Tetanus and Diphtheria Immunization Coverage in Ecuadorian Children after a National Vaccination Campaign

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Between October 1985 and June 1986, a national vaccination campaign was launched in Ecuador. Between March and November 1986, 7798 Ecuadorian children <5 years old were enrolled in a representative health survey. According to their vaccination cards, 65% of children had a complete series of three doses of diphtheria-tetanus-pertussis (DTP) vaccination. Serum samples were obtained from a subset of 1400 children; by ELISA, 80% had serologic evidence of tetanus vaccination of their mothers. For all 1400 children, median tetanus and diphtheria antitoxin titers were 1.0 and 0.6 IU/mL, respectively. These data indicate the possibility of success of such vaccination campaigns if supported by the government.

Tetanus is still a serious health problem worldwide, and in many developing countries, it is the major cause of death in newborn infants. Globally, some 1 million deaths are thought to occur annually from this disease and ~50% of these are in neonates [1]. Tetanus is a completely preventable disease. Prophylaxis can be achieved by active or passive immunization. Passive immunity conferred by equine antitoxin is of limited duration. Even in the developing world, human tetanus immune globulin for intramuscular use is beginning to supplant equine antitoxin. However, the best form of immunization is active immunization with tetanus toxoid. For developing countries, the World Health Organization (WHO)'s Expanded Programme on Immunization (EPI) [2], with its emphasis on infants, recommends a total of three doses early in the first year of life without reinforcing or booster doses. In addition, a minimum of two doses has been recommended by the EPI for pregnant women. Induction of protective levels in mothers is associated with protection from neonatal tetanus by transfer of antitoxin across the placenta.

In 1977, Ecuador became the first country in the Americas to adopt the EPI. From 1977 to 1982, immunization coverage among children increased significantly, from 22% to 65% for the first dose of diphtheria-tetanus-pertussis (DTP) vaccine [3]. However, in 1982 only 26% of children <1 year of age were immunized with the third dose of DTP, and in 1981 coverage with tetanus toxoid among pregnant women was

References


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only 12% for one dose and 4% for the recommended two doses [3]. To increase the immunization coverage, a national vaccination campaign was launched in Ecuador between October 1985 and June 1986. Between March and November 1986, 7798 Ecuadorian children, enrolled into a representative nutrition and health survey (DANS) [4], were investigated for their DTP vaccination history. For a subset of 1400 children, serum samples were obtained to analyze their antitoxin titers by ELISA. Our survey thus allowed an independent assessment of the vaccination coverage.

Materials and Methods

Routine immunization in Ecuador. Before 1977, some vaccines including DTP were routinely administered in health centers, but spontaneous demand was small, and no organized attempt was made to provide immunization services on a regular basis. In 1977, Ecuador became the first country in the Americas to adopt the EPI. Vaccination “brigades” were created for house-to-house immunizations, and mobile immunization clinics were used to promote access in larger urban areas [3].

Vaccination campaign. The plan for a national vaccination campaign was initiated by the National Institute for the Child and the Family, and it received the active support of the Ecuadorian government. The Ministry of Health and its subordinated health units at the province, region, area, and local levels were charged with the execution of the national campaign. The government mobilized all health workers in the country to do vaccinations at the local health facilities between October 1985 and June 1986. Children were to receive three doses of DTP vaccine, three doses of polio vaccine, and one vaccination with BCG (bacille Calmette-Guérin) and measles vaccine. The vaccines were provided by UNICEF. The children’s vaccination campaign was complemented by a tetanus toxoid immunization campaign to pregnant women. The Ministry of Defense and the Ministry of Education advised the soldiers and teachers, respectively, to make the campaign known in their local surroundings. In addition, the campaign was publicized by an aggressive use of the mass media, in which the active role of the First Lady was of psychological importance. The following three major external institutions participated with technical advice: US Agency for International Development, UNICEF, and Pan American Health Organization.

DANS. Between March and November 1986, 7798 children were enrolled into a representative nutritional and health survey of the ~1.2 million children < 5 years old in Ecuador. Details of the study design have been described previously [4]. The number of DTP immunizations per child was obtained from vaccination cards.

Serum samples from 1400 Ecuadorian children were available. These children were a subset of the 7798 children enrolled into the DANS study. Children ranged in age from 0 to 5 years; each 2-month age interval was represented by ~50 serum specimens. Further characteristics of the serum collection have been described previously [5].

