Tetanus toxoid coverage as an indicator of serological protection against neonatal tetanus

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Objective A Multiple-Indicator Cluster Survey (MICS) was conducted at mid-decade in more than 60 developing countries to measure progress towards the year 2000 World Summit for Children goals. These goals included the protection of at least 90% of children against neonatal tetanus through the immunization of their mothers, as measured by tetanus toxoid (TT) coverage. In the Central African Republic (CAR), serological testing was added to the MICS to understand better the relationship between survey estimates of TT coverage and the prevalence of serological protection.

Methods In the CAR MICS, mothers of children younger than one year of age gave verbal histories of the TT vaccinations they had received, using the MICS TT questionnaire. A subsample of mothers was tested for tetanus antitoxin, using a double-antigen enzyme-linked immunoadsorbent assay (ELISA). Seropositivity was defined as a titre of $\geqslant 0.01$ IU/ml, and TT coverage was defined as the proportion of mothers protected at delivery, according to their history of TT vaccinations.

Findings Among the 222 mothers in the subsample, weighted TT coverage was 74.4% (95% Confidence Interval (CI); 67.0%–81.7%) and tetanus antitoxin seroprevalence was 88.7% (95% CI; 83.2%–94.2%). The weighted median antitoxin titre was 0.35 IU/ml.

Conclusions Tetanus toxoid coverage in the CAR was lower than the prevalence of serological protection against neonatal tetanus. If this relationship holds for other countries, TT coverage estimates from the MICS may underestimate the extent to which the year 2000 goal for protecting children against neonatal tetanus was reached. We also showed that a high level of serological protection had been achieved in a country facing major public health challenges and resource constraints.

Keywords Tetanus toxoid/administration and dosage; Tetanus antitoxin; Tetanus/prevention and control; Infant, Newborn; Immunization schedule; Immunization programs/utilization; Seroepidemiologic studies; Cluster analysis; Central African Republic (*source: MeSH, NLM*).

Mots clés Anatoxine tétanique/administration et posologie; Antitoxine tétanique; Tétanos/immunologie; Nouveau-né; Calendrier vaccination; Programmes de vaccination/utilisation; Etude séroépidémiologique; Sondage en grappes; République centrafricaine (*source: MeSH, INSERM*).

Palabras clave Toxoide tetánico/administración y dosificación; Antitoxina tetánica; Tétanos/inmunología; Recién nacido; Esquema de inmunización/utilización; Estudios seroepidemiológicos; Análisis por conglomerados; República Centroafricana (*fuente: DeCS, BIREME*).

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Voir page 702 le résumé en français. En la página 703 figura un resumen en español.

Introduction

Between 1995 and 1996, a Multiple-Indicator Cluster Survey (MICS) was conducted in more than 60 developing countries to measure progress towards the year 2000 goals of the World Summit for Children and to determine if mid-decade goals had been met (*1, 2*). The mid-decade and year 2000 goals called for protecting 80% and 90%, respectively, of children against neonatal tetanus, by vaccinating their mothers. The indicator for determining if these goals were achieved is tetanus toxoid (TT) coverage, defined as the proportion of newborns whose mothers, at delivery, were protected against tetanus according to their TT vaccination history (*1*).

In the 5-dose TT vaccination schedule recommended by the World Health Organization (WHO), minimum intervals between doses and the expected duration of protection after each dose vary by the number of doses received (*3*). These features make the determination of the TT vaccination status of mothers more complicated than the determination of vaccination status of children, even when exact dates of all TT doses received by mothers are known. Often, however, exact dates of all doses are not known because mothers do not have a

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record of their TT doses, or because the record they have is incomplete (e.g. doses during different pregnancies may have been recorded in different documents and only the most recent kept). Also, the total number of doses may be difficult for mothers to recall accurately, since some doses may have been received several years in the past. For some mothers, the first doses may have been received in infancy, as a component of diphtheria-pertussis-tetanus (DPT) vaccine.

To understand better the relationship between TT coverage, based on maternal recall, and the level of serological protection achieved against neonatal tetanus, TT coverage and tetanus antitoxin seroprevalence were both measured during a national household survey in Burundi (*4*). Interviewers asked mothers the number of TT doses they received in each of their previous three pregnancies, if applicable. Despite the potential difficulties for mothers in recalling TT histories accurately, TT coverage (73% (95% CI; 66%–79%)) was close to seroprevalence (67% (95% CI; 59%–76%)). In the MICS, interviewers stopped asking questions about the TT history of mothers once it had been determined that a mother was protected at the time of her last delivery (*1*); if a mother stated she received two vaccine doses during her last pregnancy, no questions were asked about previous doses. Also, the simplifying assumption was made that all doses met the minimum interval requirements.

In 1995, the WHO Steering Committee on Epidemiology and Field Research recommended that tetanus serology be added to the MICS in 2–3 countries to determine the accuracy with which TT coverage from the MICS indicated the prevalence of serological protection (*5*). Our study was conducted in response to this recommendation, and to our knowledge was the only such study completed at mid-decade.

