

## Original Article

# Community-Based Seroepidemiology of Tetanus in Three Selected Provinces in Turkey

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**SUMMARY:** The aim of this study was to assess immunity levels against tetanus in the areas of 26 health centers in Samsun, Antalya, and Diyarbakir in Turkey in 2000-2001. The study group consisted of 2,465 healthy subjects aged 6 months old or above, randomly selected from each age group in the area. Of these, a total of 2,094 (85.0%) serum samples were assayed for tetanus antibody; 716 were from Antalya, 706 were from Diyarbakir, and 672 were from Samsun. The surveys were implemented in three steps: physical examination, interview, and blood collection. ELISA-in-house was used as a screening procedure and a particle agglutination test was used to reassess antibody titers of 1.0 IU/ml or below. It was revealed that 73.5% subjects had the full protection level ( $\geq 0.1$  IU/ml) of antibody in Antalya, 59.9% in Diyarbakir, and 75.0% in Samsun, indicating that protection against tetanus was significantly lower in Diyarbakir than in Antalya and Samsun. The results also showed that the percentage of protective levels decreased with increasing age in three provinces and was higher in rural areas than urban areas in Diyarbakir. The study indicates that the immunity levels against tetanus can be considered as satisfactory among children and adolescents but that it is necessary to increase immunity against tetanus among adults through effective vaccination of pregnant women and those in military service and also among people older than 40 years of age.

## INTRODUCTION

Tetanus is the only vaccine-preventable disease that is not communicable. Complete eradication of tetanus is not possible, because tetanus spores are widespread in soil and in the stools of people and animals, and can be transmitted without human contact. Case fatality of tetanus is between 10 and 90% (1), (Full text is available on Web site at <http://www.who.int/vaccines/en/neotetanus.shtml> [2]). Tetanus is responsible for many deaths in developing regions such as Africa, Asia, and Latin America; neonatal tetanus is also still a major global public health problem in some developing countries, with an estimated 500,000 cases occurring every year (1,2). Without specific therapy, more than 95% of infants will die of neonatal tetanus, and even with specific therapy lethality rates are between 25 and 90% (2). Neonatal tetanus cases have been reported in from four to six European countries every year, although underreporting is likely; Turkey and Albania have the highest incidence rates of 0.05 to 0.10 per 1,000 live births (3). Maternal tetanus is responsible for at least 5% of maternal deaths, with approximately 30,000 deaths annually all over the world (4). The elimination of neonatal tetanus as a public health problem by the year 2005 has been agreed to by all member states of the WHO, UNICEF, and UNFPA (2,4).

Tetanus can be prevented through the use of tetanus toxoid. The toxoid is a formaldehyde-inactivated (preparation of chemically inactivated) toxin, adsorbed onto aluminum salts

in order to increase its immunogenicity. In developed countries where infants and children are vaccinated with tetanus toxoid, the number of patients has been decreasing in the younger generation but not in adults (2,5). Some authors have reported that natural immunity against tetanus could be induced by a sublethal dose of tetanus toxin or by fragments of tetanus toxin released by digestion of ingested tetanus spores, but epidemiological evidence does not support this data (6). Thus, it is generally considered that immunity to tetanus is induced only by immunization.

The aim of the present study was to assess the current state of immunity to tetanus quantitatively in three provinces in Turkey in order to compare the immunity levels among them and to evaluate the relationships between the immunity levels and some sociodemographic variables. This is the first report on a community-based seroepidemiology against tetanus in Turkey, although some studies on limited age groups have been reported (7,8).

## MATERIALS AND METHODS

**Study design and population:** The Refik Saydam National Hygiene Center and General Directorate of Primary Health Care, Ministry of Health of Turkey implemented a project named the "Infectious Diseases Control Project in Turkey" between 1997 and 2002 with the cooperation of the Japan International Cooperation Agency (JICA). Seroepidemiological field surveys were carried out in three pilot provinces (Antalya, Diyarbakir, and Samsun) in order to quantitatively assess the current state of immunity for vaccine-preventable diseases including tetanus.

This was a cross-sectional study, and field surveys were conducted in the areas of 26 health centers selected from

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Fig. 1. Location of three selected provinces in Turkey.

Antalya, Diyarbakir, and Samsun between February 2000 and October 2001 (Fig. 1). Antalya is an area of tourism in the Mediterranean Region. Diyarbakir, which is in the East Anatolian Region of Turkey, is a province whose socio-economic level is low. Samsun, which is in the Black Sea Region of Turkey, is one of the developed provinces of Turkey. The provincial health directorates of these provinces randomized eight health centers for Antalya (four were from rural areas and four were from urban areas), eight health centers for Diyarbakir (four were from rural areas and four were from urban areas) and 10 health centers for Samsun (five were from rural areas and five were from urban areas). The study group consisted of 2,465 randomly selected healthy subjects aged 6 months old or above in three provinces. Surveys were carried out in three steps: physical examination, interview, and blood collection (Fig. 2).

