

## MASS IMMUNITY AGAINST DIPHTHERIA AND TETANUS IN SOME URBAN AND RURAL AREAS IN IRAN(\*)

F. Nazari, S. Alé-Agha, M. Mahinpoor and H. Mirchamsy

The mortality of Diphtheria in Iran was the major public health problem some 20 years ago, but it is very low at the present time. This change is mainly due to mass immunization campaigns realized by ministry of health all over the country, in urban or remote rural areas, using jointly Health Staff and Health corps. It is however not rare to observe outbreaks of Diphtheria of limited size in different parts of the country, with low mortality. In order to assess the level of immunity in the country it was decided to investigate the antitoxic titers of blood collected in a large numbers of children and to study the problem of carriers of *Corynebacterium diphtheria*. This preliminary report is an Screening hemagglutination test of some five thousand blood samples selected from a large stock of samples collected for control of Diphtheria and Tetanus Antitoxin.

### Materials and methods

#### *Children under survey*

As a rule, the children, randomly selected for this study, were, supposed to be immunized with 3 injections of Diphtheria-Tetanus-Pertussis (DTP) or combined diphtheria and tetanus (DT) during the first 3 years of life and older children had received a booster dose of DT Vaccine every 3-4 years or whenever an outbreak of Diphtheria was declared. Details of the production of DTP or DT Vaccine have been given in a previous report (2).

*Toxoids* - The purified Diphtheria and Tetanus toxoids used for sensitization of red blood cells contained 2500 Lf/mg and 1435 Lf/mg protein nitrogen of Diphtheria and Tetanus antigens respectively.

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## Preparation of sheep red blood cells

Tannic acid treatment of sheep blood cells and sensitization with the purified antigens were carried out as described by Scheibel (4) with minor modifications as follows:

The red cells were washed three times with saline by centrifugation at 2000 r.p.m. for 10 minutes. One ml of packed cell after 3rd washing was suspended in 40 ml of phosphate buffered saline pH 7.2–7.4 to obtain a 2.5 percent cell suspension. A stock solution of Tannic acid (reagent grade) 1:1000 in saline was kept at cold. For use, a solution of 1:20000 was prepared in saline. One part of 2.5 percent cell suspension was mixed with one part of a 1:20000 solution of tannic acid. After 10 minutes at room temperature the mixture was centrifuged at 1500 r.p.m. for 10 minutes, washed twice with phosphate buffer saline pH 7.2–7.4 and resuspended to a 2.5 percent suspension in saline.

One part of 2.5 percent tanned cells was added to four parts of the appropriate dilution of purified toxoid in buffered saline pH 6.4 the final concentration of both toxoids in contact with tanned cells was 30 Lf/ml. After 10 minutes at room temperature the suspension was centrifuged at 1500 r.p.m. for 10 minutes and the sediment washed once with 0.5 percent steril normal rabbit serum previously heated for 30 minutes at 56°C.

The cells were then resuspended in 0.5 percent normal rabbit serum to a 2.5 percent suspension.

*Diluent* – physiological saline containing 0.5 percent normal rabbit serum was used throughout.

*Collection of blood* – finger – blood of children under survey was absorbed on to squares of filter – paper and stored at cold as described in a previous report (3). For use, the blood was eluted from each square of filter-paper in 1.5 ml of peptone saline the evening before the tests were to be run. Elution was carried out in test tubes, the squares being soaked overnight at 4°C. According to our previous experience the initial dilution was taken as 1:10 serum dilution. Each sample of eluted blood was tested for both diphtheria and tetanus antitoxin.

*Performance of the test:* The sera were titrated in twofold dilution in tubes containing diluent in a way to have a final volume of 0.5 ml of diluted serum in each tube to ensure uniformity of the tests. The World Health Organization (W.H.O.) Standards of Diphtheria and Tetanus antitoxins were also diluted in saline containing 1: 10,000 merthiolate to contain 0.01 A. U. in 0.5 ml (Dipht. Antitoxin) or 0.0025 A. U. in 0.5 ml (Tet. Antitoxin) were included in each daily test.

