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MASS IMMUNIZATION OF CHILDREN IN IRAN WITH LIVE ATTENUATED SUGIYAMA MEASLES VIRUS ADAPTED TO CALF KIDNEY CELL CULTURES(*)

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Summary: Sugiyama live attenuated measles vaccine produced in bovine renal cells was administered during pilot studies and a mass immunization programme in Iran. Clinical observations in several of the vaccinees revealed a mild fever lasting 3–4 days which was accompanied by the appearance of a morbilliform rash which faded in 2 days. There was no significant difference in reactions from subcutaneous or intramuscular routes of inoculation. The mean of days of pyrexia was greater in plateau than in mountainous areas. The occurrence of a morbilliform rash seemed to be higher in plateau than in mountainous regions especially when vaccine was inoculated intramuscularly. When a double-dose of vaccine was administered, the adverse symptoms were more severe. The seroconversion was 95 to 100% when specific antibodies were titrated by seroneutralization or by haemagglutination-inhibition tests.

INTRODUCTION

The efficacy and the innocuity of a live measles vaccine has now been well established in Iran and large scale immunization of children is a routine programme of the Iranian Ministry of Health. Since 1965, the measles vaccines used in this country were imported from abroad and were all derived from the Edmonston measles virus (Edmonston B.,Schwarz and Beckenham Strains). Attempts were then made to locally produce a live vaccine which met the minimum requirements formulated by the WHO Expert Committee [10]. The Sugiyama Strain of measles virus adapted by Matumoto *et al.* [2, 3] to bovine renal cells and used in Japan as a live attenuated vaccine [4, 5] was selected. Before applying this vaccine to large populations, 272 children were immunized with vaccine received from the Chiba Serum Institute in Japan. Clinical and

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serological responses of children were observed and have been described in a Previous note [7]. A good serological conversion was recorded for suseptible children immunized with Sugiyama live attenuated vaccine. The severity of post-vaccinal reactions was similar to that produced by Schwarz Strain of measles virus. Following these observations, a lot of 600,000 doses of Sugiyama live measles vaccine was made by this Institute early in 1970 and was approved by the Iranian Ministry of Health to be used in a mass vaccination campaign. This report describes the clinical and serological findings of this field trial.

MATERIALS AND METHODS

Preparation of the vaccine: The Sugiyama vaccine strain of measles virus, BK–78, was kindly supplied by Dr S. Hashizume. In order to produce enough seed, this virus was passed three times in primary calf kidney cells. The vaccine was produced in the same cell system according to the procedure developed by Chiba Serum Institute. Fifty micrograms/ml of Kanamycin and Neomycin were added to all media. The control of sterility, safety and efficacy was done according to the WHO requirements for live measles vaccine [10].

The vaccine was packaged in 10-dose vials and freeze dried. These were sealed under high vacuum and stored at –20°C. It was packed in dry ice and air-transported to the main cities from where it was sent in ice by Health Office Personnel to the various missions in the rural districts of the country.

Vaccination programme: There were three field studies, each of which included a number of susceptible children who were between 9 months and 7 years and had no histories of measles. The vaccine was used subcutaneously or intramuscularly. In order to investigate the margin of safety of the Sugiyama attenuated strain at its present passage level, some children were immunized with a double-dose of vaccine. In each trial, blood samples were collected prior to vaccination and 30 days after vaccination on paper disks as described previously [6].

Study 1. The first study population was children of personnel of the Razi Institute. Follow-up examinations were performed by the medical staff of the Institute. The purpose of this study was to reassess the safety of the vaccine and its seroconversion rate. No controls were included.

Study 2. The site of this study was a privately owned textile factory located in a suburb of Karaj, a district 30 miles west of Teheran. In the Karaj area, 2,013 susceptible children were immunized with the same lot of vaccine in March 1970. Some children were selected in this trial for follow-up examination and serological studies. A total of 354 children ranging in age from 9 months to 5 years without a history of measles constituted this study group. They were

vaccinated subcutaneously with a double-dose of vaccine and were under the care of physicians and attendant personnel of the Health Department of the Teheran region.

Study 3. This was performed in several villages in a suburb of Esfahan, a central city and province of Iran. A total of 1,018 healthy children aged 9 months to 7 years and evenly distributed between the two sexes were selected for this field trial. None had a history of measles. The main purpose of this study was to compare the reactogeny of the vaccine in children living in plateau or in mountainous areas. An evaluation of differences in clinical reactions following subcutaneous or intramuscular inoculation was another purpose of this study. A total of 619 children were chosen to represent the mountainous group of the Lenjan region which is southwest of Esfahan and comprised the four villages of Bagh-Bahadoran, Tcharmihan, Kartchekan and Nowgaran. The area has a maximum elevation of 2,350 meters. A total of 353 children of the village of Dowlatabad in the Bolkhar region north of Esfahan were selected as representatives of plateau area. This area is about 1,200 meters above sea level. In both regions, a group of children was inoculated with sterile saline as a placebo.