Methods

Sampling

In the CAR MICS, conducted from January to March 1996, interviewers returned to the same 231 clusters that were used in the 1994–95 Demographic and Health Survey (DHS). For the DHS, the country was divided into 11 strata: an urban and a rural stratum in each of the five regions, and Bangui, the capital (*6*). The primary sampling units (PSUs) were census tracts, selected with a probability of selection proportional to the total population as determined by the 1988 national census. Households in each selected PSU were mapped and listed. For the MICS, 36 households from the complete DHS household list for each PSU were selected by systematic sampling. In each household, MICS questionnaires were used to obtain information on all children under 16 years of age and their mothers, including a TT questionnaire for the mothers of children younger than one year of age.

The survey team member responsible for obtaining filter-paper blood specimens visited one-quarter of the households in each cluster chosen by systematic sampling, and asked all mothers of children younger than one year of age for consent to obtain a filter-paper blood specimen for tetanus serology. The probability of selection for mothers in the seroprevalence subsample and complete MICS sample was estimated by dividing the estimated total number of households in each stratum in 1994 by the number of subsample and complete MICS sample households, respectively, in the stratum (*7*).

Questionnaire and analysis of TT coverage

The MICS TT questionnaire asked mothers about TT doses they received during the pregnancy of the child whose protection-at-birth status was in question (*1*). Mothers who answered that they received only one dose of TT, or none at all, during the pregnancy were asked if they received any dose before their last pregnancy (shortened slightly from the standard MICS question " ... at any time before your last pregnancy, either during a previous pregnancy or between pregnancies?"). If their answer was "yes," they were asked for the number of doses received previously and for the month and year of the last of these doses. If they could not remember the month and year, they were asked how many years it had been since the last of the doses. A child was considered protected at birth if the child's mother stated she received two or more doses during her pregnancy or one dose during the pregnancy and at least one dose any time previously. If she stated she received no dose during her pregnancy but 2–4 doses before the pregnancy, and could say when the last of these doses was given (month and year of the dose, or years since it was given), the child was considered protected at birth if the birth took place during the expected period of protection conferred by the last dose (*3*). If she stated she received five or more doses before the pregnancy, her child was considered protected at birth, regardless of the time since the last of these doses. All doses were assumed to meet minimum interval requirements between valid doses.

The MICS TT questionnaire gives no indication that information from a card or other document should be used, and, in the survey manual, interviewers are instructed to ask mothers these questions and record their answers (reference 1, pages A1.10 and A1.11). However, the mid-decade manual also states: "If no card is available, you must try to find out how long ago the last TT dose was received, and the total number of TT doses the mother has received in her lifetime," which may have led to the decision in some countries to use information from a card when a card was available (reference 1, page A1.10). In the CAR, information from cards was not used because the cards or other documents with TT vaccinations recorded in them may not have had a complete record of TT vaccinations and because combining TT information from cards with maternal recall would require decision-making by interviewers that would be neither recorded nor controlled.

After being asked the MICS TT questions, mothers in the CAR who did not give a history of two or more TT doses during their last pregnancy and said they had received at least one dose previously, were asked again about the number of doses received previously and when the last one was received, this time with the interviewer instructed to ask separately, in reverse chronological order, about TT injections during the pregnancy preceding each birth (including stillbirths and children who were born alive and died) and about periods when the mother was not pregnant. The purpose of these additional, probing, questions was to see how much TT vaccination histories changed (the MICS TT questionnaire owes much of its brevity to asking a single question about the number of TT doses before the last pregnancy). After these questions, mothers were asked if they had received one or more doses of TT since their last delivery and if their last child received at least one dose of DPT vaccine.

Blood samples and laboratory analysis

The pad of the mother's third or fourth finger was cleaned with an alcohol pad, dried and punctured by a sterile lancet, and the blood collected on Schleicher and Schuell #903 filter paper. Blood specimens were allowed to dry thoroughly out of direct sunlight and each filter paper was stored in a separate envelope. Tetanus antitoxin titres were assayed at North American Vaccine, Inc. (now Baxter HealthCare), Columbia, Maryland, USA, using a double-antigen ELISA. This method provides results that correlate well with in vivo testing and, unlike conventional indirect ELISA assays, does not overestimate titres in low-titre sera (*8*). Seropositivity was defined as a titre of $\geqslant 0.01$ IU/ml (*9*).

Adhesive labels with the mother's identification number were placed on the filter paper, questionnaire, and consent form of each mother who provided a blood sample. As a back-up method for matching serological results with questionnaires, the PSU number (which also identified the stratum), the household number, and the identification numbers of both mother and child were written on the filter paper.

Data entry, matching and logistic regression

All MICS questionnaires were entered and stored at the National Census Bureau. However, when the questionnaire data were entered into the database, the label numbers on the TT questionnaires of mothers who provided a blood sample were not initially entered in the database, and the questionnaires were lost during civil conflict in November 1996. Therefore, filter-paper results were matched with questionnaires using only the back-up identification numbers. The matching process did not take into account either the vaccination histories of mothers or their serological status.