ELISA. Purified tetanus and diphtheria toxoid were purchased from a WHO reference laboratory (Statens Seruminstitut, Copenhagen). The toxoids were added to carbonate-bicarbonate buffer (pH 9.6) at a concentration of 5 μg/mL and coated on microtiter plates. Control plates were coated with carbonate-bicarbonate buffer only. Toxoid-specific IgG antibodies were detected by an ELISA described previously [5]. A human serum of known tetanus antitoxin titer was obtained from Institut Pasteur (Paris), and a human serum of known diphtheria antitoxin titer was obtained from the Swiss Serum and Vaccine Institute (Bern).

Serum standards were incorporated into all assays. With the help of these reference sera, we determined that 1:100 serum dilutions gave a satisfying relationship between optical density (OD) reading in ELISA and antitoxin titers expected from DTP immunization in South American children [6]. For the prevalence analysis, a serum sample was counted as positive if the absorbance on the test plate was at least twice that on the control plate and at least 0.1 OD unit greater than the OD of the control plate. Standardization of the reagents with respect to sensitivity and specificity was done as described previously [5]. An absorbance of 0.1 OD unit corresponded to 0.2 IU/mL tetanus and 0.3 IU/mL diphtheria antitoxin in the undiluted serum sample or to 3 μg of toxoid-specific antibody/mL of undiluted serum sample.

Results

Vaccination history. A DTP vaccination history was obtained from the vaccination cards of the 7798 children enrolled into the DANS study. Vaccination coverage for the first and second doses was high, >82%, and somewhat smaller for the third dose (table 1). The coverage for each individual dose was ~8% higher in the urban than in the rural areas. Coverage for all three doses varied between 54.7% and 75.9% according to the region; urban areas served a 17% higher coverage for all three doses. As there were some doubts about the reliability of the vaccination cards, serologic analysis of the serum antitoxin levels was done in a subset of 1400 children enrolled into the DANS study.

Tetanus antitoxin. The percentage of children showing antitoxin titers ≥0.25 IU/mL, indicative of a recent immunization, was similar between urban (≥20,000 inhabitants per cantón) and rural areas (91.2% vs. 86.8%), low-altitude (<1000 m above sea level) Costa and high-altitude Sierra regions (88.5% vs. 90.2%), boys and girls (90.6% vs. 87.8%), and different social classes (data not shown). However, children living in urban high-altitude areas had significantly higher tetanus antitoxin titers than did children living in rural high-altitude or urban low-altitude areas (table 1). Serum samples were drawn from children living in 88 different geographic areas of Ecuador. A 100% coverage of tetanus immunization, with an antitoxin titer ≥0.25 IU/mL, was observed in children from 29 census areas (33%). A >90% coverage was achieved in a total of 52 census areas (59%). On the other hand, a total of 4 census areas (5%) showed a coverage of <50%. Figure 1A shows the distribution of antitoxin titers.
The frequency distribution curve showed a peak at 0.25–0.5 IU/mL antitoxin, with a long trailing “shoulder” toward higher antitoxin titers (figure 1A). The median antibody titer was 1.0 IU/mL antitoxin.

Age-related prevalence of tetanus antitoxin. More than 80% of the Ecuadorian children were born with tetanus antitoxin titers ≥0.25 IU/mL. The percentage of sera with ≥0.25 IU/mL antitoxin decreased in children 2–6 months old and reached a minimum of 36% in 4- to 6-month-old children. However, in 6- to 8-month-old children this prevalence increased once again to 72%, reached 81% in 8- to 10-month-old infants, and remained >85% in children between 10 months and 5 years of life, the age limit of our study (figure 1B). Parallel to the increase in prevalence of antitoxin in children >6 months of age was an increase in the mean OD in ELISAs for children >6 months (figure 1C). Note that the antibody means were calculated from all subjects, not just from those with results considered positive. Children >1 year old showed a mean antitoxin titer of ~1.5 IU/mL.

Diphtheria antitoxin. ELISA for the determination of diphtheria antitoxin was done in parallel. We set 0.1 OD unit, corresponding to 0.3 IU/mL diphtheria antitoxin, as the cutoff point for the prevalence analysis. Of the infants <2 months old, 31% had diphtheria antitoxin titers ≥0.3 IU/mL. This prevalence decreased to ~10% in 2- to 6-month-old infants and increased to ≥50% in children >8 months old. About 70% of the children >2 years showed ≥0.3 IU/mL diphtheria antitoxin. Sera with high-titers of diphtheria antitoxin were less prevalent than sera with high-titers of tetanus antitoxin; these differences were most evident in infants (figure 1B). While we found a good correlation between tetanus and diphtheria antitoxin ELISA readings (r = .63, P < .001; t test), the former were systematically higher than the latter (the mean difference in OD ± SD was 0.15 ± 0.32 for all 1400 sera). This difference in OD was observed in all age groups (figure 1C). The median diphtheria antitoxin titer was 0.6 IU/mL.