Surveillance. The names and addresses, as well as the previous medical histories of all children, were recorded on special card before vaccination. In Study 3, the children in each area were randomly divided into three groups and received either measles vaccine subcutaneously or intramuscularly or sterile saline. A standard card was used to record the date of vaccination, daily temperature and significant reactions. Mild respiratory diseases were common in all villages under study. Daily temperature and clinical reactions were recorded between 7 and 21 days post vaccination by public health personnel. All children with severe reactions were visited by physicians of the respective regions who communicated their findings to responsible officials of the field trial.

Serological examination: Paired blood specimens of all children under study were kept in envelopes in a dry and cool place before testing. Each pair of sera from Studies 1 and 2 were tested by seroneutralization (SN) and haemagglutination-inhibition (HI) tests as described [7]. The sera of the 3rd Study were examined only by the HI test.

RESULTS

Study 1. The general symptoms observed in this group were a mild pyrexia 7 to 9 days post-vaccination which was accompanied in some cases by a morbilliform rash. A summary of these reactions is given in Table 1. Of the 12 vaccinees with 2000 TC/ID50 of virus, 4(33.4%) developed a fever not exceeding 39.2°C and 5 (41.6%) developed a morbilliform rash on the face, thorax and abdomen. Cases of bronchopneumonia occured in two children of 10 and 12

months inoculated with a double-dose of vaccine. Both children were treated with penicilline and recovered in 3 days. Children of this group immunized with a single dose of vaccine (1000 TC/ID50) showed less reactions. Of 28 children, only 5 (17.8%) had a rise of temperature between 37.4 to 39.2°C and 4 (14.3%) had a pronounced rash which disappeared after 2 days. A few cases of Koplik spots were also recorded in vaccinees. The most common complaint of the mothers was the restlessness of children at night during the period of fever and rash. Convulsions, otitis or other complications were not observed by attending physicians. The serological findings are shown in Table 1. Seven children had natural measles antibodies and were not included in this study.

TABLE 1

Summary of finding in children of study group 1 inoculated with Sugiyama strain live mealses vaccine.

Dose of vaccine TCID 50/Dose	Inocu-lated	Ob-served	Feber 37.4–39.20 (%)	Rash (%)	Koplik (%)	Respira-tory disorder (%)	Serological response						
							Sera tested	Natural anti-body	No. sero-logical response	S.N. titre		H.I. titre	
										Mean titres *log 2*	Sero-conver-sion (%)	Mean titre *log 2*	Sero-conver-sion (%)
2000	14	12	4 (33.4)	5 (11.6)	2 (16.6)	2 (16.6)	13	0	0	8.0	100	7.6	100
1000	35	28	5 (17.8)	4 (14.3)	5 (17.8)	—	33	7	0	7.5	100	6.7	100

A rise of measles antibodies was found in all children vaccinated with a single or double dose of vaccine. While the 100% conversion rate was recorded for all children under study, the mean antibody titre was 0.5 to 0.9 *log* 2 greater in those immunized with double dose of vaccine.

Study 2. The age and sex distribution of this group is illustrated in Table 2. The variations of fever are also shown in Table 3. A mild fever (37–38° C) was observed in 158 of 211 vaccinees (55.4%); 48 children (16.8%) manifested a rise of temperature between 38–39°C. The onset of fever was 8–11 days after inoculation and the mean duration of pyrexia was 4.22 days (Table 4). The variation of the mean duration and maximum fever based on age group is reflected in Table 5 and is not significant. The fever subsided when the rash, if

TABLE 2

Age and sex distribution of inoculated children.

Total inoculated	Sex		Age (years)			
	Female	Male	9–12 months	> 1–2	> 2–5	> 5
354	165	189	62	133	121	38

TABLE 3

Number of children developing fever.

No. of children clinically assessed	Fever		
	37–38 °C	38–39 °C	> 39 °C
	158 (55.4%)	48 (16.8%)	5 (1.75%)

TABLE 4

Distribution of children by incubation period of fever.

Onset (mean days)	8.11
Mean duration of pyrexia (days)	4.22
Mean duration of maximum temperature (days)	2.6

TABLE 5

Pyrexia variation according to age group.