The amount of cell suspension added was 0.05 ml per test tube. The tubes were then shaken for 30 seconds and left overnight at room temperature. The reading was carried out by means of a mirror and magnifying glass and the end point was chosen as the highest serum dilution giving distinct agglutination.

## RESULTS

*Diphtheria Antitoxin.* From figures reflected in Table 1, the children under study were in 3 groups:

First group, 582 children (14%) have a titer of 0 – 0.015 A.U./ml; second group, 2653 children (56%) have 0.015 – 1 A.U./ml and 1433 children (30%) have shown a titer between 1 to more than 16 A.U./ ml. Since it is generally accepted that the antibody level of 0.01 A.U./ml will protect a child against diphtheria infection, we conclude that 86% of children under study have had a significant immunity and only 14% failed to show the necessary amount of antitoxin to protect them from infection.

### *Tetanus Antitoxin*

In a similar way we may analyse figures in table 2 in which the results of tetanus antitoxin titration are exposed.

It is worth mentioning that for protection of man against tetanus, it is generally admitted that, 1/500 A.U. per ml of serum would be enough. In the present experiment only 64 out of 4704 children (1,3%) have a titer below 1/500, thus 98.7% of children under study were fully protected against tetanus.

## DISCUSSION

The immunization of children in Iran against diphtheria, tetanus and pertussis is carried out under certain circumstances by health teams in urban and rural districts through mass campaigns. There are also health centers all around the country where combined vaccines are available and vaccination of children is performed, on request of their parent, free of charge. It is obvious that in the long absence of outbreaks of Diphtheria, people almost neglect to refer to the health centers for vaccination of their children and since there is not a regular compulsory immunization.

The number of susceptible children increases annually in a way that each 4–5 years a new wave of Diphtheria infection may be expected in communities with inadequate status of immunization. On the contrary, in these

Table 1.

*Diphtheria Antitoxin value of filter paper elutes of blood taken from 4668 children*

Age	Sex		Titre													Total
	M	F	0 0.001	0.007 0.015	0.015 0.031	0.031 0.062	0.062 0.125	0.125 0.25	0.25 0.5	0.5 1	1 2	2 4	4 8	8 16	16<	
3-6 M	1	6	1	2	—	—	—	—	—	1	1	1	1	—	—	7
6-12 M	12	17	7	—	—	1	2	5	4	4	1	1	3	1	—	29
1-2 Y	37	32	6	—	2	2	5	7	5	17	12	10	1	1	1	69
2-3 Y	27	29	3	2	1	5	5	10	13	6	7	1	1	1	1	56
3-4 Y	31	39	4	—	—	7	8	14	9	8	11	3	4	1	1	70
4-5 Y	34	39	8	1	3	1	7	6	11	10	6	6	8	6	—	73
5-6 Y	24	35	4	1	—	1	3	4	4	7	10	7	7	8	3	59
6-7 Y	132	228	26	1	2	8	15	31	42	45	56	68	41	15	10	360
7- Y	546	472	76	11	30	67	98	125	113	109	152	103	52	23	59	1018
8- Y	606	650	20	193	87	78	146	130	147	129	95	79	66	30	56	1256
9- Y	517	575	3	145	60	89	122	166	139	98	118	90	40	14	8	1092
10- Y	224	227	—	44	27	31	46	69	56	72	65	28	9	2	2	451
11- Y	37	40	—	17	6	5	11	12	9	4	10	2	—	1	—	77
12- Y	24	27	—	7	6	5	7	8	3	2	7	2	4	—	—	51
<b>Total</b>	<b>2252</b>	<b>2416</b>	<b>158</b>	<b>424</b>	<b>224</b>	<b>300</b>	<b>475</b>	<b>587</b>	<b>555</b>	<b>512</b>	<b>551</b>	<b>401</b>	<b>237</b>	<b>103</b>	<b>141</b>	<b>4668</b>

Table 2.  
Tetanus Antitoxin value of filter paper elutes of blood taken from 4704 children

