

**EXCESSIVE ATTENUATION OF MEASLES VIRUS  
AS A POSSIBLE CAUSE OF FAILURE IN MEASLES  
IMMUNIZATION**

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**Summary**

In order to evaluate the effectiveness of 3 further attenuated measles vaccines, *i.e.* AIK-HDC, heat-treated AIK-HDC and Edmonston-Zagreb-HDC, they were injected into 743 seronegative children aged 7-72 months. Statistically significant reductions in the seroconversion rates were recorded for heat-treated AIK-HDC as well as for Edmonston-Zagreb-HDC vaccines. Seroconversion rates in all 3 groups of vaccinees were lower for children 7-11 months old. The difference in immunogenicity is attributed to the lower antigenicity of measles viruses treated by heat or successive passages.

## Introduction

Administration of live measles vaccine has been a common practice in Iran since 1965 [30]. Primary investigations in this country have shown a high rate of fever and other severe clinical reactions following vaccination with measles vaccine, Sugiyama strain, adapted in baby calf kidney cells [19, 24,25]. Although mass campaigns in rural areas of Iran during 1970-1977 were highly successful and the disease has since been virtually under control in those areas, frequent occurrence of postvaccinal reactions in urban private clinics, as well as the side effects of the vaccine, threaten the further use of locally manufactured measles vaccine.

The introduction of a further attenuated vaccine (the AIK strain [16, 17] in chicken fibroblasts, locally adapted to the human diploid cells (HDC) MRC-5 [26, 28]) increased the acceptability of the home-made vaccine in private practice. So far, about far, about 15 million doses of this vaccine have been administered in this country. Pediatricians and practitioners using it in private clinics have expressed satisfaction, since post-vaccinal reactions are mild and severe side effects have not thus far been reported. In order to improve the stability of this vaccine, we tried, in a recent study, to isolate a variant of AIK-HDC progeny to be more heat-resistant. Two experimental batches of vaccines were manufactured with the new variant. In a field trial, routine AIK-HDC and a heat-stable vaccine derived from AIK-HDC and a third further attenuated measles vaccine also produced in our laboratory using the Edmonston-Zagreb strain were applied. The results of this comparative field trial are reflected in this report.

## MATERIALS AND METHODS

### Vaccine A.

A further attenuated live measles vaccine, AIK-HDC lot 771, was used in this field trial. Ten dose-vials of lyophilized vaccine contained

4.87 log<sub>10</sub> of virus. The contents of each vial were suspended in 5 ml of distilled water; 0.5 ml of diluted vaccine was inoculated subcutaneously. The titre of the vaccine kept for 7 days at 37°C dropped to 4.25 log<sub>10</sub> TCID<sub>50</sub>.

#### Vaccine B.

One hundred ml of seed material of AIK-HDC, with a titre of 4.5 Log<sub>10</sub> TCID<sub>50</sub>/ml, were heated for 15 min at 50°C. The titre of the seed in Vero cells after heating dropped to 2.5 log<sub>10</sub> TCID<sub>50</sub>. This suspension was subcultured (0.1 TCID<sub>50</sub>) in several Roux bottles of HDC-MRC5, as described in a previous report [26]. Six days later, the virus was harvested (titre: 4.5 log<sub>10</sub> TCID<sub>50</sub>/ml); 100 ml of the harvested virus were first centrifuged 2000 rpm/min for 20 min and then heated at 50°C for 15 min. The titre of heated virus in Vero cell culture dropped to 2.75 log<sub>10</sub>/ml. It was again subcultured as before in Roux bottles containing MRC-5 cells. This operation was repeated 4 times. At the-end of operation with this seed virus, two batches of experimental vaccines (lot 771/AIK-HDC 50/4/A and lot 771/AIK-HDC/H 50/4/B) were produced. Ten dose-vials of the lyophilized vaccines contained 4.85 and 3.87 log<sub>10</sub> TCID<sub>50</sub> seven days after heating at 37°C, respectively. In the present field trial, vials of lot 771/AIK-HDC/H 50/4/A were used as B Vaccine.

