Moderate malaise, rhinorrhoea, hoarseness or cough, were reported by 1.3% of vaccinees.

##### *Epidemiological evaluation*

Vaccine efficacy was evaluated by calculating the percentage incidence of absences due to respiratory disease among the vaccinated volunteers and the

control group. Only disease lasting more than 3 days was considered. This was done to increase the possibility of appreciating protection due to vaccination by limiting the amount of minor pathological respiratory events, through the exclusion of mild infections causing periods of absence shorter than those usually due to influenza. Absenteeism data, classified according to medical certificate, were individually collected during the months from November 1976 to March 1977. In December 1976 absenteeism was only checked from the 1st to the 20th day of the month, in order to avoid taking into account absences occurring during the period of vaccine administration in the holiday season. Absences from respiratory disease were also checked 1 year before vaccination, during the month of February 1975, when influenza virus similar to A/Victoria/3/75 first circulated in Rome. This was done in order to control how the two groups of candidates to the assay behaved in terms of absenteeism during a period of influenza prevalence.

#### *Influenza surveillance in Rome*

Analysis of mortality due to respiratory causes and to all causes has been performed on the Roman population for several years; the method of recognizing excess mortality and of defining epidemic periods has been described in detail elsewhere (Serfling, 1963; Ragona *et al.* 1978). Influenza virus prevalence was determined by prospective culturing of virus of patients with respiratory illnesses presenting to care facilities in Rome (Ragona *et al.* 1978). Serological tests were carried out from December 1976 to May 1977, in order to evaluate the spread of infection in Rome. Random groups of serum specimens collected at monthly intervals from an average number of 350 healthy adults of the general population, were checked by haemagglutination-inhibition (HAI) test for the presence of antibody to influenza A/Victoria/3/75. The viral antigen was kindly supplied by the W.H.O. Italian Influenza Centre. A standard micro-method employing 4 units of antigen was used (Sever, 1962).

### RESULTS

Virology, seroepidemiology and mortality data indicated an appreciable epidemic influenza activity in the urban area in February 1977. Influenza A Victoria was isolated in specimens from persons living in Rome at the end of January 1977. Respiratory illnesses were positive for a virus strain similar to A/Victoria/3/75 during February. Both illnesses and influenza isolations declined rapidly, but a low prevalence of influenza infection continued through the first 2 weeks of March. Random samples of adult serum specimens in December 1976 showed that, before the epidemic, almost 35% had detectable HAI antibody to influenza A Victoria virus. After the epidemic, in May 1977, about 58% had HAI antibody to this virus and the comparison of incidence with pre-epidemic sample suggests that more than 20% of the population had been infected with the virus during the epidemic (Table 1).

An excess of deaths occurred in Rome during the 4-week period corresponding

Table 1. *Haemagglutination-inhibition (HAI) antibody to influenza A/Victoria/3/75 virus in random groups of serum specimens collected before and after the A Victoria epidemic from healthy adults in Rome*

Month	No. tested	% with 10*	HAI titre						
			Cumulative % with						
			10	20	40	80	160	320	640
Dec. 1976	350	65.1	34.9	16.6	8.2	4.7	2.6	1.2	0.6
May 1977	350	42.3	57.7	39.5	28.4	16.8	9.0	4.6	1.4

\* Reciprocal of titre.

Table 2. *Morbidity from respiratory diseases in vaccinated (V) and non-vaccinated (P) volunteers during cold-weather months, 1976-1977*

Month	Morbidity rate (%)		Days of absence (%)	
	V	P	V	P
Nov.	5.14	4.41	46.38	37.93
Dec.*	3.23	4.13	28.00	36.05
Jan.	6.76	5.54	56.00	49.29
Feb.	9.62	12.30	78.28	105.16
Mar.	5.62	6.95	40.00	42.91

\* Data refer to days 1-20.

to February 1977. The rate of excess mortality attributed to respiratory causes and to all causes was 54.1 and 118.2 per million respectively, during this period. No other peak of excess mortality occurred during the year. Absenteeism from respiratory disease recorded among the placebo group of volunteers participating in the trial also showed a significant peak in February 1977 ( $P < 0.001$ ) with an estimated influenza morbidity rate  $\geq 6\%$ ; at that time the number of illnesses and of absences was twice as much as that observed in November to January and in March (Table 2).

Morbidity due to respiratory disease showed the largest difference, nearly 2.7% ( $P < 0.05$ ), between the two groups of volunteers during the A Victoria epidemic in February 1977; the rate of increase of morbidity, compared with January, was significantly greater ( $P < 0.001$ ) in the control group (from 5.54% to 12.30% morbidity, i.e. some 122% increase), than in vaccinees (from 6.76% to 9.62% morbidity, i.e. some 42% increase). No other significant difference of morbidity between the two groups was noted during the surveillance period.

However, in several months the two groups significantly differed in absenteeism ( $P < 0.001$ ); in November and in December 1976 either group prevailed on the other one by 8.5 and 8.0% days of absence. In February 1977 the incidence of absences of the vaccinees was nearly 27% lower than that of the control group (Table 2). The rate of increase of absenteeism in February, compared with January, was also significantly lower ( $P < 0.001$ ) in vaccinees (from 56% to 78% absen-

teism, i.e. some 39% increase) than in controls (from 49% to 105%, i.e. some 113% increase).

The study on absenteeism during the preceding period of prevalence of A Victoria infection in February 1976 showed that before the trial the two groups of candidates had an equal morbidity rate and that the difference in terms of absenteeism was at that time only 7.3%.

#### DISCUSSION

Immunogenicity of the live influenza virus vaccine used in this trial was consistently demonstrated in studies showing high incidence of serum and secretory antibody rise following vaccination (Lobmann, Delem & Jovanovic, 1977; Kunz & Hofmann, 1977; Nicholson, Lawford & Tyrrell, 1977; Crifò *et al.* 1978). The experimental design of this trial, instead, required the occurrence of epidemic influenza in order to verify any protection due to immunization.

Surveillance methods indicated that a small-sized influenza epidemic due to A Victoria virus occurred in February 1977, 1 month after the vaccination was completed. The epidemic produced a limited peak of excess mortality in Rome, and determined a respiratory disease excess morbidity rate of 6% among the participants who served as control to the study. Even though the impact of the epidemic into the study population was rather limited, the epidemic itself occurring during cold weather period when respiratory agents other than influenza virus were also active, the data on absenteeism indicate that morbidity from respiratory infection was lower in vaccinees than in controls during the influenza epidemic. The rate of increase of morbidity during the epidemic month compared with the preceding month, was three times lower in vaccinees than in controls, and the difference in absenteeism between vaccinees and controls greatly exceeded the ordinary fluctuation that was observed during the months preceding the epidemic.

Since the influenza virus strain which caused the epidemic was antigenically homologous to the live attenuated vaccine strain, it can be assumed that the reduction in absenteeism presented by vaccinees was due to immunization.

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