**Physical examination:** The physicians of 26 health centers examined subjects, and if the subjects were free of acute febrile diseases, they were enrolled in the study.

**Interview:** The physicians interviewed face-to-face with subjects by using a questionnaire. The questionnaire included 20 questions regarding sociodemographic characteristics, vaccination history, infectious diseases history, etc.

**Blood collection:** The nurses from the health centers collected 5 - 10 ml venous blood samples from the subjects. After keeping the samples overnight at 4°C in the regional branch laboratory, the sera were separated from blood samples and transferred to the Refik Saydam National Hygiene Center by coldchain in order to assay tetanus antibody titers. Of 2,465 subjects randomly selected for the survey, 2,094 sera (85.0%) were assayed for the tetanus antibody; 716 were from Antalya (86.7%), 706 were from Diyarbakir (79.0%), and 672 were from Samsun (90.2%) (Fig. 2).

**Laboratory methods:** Enzyme linked immunosorbent assay (ELISA-in-house) and particle agglutination test (Particle Agglutination Kit, KPA, Chemo-Sero-Therapeutic Research Institute, Kumamoto) were selected to determine the immunity level against tetanus. Purified tetanus toxoid (Research Foundation for Microbial Diseases of Osaka University, Kagawa, 400 Lf/ml 122 µgPN/ml) was used for ELISA-in-house. The International Unit (IU) of the in-house

reference antiserum was determined by parallel line assay with the reference antitoxin from the National Institute for Biological Standards and Control (Anti-tetanus immunoglobulin, human, Lyophilized, 120 IU/ampoule). ELISA-in-house was used as a screening procedure, and then antibody titers, which were equal to 1.0 IU/ml or below, were reassessed by KPA (Coplu et al., unpublished data).

**Tetanus antitoxin levels:** Tetanus antitoxin levels were classified according to the generally adopted protective levels, <0.01 IU/ml no protection, 0.01-<0.1 IU/ml uncertain, short term protection, and ≥0.1 IU/ml, full protection (6,9).

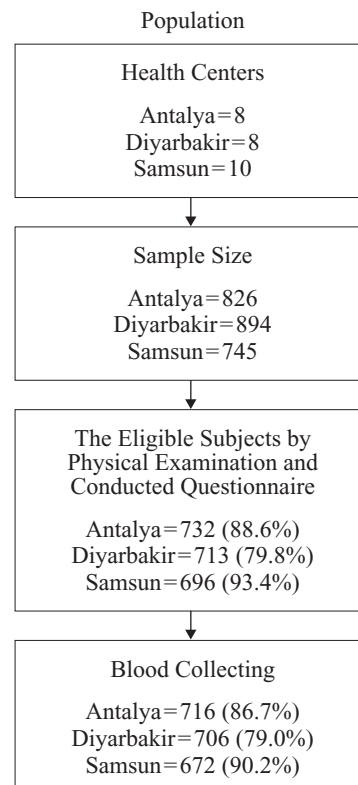


Fig. 2. Study population.

**Statistical analysis:** Differences among three provinces in regard to age group, gender, and residence were assessed by  $\chi^2$ -test. Associations between the antibody levels and variables (age, gender, residence, educational status, occupational status among subjects aged 15 years old or above, doses of tetanus vaccine, and the period from last tetanus vaccination) were assessed by logistic regression analysis. For this analysis, the outcome variables were used as binary variables for the protection (full protection versus uncertain or no protection). Odds ratios (OR) with 95% confidence intervals (CI) were calculated. Statistical analyses were performed

using the SPSS 10.0 for Windows and software which was prepared by the JICA expert using Visual Basic 6.0 and Access.