**Discussion**

Serologic surveys of tetanus antitoxin serve the important purpose of measuring the impact of tetanus immunization programs. However, only few serosurveys have been reported for children from developing countries, where the burden of tetanus is the highest [7, 8]. For the prevalence analysis, we set 0.1 OD unit, corresponding to 0.25 IU/mL antitoxin, as the cutoff point. This antitoxin titer is higher than even conservative estimates of the protective level for tetanus antitoxin immunity [9]. It is well above the levels for natural immunity to tetanus toxin reported in serosurveys from developing countries [10] and corresponds to the mean antitoxin titer obtained in South American infants after the first dose of DTP immunization [6]. Thus, we have chosen an antibody level that reflects recent immunization rather than protection.

Of the Ecuadorian children enrolled into this study, 89% showed tetanus antitoxin titers ≥0.25 IU/mL. This high coverage compares favorably with the antitoxin status of South American children before implementation of the EPI. In 1979, only 30% of children from poor areas of São Paulo had antitoxin levels ≥0.1 IU/mL [7]. The high antitoxin titer in Ecuadorian children is gratifying in view of the fact that a sizable percentage of the Ecuadorian children showed signs of undernutrition: 34% of the children studied had height-for-age values 2 SD below WHO reference values [4]. A depressed immune response to tetanus has been reported in children suffering from nutritional deficiency [11].

The percentage of children with high antitoxin titers substantially exceeded the percentage of children who received three doses of DTP vaccine (89% vs. 65%), indicating that two doses of DTP vaccine might be sufficient. Unfortunately we did not have vaccination records correlated with individual children who gave serum samples. This prevented an analysis of the antitoxin titers as a function of the number of vaccine doses. Evaluation of the vaccination cards for com-
Figure 1. **A**, Frequency distribution of Ecuadorian children's sera showing indicated tetanus antitoxin titer. n, number of sera. Antitoxin titer is given as optical density (OD) units in our ELISA. Several corresponding antitoxin titers are shown in IU/mL. %, cumulative percentage of sera showing antitoxin concentration equal to or less than indicated antitoxin titer. **B**, Age-related prevalence of tetanus and diphtheria antitoxin (●), only tetanus (■), or only diphtheria (□) antitoxin titers ≥0.25 and ≥0.3 IU/mL, respectively. n, number of children in each 2-month age interval. **C**, Mean OD measured by ELISA for serum IgG antibody to tetanus (—) and diphtheria (---) toxoid in 1400 Ecuadorian children of indicated ages. OD of 0.1, 0.2, and 0.4 corresponds to tetanus antitoxin titer of 0.25, 0.5, and 1 IU/mL and diphtheria antitoxin titer of 0.3, 0.7, and 2.0 IU/mL, respectively.

Completed immunization series thus underestimates the impact of vaccination campaigns. The coverage for two doses might be a more realistic measure for protection against tetanus disease. The national campaign has raised the coverage for three doses from 26%, obtained in 1982 with the routine EPI, to 65%. The WHO EPI promotes the control of neonatal tetanus by immunizing women of childbearing age, particularly pregnant women, with the aim of conferring passive immunity to the child [12]. Our serosurvey showed that >80% of the Ecuadorian infants studied were born with high tetanus antitoxin titers while <30% were born with high diphtheria antitoxin titers. This difference between tetanus and diphtheria antitoxin prevalence is a serologic indication that a substantial percentage of Ecuadorian women underwent tetanus toxoid vaccination during pregnancy. During a cross-sectional study conducted between 1982 and 1983, only 37% of Ecuadorian women showed protective tetanus antitoxin levels [13].
The national vaccination campaign thus achieved substantially higher vaccination coverages both in infants and children and in pregnant women than did the routine EPI. There has been some concern that the transfer of passive immunity to tetanus toxoid might decrease the “take” rate of the DTP vaccination [14]. In Ecuador, the high level of passively acquired maternal antitoxin apparently did not prevent a good immune response in the vaccinees.

In the Ecuadorian vaccinees, we consistently found higher antibody levels to tetanus toxoid than to diphtheria toxoid. Similar observations were made in two American studies [6, 15], while no difference was detected in British [16] and Iranian [8] vaccinees. The difference in response to various preparations of DTP is not surprising. It could result from different amounts of antigen used or from the pertussis adjuvant effects in the different vaccines, but we do not have data to substantiate such hypotheses.

In summary, the present analysis of a national vaccination campaign demonstrated that highly successful immunization programs can be run in developing countries. It remains to be seen whether the governments of other developing countries are able to support similar vaccination programs and whether the Ecuadorian government will be able to sustain such efforts.

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