Age	Mean duration (days)	Mean maximum fever (°C)
9–12 months	4.14	37.9
> 1–2 years	4.4	37.6
> 2–5 years	4.7	37.2
> 5 years	4.0	37.4

present, was first observed. Of the 285 vaccinated children, 118 (41%) had a moderate morbilliform rash and 57 (20%) a marked rash (Table 6). The rash consisted of macular and sometimes papular areas and occuring behind the ears, on the face, thorax and abdomen. In most instances, the rash was much less extensive than that found in natural measles. Koplik spots were observed on 17 vaccinees (6%). A slight cough or coryza were also seen in many of subjects (Table 6). Anxiety was the main disorder observed by mothers during the

TABLE 6

Major symptoms observed in 285 children vaccinated with double doses of live Sugiyama strain, measles vaccine.

Symptom	Number	Percentage
Total inoculated	354	—
Total observed	285	80
Total reacting	175	61.4
Slight or moderate rash	118	41
Marked rash	57	20
Koplik spots	17	6
Coryza	153	54

TABLE 7

Serological findings, 1 month after immunization of children, study group 2 with double-dose live Sugiyama measles vaccine.

Type of test	No. of sera tested	No. positive before immunization	No. response	Titres (*log 2*) 4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10	Mean titre	Seroconversion %
S.N	221	18	5	11	11	15	19	33	23	44	20	14	12	15	–	7.1	97.70
H.I			11	7	7	8	15	32	18	40	23	29	8	13	3	7.5	95.02

TABLE 8

Age and sex distribution of inoculated children in mountainous area.

Route of inoculation	Inoculated	Sex: Female	Sex: Male	Age: 9–12 months	Age: > 1–2 years	Age: > 2–5 years	Age: > 5 years
Sc.*	229	110	119	52	75	91	11
Im.**	210	92	118	43	61	99	77
Placebo	180	103	77	42	60	71	7

* Sc. = Subcutaneous
** Im. = Intramuscular

TABLE 9

Age and sex distribution of inoculated children in plateau area.

Route of inoculation	Inoculated	Sex: Female	Sex: Male	Age: 9–12 months	Age: > 1–2 years	Age: > 2–5 years	Age: > 5 years
Sc.	127	56	68	32	47	41	7
Im.	118	55	63	34	37	42	5
Placebo	108	44	64	34	35	37	2

second week after vaccination. Other signs such as otitis, diarrhoea or convulsions were not recorded during the period of follow-up studies. The results of serological studies are summarized in Table 7. In sera tested by the SN test, the

TABLE 10

Intensity and percentage of febrile reaction in children in mountainous area.

Route of inoculation	Inoculated	37.4–38 °C	38–39 °C	> 39 °C
Sc.	229	103 (44.9%)	28 (12.2%)	5 (2.1%)
Im.	210	89 (42.3%)	35 (16.6%)	10 (4.7%)
Placebo	180	57 (31.6%)	12 (6.6%)	1 (0.5%)

TABLE 11

Intensity and percentage of febrile reaction in children in plateau area.

Route of inoculation	Inoculated	37.4–38°C	38–39°C	>39°C
Sc.	127	31 (24.4%)	16 (12.5%)	5 (3.9%)
Im.	118	28 (23.7%)	17 (14.4%)	7 (5.9%)
Placebo	108	17 (15.7%)	6 (5.5%)	2 (1.8%)

TABLE 12

Pyrexia variations according to age group, altitude and route of vaccine administration.

Route of inoculation	Region	9–12 month			>1–2 years			>2–5 years			>5 years		
		M.D. days	M.M.F. (°C)	M. days	M.D. days	M.M.F. (°C)	M.I. days	M.D. days	M.M.F. (°C)	M.I. days	M.D. days	M.M.F. (°C)	M.I. days
Sc.	M.	3.3	37.9	7.4	3	37.8	8.3	3.0	37.8	7.8	1	37.8	8
	P.	3.1	38.2	8.9	2.8	38.2	8.9	2.0	37.8	8.2	1	38.2	9
Im.	M.	2.5	38	8.1	2.6	38.0	7.7	3.2	38	7.4	5	38	6.3
	P.	2.9	38.3	8.1	2.9	38.4	9.0	1.7	38.1	9.3	1	37.5	10
Placebo	M.	2.2	37.3	8.2	3.1	38.0	6.8	2.3	37.7	7.5	1	37.5	12
	P.	1.6	38.1	8.7	1.1	37.9	7.4	2.1	38.0	8.3	–	–	–

M. = Mountainous region M.D. = Mean duration M.M.F. = Mean maximum fever
P. = Plateau region M.I. = Mean incubation

TABLE 13

Clinical findings in children under 2 years in mountainous area.