#### Vaccine C: Edmonston-Zagreb-HDC vaccine.

Originally, this strain was attenuated by Enders [12] in primary culture of human kidney (24 passages) and human amnion cells (28 passages). Musser [29] subcultured the attenuated strain of Enders in chicken fibroblast cells (CFB) 22 times and in primary dog kidney cells 15 times. The strain was finally adapted by Ilic *et al.* [15] to HDC(WI-38) and subcultured 19 times in this cell system. We isolated this strain

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CFB = chicken fibroblast (cells).  
GMT = geometric mean titre.  
HA = haemagglutinin.

HDC = human diploid cell.  
HI = haemagglutination inhibition.  
NA = neutralizing antibody.

(IKIC Edmonston-Zagreb) from a batch of vaccine imported by the Ministry of Public Health of the Islamic Republic of Iran, in HDC (MRC-5). After 4 passages in this cell culture, we produced an experimental batch of vaccine, lot n<sup>o</sup> 791 Edmonston-Zagreb/HDC/5. The titre of 10 dose-vials of this vaccine was  $5.0 \log_{10} \text{TCID}_{50}$  after lyophilization and  $2.75 \log_{10} \text{TCID}_{50}$  when samples of vaccine were kept for 7 days at 37°C. This vaccine was used as vaccine C in the present field trial.

### **Study population.**

Rajai-Shahr (ex-Dowlat Abad) and Islam-Shahr, with about 150,000 and 50,000 inhabitants, respectively, are two working-class communities of the east suburban residential area of Tehran. This field trial was conducted by the Department of Preventive Medicine of the eastern region of Tehran. No cases of measles had been reported in these areas during the previous 12 months and children – according to their parents – had not been vaccinated against measles.

### **Surveillance.**

Before vaccination, the names and addresses of all children were recorded on a special card by a mobile team of health workers headed by one or two physicians. Each working day, children were randomly divided into three groups and received one of the three measles vaccine A, B or C. Mobile teams from the Ministry of Public Health were equipped with a proper cold-chain system and vaccines were diluted with chilled sterile distilled water and used within a short time after rehydration. Mild respiratory and gastrointestinal diseases were common in the area under study. Daily temperature and clinical reactions were recorded between 7 and 21 days postvaccination by health workers in charge of field trials. Physicians were available to visit children if severe reactions occurred.

### **Serological examination.**

In order to evaluate the antibody response of vaccination, blood samples were collected immediately before inoculation of vaccine and 6

weeks later. The blood was collected from finger pricks using paper disks as described previously [23]. The blood samples were kept at  $-20^{\circ}\text{C}$  before use. As previously noted, 0.16 ml of blood was needed on the filter paper in order to cover the surface of a square measuring 3 X 3 cm.

#### **Serology.**

The blood was eluted from each square in 0.8 ml of saline the evening before the tests were to be run. Elution was carried out in test tubes, with the square soaked overnight at  $4^{\circ}\text{C}$ . It was arbitrarily accepted that each blood sample would contain 50% cells and 50% serum; hence, the initial dilution was 1/10. To remove non-specific inhibitors, the diluted sample was mixed with 25% acid-washed kaolin in PBS pH 7.2, shaken for 20 min at room temperature and centrifuged at 2000 rpm/min for 15 min. The clear supernatant was used as 1/20 treated serum.

#### **Serological method.**

Haemagglutinin (HA) was produced in our laboratory according to the Norrby technique [31]. Measles antibody was measured by the haemagglutination inhibition (HI) test using 4 units of HA antigen. Positive and negative sera were introduced in each test.

## **RESULTS**

#### **Study of residual maternal antibodies in 3-12 month old children.**

This survey conducted in Tehran city [27] showed that about one-third of children at 3-4 months of age possessed detectable amount of maternal antibodies. The average of the positive rate dropped to 8.6% at 5-12 months (table I).

**Tab. I. Pattern of maternal antibodies in  
3-12-month old Iranian children (1977)**

Age (months)	Nb tested	Positive maternal antibodies	
		Nb	%
3	22	8	36
4	53	18	34
5	44	5	12
6	41	3	7
7	49	7	14
8	38	1	3
9	42	2	5
10	42	4	10
11	13	1	8
12	20	2	10
Total	289	25	8.6

(5-12 months)

**Clinical findings.**

The clinical follow-up was organized 7 days after vaccination for a period of 3 weeks, by a team of physicians and health workers from the Department of Preventive Medicine of the eastern region of Tehran. Daily home visits were performed during which axillary temperature, presence or absence of rashes, otitis, coryza, Koplik spots or any other clinical abnormalities were recorded. Fever and rash occurred mainly 8 to 15 days after vaccination; moderate fever, lasting on average 1.5 days, was observed in about 50 to 60% of children, regardless of the type of vaccine used. Vaccine A produced more discrete and morbilliform rash than vaccines B and C ( $P < 0.05$  table II). The incidence of illness (otitis and coryza) was equal in all 3 groups. Koplik sign and convulsions were not observed. On the whole, illness following vaccination was mild and no child was reported as being seriously ill.