Age	Sex		Titre													Total
	M	F	0 0.001	0.007 0.015	0.015 0.031	0.031 0.062	0.062 0.125	0.125 0.25	0.25 0.5	0.5 1	1 2	2 4	4 8	8 16	16<	
3-6 M	1	6	—	2	—	—	—	—	1	1	1	1	1	—	—	7
6-12 M	12	17	2	—	5	3	4	3	7	1	1	1	2	—	—	29
1-2 Y	37	32	7	—	8	6	7	7	9	4	8	1	1	3	1	69
2-3 Y	27	29	1	—	6	6	4	10	6	8	7	2	3	2	1	56
3-4 Y	30	39	2	—	5	3	4	11	11	16	5	5	5	1	1	69
4-5 Y	34	39	3	2	3	1	6	4	3	24	16	5	5	1	—	73
5-6 Y	24	35	1	1	2	2	—	2	3	16	21	9	2	—	—	59
6-7 Y	133	228	12	1	4	21	40	42	43	59	71	23	21	4	13	361
7- Y	562	472	31	45	66	68	99	112	130	121	131	73	40	12	106	1034
8- Y	606	658	5	85	121	99	116	141	140	160	163	91	46	26	71	1264
9- Y	517	578	—	62	85	67	75	119	118	167	127	130	67	31	44	1092
10- Y	224	227	—	24	62	29	38	46	51	50	55	42	31	11	12	451
11- Y	37	40	—	8	14	4	13	12	12	2	3	3	2	1	3	77
12- Y	38	25	—	7	8	7	4	8	9	6	6	1	5	1	1	63
<b>Total</b>	<b>2282</b>	<b>2422</b>	<b>64</b>	<b>237</b>	<b>396</b>	<b>316</b>	<b>410</b>	<b>517</b>	<b>543</b>	<b>642</b>	<b>615</b>	<b>389</b>	<b>231</b>	<b>93</b>	<b>253</b>	<b>4704</b>

regions where a programme of regular immunization of susceptible children is carried out, the Diphtheria infection should normally not be observed. Tasman and Lansberg (5) believe that the immunization reduces the number of carriers and avoid infection. It is also worth mentioning that the old concept of most of health authorities who suggest that «if 70% of a population is fully immunized against diphtheria, the disease will disappear» is no more applicable in the prevention of diphtheria. As a matter of fact, with the increase in the number of cases of diphtheria in the United States of America during the past few years, the value and the effect of immunization on the control of outbreaks has become a question(1).

Although morbidity and mortality rate of diphtheria in Iran, comparing with 20 years ago when large scale immunization was not yet started, is much reduced; however the existing small outbreaks of diphtheria indicate either lack of enough immunization covering school age children or persistence of large reservoirs of carriers of corynebacterium diphtheriae in heavily populated districts. May be, similar to U.S.A., the disease continue its spread in the presence of an adequate herd immunity. To investigate the situation, it was decided to evaluate from one side the existing immunity levels, on a nation wide basis, and to study from another side the rate of carriers of C. diphtheriae in populated regions.

The present report is a preliminary survey of 4700 samples of blood selected at random from a very large number of samples collected all around the country. The carrier status has not yet been investigated but small outbreaks of diphtheria with limited size of infection has been observed during the recent years in well immunized districts of the country.

From data presented in tables 1 and 2 it is evident that there exist a high level of immunity against diphtheria and tetanus in most of regions under study, since 86% of the total children in this survey showed a titer over 0.01 A.U./ml for diphtheria. The percentage of protection against tetanus was much higher. 98.7% of children had already a titer of tetanus antitoxin over 0.01 A.U./ml.

#### **Summary**

4700 blood samples collected by finger – blood of children, 3 months to 12 years old, previously immunized against diphtheria and tetanus were tested by hemagglutination with tanned sheep red blood cells sensitized with diphtheria or tetanus toxoids. 86% of children had shown solid immunity against diphtheria; also 98.7% were protected against tetanus. Sporadic outbreaks of diphtheria among immunized children were observed.

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