## RESULTS

A total of 2,094 subjects above 6 months of age were assayed for the immunity level of tetanus. Of these, 716 were from Antalya, 706 were from Diyarbakir, and 672 were from Samsun. Distribution of the study group by sociodemographic variables is shown in the Table 1. The significance was not

Table 1. Distribution of the study group in regard to certain sociodemographic variables in Antalya, Diyarbakir, and Samsun

|   | Provinces         |       |                   |       |                   |       |
|---|-------------------|-------|-------------------|-------|-------------------|-------|
|   | Antalya           |       | Diyarbakir        |       | Samsun            |       |
|   | <i>n</i>          | %     | <i>n</i>          | %     | <i>n</i>          | %     |
| Age group                                     |                   |       |                   |       |                   |       |
| 0   | 30                | 4.2   | 38                | 5.4   | 31                | 4.6   |
| 1-4   | 134               | 18.7  | 133               | 18.8  | 132               | 19.6  |
| 5-9   | 158               | 22.1  | 156               | 22.1  | 149               | 22.2  |
| 10-14   | 32                | 4.5   | 36                | 5.1   | 34                | 5.1   |
| 15-19   | 31                | 4.3   | 32                | 4.5   | 30                | 4.5   |
| 20-29   | 84                | 11.7  | 75                | 10.6  | 70                | 10.4  |
| 30-39   | 80                | 11.2  | 62                | 8.9   | 70                | 10.4  |
| 40-49   | 71                | 9.9   | 65                | 9.2   | 64                | 9.5   |
| 50+   | 96                | 13.4  | 109               | 15.4  | 92                | 13.7  |
| Gender  |                   |       |                   |       |                   |       |
| Male  | 325               | 45.4  | 342               | 48.4  | 326               | 48.5  |
| Female  | 391               | 54.6  | 364               | 51.6  | 346               | 51.5  |
| Residence                                     |                   |       |                   |       |                   |       |
| Rural   | 360               | 50.3  | 355               | 50.3  | 336               | 50.0  |
| Urban   | 356               | 49.7  | 351               | 49.7  | 336               | 50.0  |
| Total   | 716               | 100.0 | 706               | 100.0 | 672               | 100.0 |
| Graduates of educational institutions         |                   |       |                   |       |                   |       |
| Preschool                                     | 217               | 30.4  | 227               | 32.2  | 210               | 31.3  |
| Primary school children                       | 118               | 16.5  | 115               | 16.3  | 107               | 15.9  |
| Illiterate                                    | 55                | 7.7   | 169               | 24.0  | 48                | 7.1   |
| Literate                                      | 17                | 2.4   | 52                | 7.4   | 19                | 2.8   |
| Primary school                                | 197               | 27.6  | 88                | 12.5  | 165               | 24.6  |
| Junior high school                            | 28                | 3.9   | 27                | 3.8   | 33                | 4.9   |
| High school                                   | 44                | 6.2   | 18                | 2.6   | 51                | 7.6   |
| Higher educational institutions               | 37                | 5.2   | 9                 | 1.3   | 39                | 5.8   |
| Total   | 713               | 100.0 | 705               | 100.0 | 672               | 100.0 |
| Occupation status among 15 years old and more |                   |       |                   |       |                   |       |
| Not in labor                                  | 210               | 58.0  | 198               | 57.7  | 182               | 55.8  |
| In labor                                      | 152               | 42.0  | 145               | 42.3  | 144               | 44.2  |
| Total   | 362               | 100.0 | 343               | 100.0 | 326               | 100.0 |
| Marital status                                |                   |       |                   |       |                   |       |
| Single  | 407               | 56.8  | 423               | 59.9  | 402               | 59.8  |
| Ever married                                  | 309               | 43.2  | 283               | 40.1  | 270               | 40.2  |
| Total   | 716               | 100.0 | 706               | 100.0 | 672               | 100.0 |
| Household                                     |                   |       |                   |       |                   |       |
| 1-4   | 495               | 69.8  | 111               | 15.7  | 297               | 44.5  |
| 5-9   | 210               | 29.6  | 401               | 56.8  | 330               | 49.4  |
| 10+   | 4                 | 0.6   | 193               | 27.4  | 41                | 6.1   |
| Average (SD), Median                          | 1.3 ( $\pm 0.5$ ) | 1.0   | 2.1 ( $\pm 0.7$ ) | 2.0   | 1.6 ( $\pm 0.6$ ) | 2.0   |
| Total   | 709               | 100.0 | 705               | 100.0 | 668               | 100.0 |
| Social security                               |                   |       |                   |       |                   |       |
| No  | 206               | 28.8  | 323               | 45.8  | 99                | 14.7  |
| Yes   | 510               | 71.2  | 383               | 54.2  | 573               | 85.3  |
| Total   | 716               | 100.0 | 706               | 100.0 | 672               | 100.0 |

found statistically in the distribution of age, gender, and residence among the survey subjects from the three provinces, Antalya, Diyarbakir, and Samsun ( $P > 0.05$ ).

Table 2 shows a comparison of immunity levels against tetanus in terms of certain variables from survey results of 2000-2001. Overall, 73.5% in Antalya had full protection (antibody level  $\geq 0.1$ /ml), in Diyarbakir 59.9%, and in Samsun 75.0%. The average immunity level of the survey subjects

in Diyarbakir was lower than the averages of the other two provinces ( $P < 0.05$ ).