TABLE II. — Febrile reaction and clinical responses in serum-negative children.

	Vaccine A (%)	Vaccine B (%)	Vaccine C (%)
Fever (37.5-39°C)	59	48	55
Rash	14	7	7
Otitis	22	23	15
Cough or coryza	30	25	27
Koplik sign	0	0	0
Convulsions	0	0	0

#### Initial measles immunity status of the child population.

As previously mentioned, blood samples were collected from all children at the beginning of the study (*i.e.*, Just before injection of vaccines A, B or C) and again 6 weeks later. Table III gives measles seropositive rates in different age groups. While it was not possible to detect residual maternal antibodies in 99% of children under one year of age, HI antibody was found in about 15% of older children due to natural measles infection or following measles immunization.

TABLE III. — Initial measles immunity status of child population.

Age (months)	Nb	HI antibody		% Susceptible
		negative	positive	
7-11	102	101	1	99
12-72	292	248	44	85

HI-antibody titre  $\geq 1/20$ .

Positive numbers indicate persons with a history of measles or prior measles vaccination.

## Measles HI antibody study.

In table IV, measles HI antibody titres are analysed in terms of the type of vaccine used and the age of the vaccinees.

The seroconversion rates – at a titre of  $\geq 1/20$  – varied, for the age group 7-11 months, between 35 and 63 % and, for the age group 12-72 months, between 50 and 91%. It is known that the HI test is relatively insensitive compared with seroneutralization, ELISA or haemolysis in gel [11], and we found neutralizing antibody (NA) in some sera in which HI antibody was not detected. It is also worth mentioning that, for most investigators who used the HI test, the titre of  $\geq 1/10$  was accepted as positive, while in our study, the titre of  $\geq 1/20$  was considered positive; therefore, many sera which we believed to be free of HI antibody may contain detectable amounts of HI at a titre of  $\geq 1/10$  or lower. Similarly, Bass [4] showed that two-thirds of sera that would have been stated as being negative if tested at  $\geq 1/10$  were, in fact, positive.

Tab. IV. Post-vaccination measles immunity status  
of vaccinated children.

Measles vaccine	Age (months)	Nb of sera tested	Seroconversion		GMT Log <sub>2</sub>
			Nb	%	
A	7-11	19	12(*)	63	5.38
	12-72	77	70	91	6.67
B	7-11	43	25	42	5.28
	12-72	70	49	70	6.10
C	7-11	40	14	35	4.87
	12-72	101	51	50	5.90

(\*) HI antibody titre of  $\geq 1/20$



The reason for the lower seroconversion rates in children aged 7-11 months is probably due to the persistence of measles antibody of maternal origin (table I) not detected by the HI test. The differences in seroconversion rates of children 12-72 months old may be due to the fact that these children were randomly selected and immunized with almost equal amounts of TCID<sub>50</sub> of one of 3 vaccines used in this field trial, and there was no lapse in vaccine handling; the progressive failure rate of seroconversion may thus be attributed to the lower quality of vaccines B and C in comparison with vaccine A. As mentioned before, the seed material of B vaccine was heated 4 times for 15 min at 50°C. This handling may have affected some of the structural proteins of measles virus (HA or M protein) which led to the poor replication of virus in human body. The immunogenicity of Edmonston-Zagreb virus (vaccine C) was probably also impaired by further passages in HDC (Beck, personal communication).