Route of inoculation	Inoculated	Temperature after vaccination (°C) 37.4–38	38–39	>39	Cough or coryza	Koplik spots	Rash	Diarrhoea	Otitis	Convulsion	Conjunctivitis	Pharyngitis
Sc.	127	55 43.3%	16 12.5%	3 2.3%	30 23.6%	7 5.5%	72 56.6%	44 34.5%	27 21.2%	—	54 42.5%	1 0.7%
Im.	104	49 47.1%	16 15.3%	6 5.7%	14 13.4%	2 1.9%	59 56.7%	40 38.4%	17 16.3%	—	39 37.5%	—
Placebo	102	34 33.3%	9 8.8%	1 0.9%	10 9.8%	—	23 22.5%	26 25.4%	19 18.6%	—	27 26.4%	1 0.9%

seroconversion was 97.7% with a mean titre of 7.1 *log* 2. With the HI test, a seroconversion of 95.02% with a mean titre of 7.5 *log* 2 was recorded.

Study 3. The age and sex distribution of the children living in the plateau and in mountainous areas is given in Tables 8 and 9. According to the data obtained, there were no significant differences between the plateau or mountainous regions in the degree of maximum temperatures observed (Tables 10 and 11) and between the subcutaneous or intramuscular routes of inoculation. The mean of the incubation days of pyrexia in the plateau area was, however, longer

TABLE 14

Clinical findings in children under two years in plateau area.

Route of inoculation	Inoculated	Temperature after vaccination (°C) 37.4–38	38–39	> 39	Cough or coryza	Koplik spots	Rash	Diarrhoea	Otitis	Convulsion	Conjunctivitis	Pharyngitis
Sc.	79	22 27.8%	14 17.7%	5 6.3%	34 43%	1 1.2%	32 40.5%	16 20.2%	15 18.9%	1 1.2%	26 32.9%	3 3.7%
Im.	71	17 23.9%	13 18.3%	6 8.4%	32 45%	—	41 57.7%	20 28.1%	13 18.3%	—	26 36.6%	6 8.4%
Placebo	69	13 18.8%	4 5.7%	1 1.4%	21 30.4%	—	10 14.4%	12 17.3%	14 20.2%	—	19 27.5%	—

TABLE 15

Clinical findings in children over 2 years in mountainous area.

Route of inoculation	Inoculated	Temperature after vaccination (°C) 37.4–38	38–39	> 39	Cough or coryza	Koplik	Rash	Diarrhoea	Otitis	Convulsion	Conjunctivitis	Pharyngitis
Sc.	102	49 48%	12 11.7%	2 1.9%	11 10.7%	—	41 40.1%	11 10.7%	1 0.9%	—	27 26.4%	—
Im.	106	40 37.7%	19 17.9%	4 3.7%	11 10.3%	2 1.8%	45 42.4%	19 17.9%	5 4.7%	—	26 24.5%	1 0.9%
Placebo	78	23 29.4%	3 3.8%	—	5 6.4%	—	8 10.2%	12 15.3%	1 1.2%	—	17 21.7%	1 1.1%

TABLE 16

Clinical findings in children over 2 years in plateau area.

Route of inoculation	Inoculated	Temperature after vaccination (°C) 37–38	38–39	> 39	Cough or coryza	Koplik	Rash	Diarrhoea	Otitis	Convulsion	Conjunctivitis	Pharyngitis.
Sc.	48	9 18.7%	2 4.1%	—	15 13.2%	—	11 22.9%	7 14.5%	3 6.2%	1 2%	10 20.8%	—
Im.	47	13 37.6%	2 4.2%	1 2.1%	8 17%	2 4.2%	15 31.9%	3 6.3%	2 4.2%	—	12 25.5%	—
Placebo	39	6 15.3%	2 5.1%	1 2.5%	11 28.2%	—	3 7.6%	7 17.9%	4 10.2%	—	6 15.3%	—

TABLE 17

Results of measles H.I. antibody titres obtained on post-immunization sera from populations of different altitudes, inoculated subcutaneously or intramuscularly.

Region	Group	No. of sera	Natural immunity	No. response	No. conversion %	H.I mean titre (*log 2*)
Mountain	Im.	126	1	6	95.05	7.54
	Sc.	115	3	5	95.53	7.49
	Placebo	81	2	79	—	—
Plateau	Im.	69	5	3	95.31	7.36
	Sc.	61	2	2	96.61	7.78
	Placebo	50	3	47	—	—

and the occurence of morbilliform rash seemed to be more in the plateau area than in the mountainous region especially when the vaccine was inoculated intramuscularly (Tables 12 to 16). The other symptoms were not significantly different in both groups. The conversion rate for subjects inoculated subcutaneously or intramuscularly and living in plateau or mountainous regions was between 95 to 100% and the mean titre of HI antibodies was identical (Table 17).