The highest immunity level among age groups was observed in the groups of 10-14 and 15-19 years in three provinces and also in the 0 year age group in Antalya. Full protection against tetanus was found in 74.7% of the females and in 72.0% of the males in Antalya, in 58.2% of the females and in 61.7% of the males in Diyarbakir, and in 74.3%

Table 2. Immunity levels against tetanus in regard to certain variables among the study group; Antalya, Diyarbakir, and Samsun

|  | Tetanus Antibody Level (ELISA + KPA) |               |                |           |            |               |                |           |           |               |                |           |
|--|--------------------------------------|---------------|----------------|-----------|------------|---------------|----------------|-----------|-----------|---------------|----------------|-----------|
|  | Antalya                              |               |                |           | Diyarbakir |               |                |           | Samsun    |               |                |           |
|  | <0.01 (%)                            | 0.01-<0.1 (%) | $\geq 0.1$ (%) | Total (n) | <0.01 (%)  | 0.01-<0.1 (%) | $\geq 0.1$ (%) | Total (n) | <0.01 (%) | 0.01-<0.1 (%) | $\geq 0.1$ (%) | Total (n) |
| <b>Age group</b>   |                                      |               |                |           |            |               |                |           |           |               |                |           |
| 0  | –                                    | –             | 100.0          | 30        | 18.4       | 15.8          | 65.8           | 38        | 3.2       | 6.5           | 90.3           | 31        |
| 1-4  | 0.7                                  | 10.4          | 88.8           | 134       | 9.0        | 15.8          | 75.2           | 133       | 0.8       | 6.1           | 93.2           | 132       |
| 5-9  | 3.8                                  | 10.8          | 85.4           | 158       | 2.6        | 16.0          | 81.4           | 156       | –         | 4.0           | 96.0           | 149       |
| 10-14  | –                                    | –             | 100.0          | 32        | –          | 11.1          | 88.9           | 36        | –         | –             | 100.0          | 34        |
| 15-19  | –                                    | –             | 100.0          | 31        | –          | 9.4           | 90.6           | 32        | –         | –             | 100.0          | 30        |
| 20-29  | 1.2                                  | 9.5           | 89.3           | 84        | 14.7       | 26.7          | 58.7           | 75        | 1.4       | 7.1           | 91.4           | 70        |
| 30-39  | 5.0                                  | 21.3          | 73.8           | 80        | 21.0       | 22.6          | 56.5           | 62        | 8.6       | 32.9          | 58.6           | 70        |
| 40-49  | 26.8                                 | 42.3          | 31.0           | 71        | 30.8       | 41.5          | 27.7           | 65        | 20.3      | 31.3          | 48.4           | 64        |
| 50+  | 40.6                                 | 35.4          | 24.0           | 96        | 51.4       | 36.7          | 11.9           | 109       | 67.4      | 21.7          | 10.9           | 92        |
| <b>Gender</b>  |                                      |               |                |           |            |               |                |           |           |               |                |           |
| Male   | 12.0                                 | 16.0          | 72.0           | 325       | 17.0       | 21.3          | 61.7           | 342       | 12.3      | 12.0          | 75.8           | 326       |
| Female   | 7.9                                  | 17.4          | 74.7           | 391       | 17.9       | 23.9          | 58.2           | 364       | 12.7      | 13.0          | 74.3           | 346       |
| <b>Residence</b>   |                                      |               |                |           |            |               |                |           |           |               |                |           |
| Rural  | 9.2                                  | 15.3          | 75.6           | 360       | 14.9       | 20.0          | 65.1           | 355       | 11.9      | 14.3          | 73.8           | 336       |
| Urban  | 10.4                                 | 18.3          | 71.3           | 356       | 19.9       | 25.4          | 54.7           | 351       | 13.1      | 10.7          | 76.2           | 336       |
| Total  | 9.8                                  | 16.8          | 73.5           | 716       | 17.4       | 22.7          | 59.9           | 706       | 12.5      | 12.5          | 75.0           | 672       |
| <b>Graduates of educational institutions</b>                   |                                      |               |                |           |            |               |                |           |           |               |                |           |
| Preschool  | 0.9                                  | 11.1          | 88.0           | 217       | 8.8        | 15.4          | 75.8           | 227       | 1.0       | 7.1           | 91.9           | 210       |
| Primary school children  | 4.2                                  | 5.9           | 89.8           | 118       | 2.6        | 16.5          | 80.9           | 115       | –         | 0.9           | 99.1           | 107       |
| Illiterate   | 38.2                                 | 36.4          | 25.5           | 55        | 33.1       | 36.7          | 30.2           | 169       | 58.3      | 20.8          | 20.8           | 48        |
| Literate   | 47.1                                 | 35.3          | 17.6           | 17        | 36.5       | 15.4          | 48.1           | 52        | 42.1      | 31.6          | 26.3           | 19        |