### Discussion

The occurrence of measles in children who had been previously vaccinated has been recorded in several studies [1, 3, 38, 40]. According to recent US Weekly Reports [9, 10], 44% of measles patients in 1985 and 1986 in the USA had been appropriately vaccinated (on or after the first birthday). Failures in vaccination have been attributed to a number of factors, including the use of killed vaccine [13], the expected failure rate in children given the vaccine under proper conditions [14, 24], neutralization of the live virus in the vaccine by maternal measles antibody [5, 6, 41], immunological immaturity [37], loss of immunity induced by the vaccine [32], administration of the vaccine concomitantly with human immunoglobulin in infants less than one year of age, vaccine storage temperature, overexposure to light and, finally, the use of live vaccine of low or no potency. For many investigators, the latter factor is mainly due to improper handling of vaccine. According to Buy-

nak *et al.* [8], the attributes of the live vaccine itself are far less important in producing and maintaining immunity against the disease than proper handling of the vaccine. We believe, however, that the immunogenicity (potency) of the live virus particles in the vaccine should not be overlooked. The efficacy of measles vaccine strains, according to the World Health Organization's minimal requirements formulated in 1966, corresponds to virus titre, as determined in tissue culture [33]. It is predicted in the requirements that, as new strains of attenuated virus and new methods for producing vaccines are developed, it will be necessary in each case to investigate the correlation between the laboratory estimation of virus titre and efficacy for man. In a recent amendment to the requirements, a lyophilized measles vaccine is now considered heat-stable if, after exposure to 37°C for 7 days, at least 3 log<sub>10</sub> of virus is retained and the loss of titre does not exceed 1.0 log<sub>10</sub> [34]; that is, according to WHO requirements, a titre of about 1,000 TCID<sub>50</sub> of a given measles vaccine will immunize a seronegative child. The amount of TCID<sub>50</sub> of virus in different measles vaccines which can initiate seroprotection in susceptible persons, to our knowledge, has not thus far been fixed. Indeed, live attenuated measles viruses which are all derived from Enders virus have different immunizing capabilities. For Meyer *et al.* [20], using the Edmonston vaccine, seroconversion was achieved with less than 10 TCID<sub>50</sub>; for Buynak *et al.* [7] with the Moraten strain, the number of infectious doses of virus required to immunize a susceptible human being were 20 TCID<sub>50</sub>; this figure was 55 TCID<sub>50</sub> for Rosenblum *et al.* [35] and 3,000 for Wallace *et al.* [42], who used the Schwarz strain. Finally, Sabin *et al.* [36] found that 5,000 TCID<sub>50</sub> of Edmonston-Zagreb virus were necessary for immunization of a susceptible child. This figure rose to 10,000-15,000 TCID<sub>50</sub> for Makino [18], who developed the AIK-C vaccine which was also derived from Enders virus. This striking difference is probably the result of the mode of attenuation of measles virus. It is generally admitted that excessive attenuation of a virus by repeated passages in a given host cell, especially if subcultures are made in a heterogeneous alien cell system at low temperature, will lead to a new progeny of virus highly attenuated for the original host. Since live measles vaccine

does not immunize by virtue of its bulk of antigen, but rather by viral replication in a susceptible host [14], it can generally be said that attenuated viruses do not replicate in the human host to the same extent as their virulent counterparts [21], and the more attenuated strains of measles virus evoke lower levels of antibodies than natural measles [22]; it is therefore possible that successive passages of the Edmonston-Zagreb strain in primary dog kidney cell or the AIK strain in primary sheep kidney cell at 33°C are the basis for the low replication character of these viruses in the human body, a phenomenon suggested by Makino [18]. It has also been pointed out that thermal inactivation of measles virus results from denaturation of structural viral protein [2]. We can therefore assume that repeated heatings of seed materials of B vaccine used in this field trial have affected HA and especially the matrix protein of measles virus which, unlike the other proteins of the measles virus, shows substantial variations [39]. However, there is no definite correlation between structural changes in the M protein and the immunizing ability of measles virus.

*The conclusion* drawn from this study is that, for establishment of immunity, the immunogenic quality of the measles virus strain is more important than the amount of TCID<sub>50</sub> of the virus used. The accepted criteria for a good vaccine should not be based simply on the titre of the virus; an acceptable antibody response and a reasonable seroconversion rate should also be part of the criteria of a qualified measles vaccine. Measles is the second in febrile disease, after smallpox, to be considered as globally eradicated. But the continuing presence of measles as a problem 23 years after the introduction of measles vaccine creates unanswerable questions. In agreement with Brunell [6], vaccine failures in school-age children with a history of previous immunization in the second year of life cannot be attributed to the neutralization of vaccine virus by passively acquired maternal antibody. In order to solve the existing problems of measles eradication, more attention should be paid to other factors, and particularly to the potency of vaccines currently in use.

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