DISCUSSION

A safe and potent measles vaccine is of special interest in Iran where the mortality resulting from the disease is high. The mortality is estimated to be at least 10,000 per year. A mass immunization programme in the rural areas of the country was organized in 1965. The high cost of imported vaccines was one of the reasons which justified local production of measles vaccine. There are few specifications for the production of live attenuated measles vaccine. Primary monkey kidney cells and chick embryo fibroblasts are the main host cell systems used. Because of the high risk of extraneous viruses in monkey renal cultures and due to a heavy contamination of local poultry industry with avian leukosis, it was decided to produce the Japanese Sugiyama live attenuated measles vaccine developed by Matumoto *et al.* [4]. The cell system used for production of this vaccine was a monolayer of primary calf kidney cells obtained by trypsinization of kidney tissue of newborn healthy calves and the cultivation of dispersed cells in a proper growth medium in Roux bottles. One of the advantages of this cell system for growth of measles virus is that known human pathogens or latent or oncogenic viruses have not yet been isolated from it. The safety of Sugiyama measles vaccine has been shown previously [7] but observations on large populations were needed to evaluate if cases with severe symptoms following administration of this vaccine may be observed such as those frequently observed when natural measles has affected poor nutritional subjects. Double-doses of vaccine containing at least 2000 TC/ID50 of virus were administered to some children in order to evaluate possible side reactions which could occur following vaccinal over-dosage. From data presented in this report, one can conclude that fever or other post-vaccinal symptoms are mild and acceptable to the public.

The average incubation period and the mean duration of maximum temperature seem to have a close relationship with the amount of virus inoculated. While the average incubation time was 18.5, 13.4, 11.1 and 9.11 days following inoculation of 1, 10, 400 or 500 TC/ ID50 of virus [4, 7, 8], this period was reduced to 8.11 days when 2000 TC/ID50 was administered. The mean duration of maximum temperature which was 2.7 days when 500 TC/ID50 was administered [7] was increased to 4.22 days following inoculation of 2000 TC/ID50 of virus (Table 4). It is also interesting to note that in the plateau area

there was a longer incubation period following administration of vaccine with shorter duration of fever and more skin rashes as compared to the symptoms observed in vaccinees in mountainous areas. This agrees with the findings of Nafyci *et al.* [1]. The average maximum fever was not significantly different in various altitudes. The route of inoculation apparently had no effect on the nature and severity of responses and the virus showed the same degree of attenuation when tested by either route. The attenuation of Sugiyama strain of measles virus after 82 subcultures in bovine renal cells seems to be satisfactory since at its present level of passage this strain possesses all requirements of reactogeny and immunogeny needed for an attenuated strain of measles virus. In the earlier passages, the Sugiyama virus produced more symptoms when fewer viral particles were inoculated. Matumoto *et al.* [4] have observed that after 27 passages in bovine kidney cells 1 TC/ID50 produced delayed fever and rash in susceptible children. They further demonstrated that if 10 TC/ ID50 of virus were administered, about 70% of children showed a fever of 37.9–40.1 after 8–13 days which lasted 2–4 days. Approximately 65% of the seronegative children had a morbilliform rash 12–16 days after inoculation of the vaccine. The mean duration was 3 days. A seroconversion of 75–85% was noticed following immunization with 10 TC/ ID50 of virus. The data reflected in this report indicated that when 1000 TC/ID50 of Sugiyama virus at the 82nd subculture in bovine renal cells was inoculated into susceptible children, mild fever and rash spots were observed in about 40% of vaccinees which did not produce respiratory or other complications. Following this pilot study, a mass immunization programme was conducted and 600,000 doses of the same batch of vaccine was administered by Iranian Health Corps Personnel in rural regions of the country where natural infection is associated with a high mortality. The records of clinical responses suggest that the results are satisfactory. Severe symptoms necessitating hospitalization of children have not been observed. The seroconversion rate of 95 to 100% recorded in this report demonstrates the specific immunity obtained following administration of live Sugiyama vaccine.

Based on the above considerations and in accordance with the findings of the Japan Measles Vaccine Research Commission [8], we may conclude that the Sugiyama live measles vaccine is a further attenuated vaccine which can safely be used on a large scale basis without gamma globulin or prior administration of killed vaccine.

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