| Primary school   | 14.7                                 | 21.3          | 64.0           | 197       | 25.0       | 30.7          | 44.3           | 88        | 21.8      | 18.2          | 60.0           | 165       |
| Junior high school   | –                                    | 7.1           | 92.9           | 28        | 7.4        | 14.8          | 77.8           | 27        | 6.1       | 3.0           | 90.9           | 33        |
| High school  | 2.3                                  | 29.5          | 68.2           | 44        | –          | 22.2          | 77.8           | 18        | 5.9       | 17.6          | 76.5           | 51        |
| Higher   | 10.8                                 | 13.5          | 75.7           | 37        | 11.1       | –             | 88.9           | 9         | 12.8      | 30.8          | 56.4           | 39        |
| Total  | 9.8                                  | 16.7          | 73.5           | 713       | 17.4       | 22.6          | 60.0           | 705       | 12.5      | 12.5          | 75.0           | 672       |
| <b>Occupation status among subjects aged 15 years or above</b> |                                      |               |                |           |            |               |                |           |           |               |                |           |
| Not in labor   | 21.4                                 | 22.4          | 56.2           | 210       | 33.3       | 30.8          | 35.9           | 198       | 30.8      | 20.3          | 48.9           | 182       |
| In labor   | 11.8                                 | 27.6          | 60.5           | 152       | 23.4       | 29.7          | 46.9           | 145       | 18.1      | 21.5          | 60.4           | 144       |
| Total  | 17.4                                 | 24.6          | 58.0           | 362       | 29.2       | 30.3          | 40.5           | 343       | 25.2      | 20.9          | 54.0           | 326       |
| <b>Doses of tetanus vaccine (DPT, Td and TT)</b>               |                                      |               |                |           |            |               |                |           |           |               |                |           |
| 0  | 31.0                                 | 31.8          | 37.2           | 129       | 34.5       | 34.9          | 30.6           | 232       | 43.3      | 20.2          | 36.5           | 104       |
| 1  | 23.1                                 | 28.8          | 48.1           | 52        | 14.8       | 27.0          | 58.2           | 122       | 35.7      | 25.0          | 39.3           | 56        |
| 2  | 6.1                                  | 25.8          | 68.2           | 66        | 19.0       | 19.0          | 61.9           | 63        | 16.1      | 23.2          | 60.7           | 56        |
| 3  | 3.3                                  | 13.3          | 83.3           | 90        | 8.0        | 9.1           | 83.0           | 88        | 4.5       | 16.7          | 78.8           | 132       |
| 4  | 1.9                                  | 12.1          | 85.9           | 206       | 5.1        | 15.3          | 79.7           | 118       | 1.1       | 5.6           | 93.3           | 178       |
| 5  | 2.7                                  | 2.7           | 94.7           | 113       | –          | 14.5          | 85.5           | 55        | 2.1       | –             | 97.9           | 97        |
| 6  | 14.3                                 | 3.6           | 82.1           | 28        | –          | –             | 100.0          | 17        | –         | 5.7           | 94.3           | 35        |
| 7  | –                                    | 16.7          | 83.3           | 18        | –          | –             | 100.0          | 7         | –         | 10.0          | 90.0           | 10        |
| 8  | –                                    | 33.3          | 66.7           | 9         | –          | –             | 100.0          | 2         | –         | 33.3          | 66.7           | 3         |
| 9  | –                                    | –             | 100.0          | 2         | –          | –             | –              | 0         | –         | –             | 100.0          | 1         |
| 10+  | –                                    | –             | 100.0          | 3         | –          | –             | 100.0          | 2         | –         | –             | –              | 0         |
| Total  | 9.8                                  | 16.8          | 73.5           | 716       | 17.4       | 22.7          | 59.9           | 706       | 12.5      | 12.5          | 75.0           | 672       |
| <b>Period of last tetanus vaccine</b>                          |                                      |               |                |           |            |               |                |           |           |               |                |           |
| Before 1991  | 25.9                                 | 29.6          | 44.4           | 54        | 26.3       | 29.8          | 43.9           | 57        | 32.4      | 32.4          | 35.1           | 37        |
| 1992-1996  | 10.9                                 | 23.9          | 65.2           | 46        | 11.5       | 19.2          | 69.2           | 26        | 3.6       | 28.6          | 67.9           | 28        |
| 1997-2001  | 1.5                                  | 10.6          | 87.9           | 454       | 7.6        | 15.6          | 76.8           | 409       | 1.0       | 5.6           | 93.4           | 392       |
| Total  | 4.7                                  | 13.5          | 81.8           | 554       | 10.0       | 17.5          | 72.6           | 492       | 3.7       | 9.2           | 87.1           | 457       |

Table 3. Full protection levels ( $\geq 0.1$  IU/ml) against tetanus (Logistic Regression) in regard to certain variables among the study group; Antalya, Diyarbakir, and Samsun

|   | Antalya |          |                   | Diyarbakir |          |        | Samsun |          |                   |
|---|---------|----------|-------------------|------------|----------|--------|--------|----------|-------------------|
|   | OR      | 95.0% CI |                   | OR         | 95.0% CI |        | OR     | 95.0% CI |                   |
| Age   | 0.93    | 0.89     | 0.96              | 0.94       | 0.91     | 0.97   | 0.86   | 0.80     | 0.93              |
| Gender  |         |          |                   |            |          |        |        |          |                   |
| Male  | 1       |          |                   | 1          |          |        | 1      |          |                   |
| Female  | 1.17    | 0.68     | 2.02              | 1.05       | 0.64     | 1.73   | 0.72   | 0.32     | 1.60              |
| Residence   |         |          |                   |            |          |        |        |          |                   |
| Rural   | 1       |          |                   | 1          |          |        | 1      |          |                   |
| Urban   | 0.81    | 0.49     | 1.35              | 0.48       | 0.30     | 0.77   | 2.28   | 1.03     | 5.04              |
| Graduates of educational institutions                   |         |          |                   |            |          |        |        |          |                   |
| Preschool   | 0.19    | 0.02     | 1.63              | 0.48       | 0.12     | 1.92   | 0.06   | 0.00     | 2.44              |
| Primary school children                                 | 0.32    | 0.04     | 2.49              | 0.78       | 0.21     | 2.89   | 1.21   | 0.02     | 60.94             |
| Illiterate  | 1       |          |                   | 1          |          |        | 1      |          |                   |
| Literate  | 0.00    | 0.00     | $2.8 \times 10^9$ | 1.91       | 0.52     | 6.97   | 4.01   | 0.01     | $3.5 \times 10^3$ |
| Primary school  | 1.48    | 0.32     | 6.88              | 0.94       | 0.29     | 3.01   | 4.66   | 0.36     | 59.85             |
| Junior high school                                      | 1.82    | 0.21     | 16.04             | 3.58       | 0.69     | 18.72  | 9.33   | 0.24     | 365.75            |
| High school   | 2.54    | 0.43     | 15.16             | 6.05       | 0.57     | 64.25  | 0.79   | 0.04     | 14.91             |
| Higher education  | 3.45    | 0.52     | 22.79             | 7.64       | 0.55     | 105.64 | 0.64   | 0.03     | 13.66             |
| Occupation status among subjects aged 15 years or above |         |          |                   |            |          |        |        |          |                   |
| Not in labor  | 1       |          |                   | 1          |          |        | 1      |          |                   |
| In labor  | 0.69    | 0.30     | 1.59              | 2.17       | 0.67     | 6.98   | 3.56   | 0.69     | 18.29             |
| Doses of tetanus vaccine (DPT, Td and TT)               | 1.12    | 0.95     | 1.32              | 1.47       | 1.29     | 1.69   | 0.96   | 0.69     | 1.34              |
| Period of last tetanus vaccine                          |         |          |                   |            |          |        |        |          |                   |
| Before 1991   | 1       |          |                   | 1          |          |        | 1      |          |                   |
| 1992-1996   | 1.38    | 0.53     | 3.58              | 1.23       | 0.35     | 4.39   | 2.38   | 0.54     | 10.53             |
| 1997-2001   | 3.07    | 1.24     | 7.65              | 2.00       | 0.61     | 6.57   | 7.19   | 1.53     | 33.90             |

of the females and in 75.8% of the males in Samsun. The immunity levels in the urban and rural areas were 71.3 and 75.6% in Antalya, 54.7 and 65.1% in Diyarbakir, and 76.2 and 73.8% in Samsun, respectively.

For categorizing educational status, children of 10 years or under were divided into two categories: preschool children and primary school children. The subjects aged above 10 years were then grouped into six categories according to the educational institutions where they were in or graduated from. Subjects who had no history of school education were divided into two groups: illiterate and literate. In a comparison of these eight groups, the rate of the full protection level was high among the groups of primary school children (89.8% in Antalya, 80.9% in Diyarbakir, and 99.1% in Samsun), the group of junior high school children (92.9% in Antalya, 77.8% in Diyarbakir, and 90.9% in Samsun), and also preschool children (88.0% in Antalya and 91.9% in Samsun). The immunity level was lower among subjects who were illiterate than literate in Diyarbakir, 30.2 and 48.1%, respectively ( $P < 0.01$ ). Among the group aged 15 years or above and employed the full protection percentages were 60.5% in Antalya, 46.9% in Diyarbakir, and 60.4% in Samsun. Since immunization against tetanus was being provided with three kinds of tetanus toxoid containing vaccines such as DPT (diphtheria, whole cell pertussis and tetanus toxoid), Td (tetanus toxoid with adult-type diphtheria) and TT (single-type tetanus vaccine), the doses of vaccination in this study included total doses given by these three kinds of vaccines. Results showed that the rate of the full protection level increased up to more than 90% after the 5th dose of tetanus in Antalya and Samsun and after the 6th dose of tetanus in Diyarbakir.

The results of the logistic regression analysis are shown in Table 3. Categorical variables were age, gender, residence, educational status, occupation status, period from last tetanus vaccination, the three provinces, and doses of tetanus vaccination. The findings of the study showed an inverse association between the immunity levels and age in three provinces with an OR of 0.93 (95%CI: 0.89-0.96) in Antalya, with an OR 0.94 (95%CI: 0.91-0.97) in Diyarbakir and with an OR 0.86 (95%CI: 0.80-0.93) in Samsun. By logistic regression analysis on the full protective level, no significant difference was shown in a comparison of categories of gender, education status, and occupation status in all three provinces. Full protection was significantly lower among the subjects living in an urban area in Diyarbakir with an OR of 0.48 (95%CI: 0.30-0.77), but on the other hand it was significantly higher among subjects living in an urban area in Samsun with an OR 2.28 (95%CI: 1.03-5.04), and no significant difference was found between rural and urban areas in Antalya. The survey results showed the immunity level is strongly associated with the doses of tetanus toxoid in Diyarbakir (OR = 1.47 95%CI: 1.29-1.69). The logistic regression analysis again showed that the overall protective level was significantly higher in Samsun (OR = 1.99, 95%CI: 1.45-2.75) and Antalya (OR = 1.75, 95%CI: 1.28-2.39) than in Diyarbakir. The full protection level of the group who had the last tetanus toxoid between 1997 and 2001 was significantly higher in Antalya (OR = 3.07, 95% CI: 1.24-7.65) and Samsun (OR = 7.19, 95%CI: 1.53-33.90).

## DISCUSSION

Different laboratory methods are available to determine



tetanus antibody titer. In some studies, ELISA has been used to assess the immunity level against tetanus (10-12). We have compared the ELISA-in-house and KPA methods with the mouse neutralization method, which is a golden standard. The results showed that for the level of 1.0 IU/ml or below, the results of our ELISA-in-house were not definite (Coplu et al. unpublished data). For that reason, the tetanus antibody titers in this study were determined by a combination of ELISA and KPA methods. ELISA-in-house was used for screening and titers 1.0 IU/ml or below were reassessed using the KPA method.

The "Immunization Program of Ministry of Health of Turkey" includes three doses of DPT in the first year of life, followed by one DPT at 16-24 months of age, and two doses of Td at the primary school period, and one TT at the high school period. In addition, tetanus vaccination for pregnant women has been accelerated since 1990 as a target of the neonatal tetanus elimination program. Men also receive additional doses of monovalent tetanus vaccine when doing military service (13). According to Ministry reports (14,15), the coverage of DPT<sub>3</sub> (the third dose of DPT) in 2000 and 2001 were 87-91% in Antalya, 44-56% in Diyarbakir, and 90-87% in Samsun.

The provinces surveyed in the present study had different socio-economic levels, but the study groups of these three provinces consisted of subjects not significantly different in age, gender, and residence ( $P > 0.05$ ). The survey results showed that full protection against tetanus was significantly lower ( $P < 0.05$ ) in Diyarbakir, which is located in the south-eastern region of Turkey with the lowest vaccination coverage among three provinces surveyed.

The results of the present study showed that a high proportion of children and adolescents had sufficient protection against tetanus in Antalya, Diyarbakir, and Samsun. The full protection among 0 age were 100.0% in Antalya, 65.8% in Diyarbakir, and 90.3% in Samsun. The full protection ranges among those 1 to 19 years old varied between 85.4 to 100.0% in Antalya, 75.2 to 90.6% in Diyarbaki, and 93.2 to 100.0% in Samsun, respectively. Routine childhood immunization against tetanus, diphtheria and pertussis has been launched in Turkey since 1968. Turkey participated in the Expanded Program on Immunization (EPI) of the WHO in 1981, but immunization was accelerated in 1985 with the "National Vaccination Campaign", and according to the data of the Ministry of Health, the immunization rate of DPT<sub>3</sub> increased from 20-30% to 83% through this campaign. In this study we found that among age groups, the proportion of full protective levels was the highest in groups of those 10 to 19 years of age in all three provinces. This finding should reflect the effect of the National Vaccination Campaign introduced in 1985 in Turkey.

The immunity levels against tetanus can be considered as satisfactory among children, but it is difficult to consider the same among adults. For those of childbearing age, the results of the present study showed that full protection in three provinces ranged between 58.7-91.4% among groups aged 20-29 years and 56.5-73.8% among those aged 30-39 years. In this connection, a study on pregnant women in Turkey (16) reported that full protection was found in 93.7% of the subjects aged less than 21 years, in 75% of subjects among group of age 21-30, and in 44.6% of those aged more than 30 years. According to the Ministry, reported coverages of vaccination for pregnant women with TT<sub>2</sub> (the second dose of TT) in 2000 and 2001 were 52 and 50% in Samsun, 65 and

65% in Antalya, and 8 and 11% in Diyarbakir, respectively (14,15). These findings showed that it is necessary to expand immunization services for pregnant women in order to achieve the goals of the neonatal tetanus elimination program. According to the global program for elimination of neonatal tetanus, all women of childbearing age are included in the target population for routine tetanus toxoid immunization. Since 1989, the World Health Assembly has called for the elimination of neonatal tetanus, and UNICEF, WHO, and UNFPA recently (in December 1999) agreed to set the year 2005 as the target date for worldwide elimination (4). As of June 2000, 57 countries have not yet eliminated of neonatal tetanus. Turkey is included in six of 22 countries which have potentially eliminated maternal and neonatal tetanus, but district assessments of the coverage must confirm the achievement of this goal (2,4).

In the present study, the immunity level was lower among subjects who were illiterate in Diyarbakir. This finding indicates the importance of school vaccination. It has been reported that immunization of boys and girls with Td or TT during the first 3 years of school provided protection from tetanus for the next 10-15 years (4) and that in the young group which had already received basic immunization with combined vaccine and the additional booster given at the age of 12 years, the protective antitoxin level appears to be maintained until 20 years old (17) and also that the national immunization program in the United Kingdom provided adequate protection against tetanus for the 17 to 25 year age group (18). The present study also showed that the level of protective antibodies against tetanus was significantly higher among subjects who received their last tetanus toxoid between 1997-2001 than those who received before that period in Antalya (OR = 3.07, 95% CI: 1.24-7.65) and Samsun (OR = 7.19, 95% CI: 1.53-33.90), but the difference was not statistically significant in Diyarbakir (OR = 2.00, 95% CI: 0.61-6.57). This finding may indicate the insufficiency of the immunization service in Diyarbakir during last years.

The present study also indicates a need for improving the immunization status of the people older than 40 years of age. More than 50% of them had no protective antibodies against tetanus. Full protection in three provinces ranged between 27.7-48.4% among the age group of 40-49 and 10.0-24.0% among the aged 50 years and above. This is an expected situation, because there is no routine immunization against tetanus for adults except pregnant women and men performing military duty in Turkey. Our results were in accordance with those by Ozturk et al. (8) who reported the immunity level in Central Anatolia, Turkey, as 25.3% among people older than 40 years of age. The decrease in the full protection level against tetanus among adults has also been noted in various countries. McQuillan et al. (19) reported that the proportion of protective tetanus antibody level of Americans decreased from 91% among children 6-11 years of age to 30% among persons 70 years of age. Stark et al. (9) reported that 72% of blood donors in Berlin had full protection against tetanus with a decreasing level by age. Gergen et al. (20) reported that the protective levels of tetanus antibodies in the United States have started to decline at the age of 40 years and the rate decreased to 27.8% among those 70 years of age or older. Kjeldsen et al. (21) reported that 51% of a study group aged 30-70 years in Denmark was unprotected against tetanus and the highest numbers of unprotected subjects were found among those 60-69 years old. These findings reported by other investigators are in accordance with our results in

Turkey, indicating that the decline of tetanus immunity among adults occurs worldwide, probably because of the waning of immunity for a long period after immunization, or incomplete or lack of immunization during childhood.

The high antibody levels against tetanus achieved in childhood may not last throughout an individual's life. Therefore, it is necessary to provide routine tetanus immunization not only for children but also for pregnant women and men performing military duty, and additionally to elderly people who are vulnerable to accidents and/or injuries.

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