We used Epi Info, version 6.03 (*10*) to edit data and define variables, and Software for Survey Data Analysis (SUDAAN), version 7.50 (*11*) for logistic regression and to estimate the precision of proportions, taking into account the cluster sampling design.

Human subjects review and informed consent

The study protocol was approved by a Human Subjects Review Committee at the Centers for Disease Control and Prevention, and by the CAR Ministry of Population and Public Health. Informed consent was obtained from all mothers who participated in the study.

Results

A total of 8324 households were selected for the MICS sample from the maps and lists created for the 1994–95 Demographic and Health Survey (*12*). No information was obtained for 1542 (18.5%) of these households, the principal reasons being that the structure in which the household residents had lived was destroyed (6.3%), no longer occupied (6.3%), or that the inhabitants were absent (4.6%). The percentage of households from which no information was obtained was 21.8% (974/4462) in rural areas and 14.7% (568/3862) in urban areas.

A total of 1156 mothers (96.9%) of the 1193 children less than one year of age in the MICS sample were interviewed with the TT questionnaire, and 286 filter-paper blood samples were obtained from them. Survey records that would have shown the number of mothers who refused to provide a blood sample were stolen from the National Census Bureau. However, we estimated that filter-paper blood specimens would have been obtained from 289 (1156/4) mothers if there had been no refusals, since the mothers of children less than one year of age in a fourth of all MICS sample households were asked if they would provide a blood sample for serology. A total of 286 filter-paper blood specimens were obtained, suggesting that the refusal rate was small. In all, 259 (90.6%) of the 286 blood samples obtained could be matched with a TT questionnaire using the back-up matching numbers. Thirty-seven (14.3%) mothers who provided the matching blood samples stated that they had received a TT injection since their last delivery. The records of these mothers were removed from the analysis, since the mothers may have become seropositive since their delivery.

The analysis of TT coverage and tetanus antitoxin seroprevalence was confined to the remaining 222 mothers. Among these, 75.7% stated they had a TT vaccination card or booklet, but only 57.7% were able to show one to the interviewer (Table 1). In all, 77.9% said they had been seen for antenatal care at least once during their last pregnancy, while 63.5% stated that their last child was born in a medical facility or in the presence of a trained birth attendant. A total of 186 (83.8%) of the mothers knew, or were able to show, an exact date of birth for their child.

According to their verbal histories, 41.0% (91/222) of mothers in the sample were considered to be protected at the time of their delivery because of two or more injections of TT during their last pregnancy; 21.6% (48/222) by the single dose received during their last pregnancy and at least one dose

Table 1. **Characteristics of mothers of children younger than one year of age, Central African Republic (CAR) 1996**[a]

Characteristic	*n*
When asked for her vaccination card, the mother	
showed it to the interviewer	128 (57.7)[b]
stated she had one, but was unable to show it to the interviewer	40 (18.0)
stated she did not have one	54 (24.3)
Number of pregnancies	
1	44 (20.1)
2	26 (11.9)
3	36 (16.4)
$\geqslant 4$	113 (51.6)
Missing	3
Number of antenatal visits, last pregnancy	
0	46 (20.7)
1	20 (9.0)
2	27 (12.2)
3	64 (28.8)
4	62 (27.9)
Not remembered	3 (1.4)
Last delivery in a health facility or with a trained birth attendant	
Yes	141 (63.5)
No	81 (36.5)

[a] Data taken from the seroprevalence subsample of the CAR MICS, 1996. The percentages were calculated from the total number of mothers in the subsample ($n = 222$).

[b] Figures in parentheses are percentages.

previously; and 13.5% (30/222) by doses received before their last pregnancy (Table 2). Questionnaire answers were missing for four mothers (1.8%) that, if not missing, could have resulted in the mothers' being considered protected. Since the MICS analysis does not exclude mothers from the analysis for missing information, these mothers were kept in the analysis and classified as unprotected. Weighted TT coverage among the 222 mothers was 74.4% (95% CI; 67.0%–81.7%). Weighted tetanus antitoxin seroprevalence was 88.7% (95% CI; 83.2%–94.2%).

Three factors were tested as potential risk factors for tetanus antitoxin seronegativity and for a nonprotective TT vaccination history: having been pregnant only once, residence in a rural area and having received no antenatal care during the last pregnancy (Table 3). In multivariable modeling, having received no antenatal care during the last pregnancy was the only factor significantly associated with a nonprotective vaccination history, while all three were associated with seronegativity. Only one (0.9% (95% CI; 0.0%–2.5%), weighted analysis) mother in the urban strata and Bangui was seronegative.

Five of the 20 seronegative mothers stated they delivered at a health facility or with a trained birth attendant (24.4% (95% CI; 3.0%–45.7%), weighted analysis); five stated their child received at least one dose of DPT (26.7% (95% CI; 2.0%–51.4%), weighted analysis); and eight had at least one of these opportunities to be vaccinated (39.5% (95% CI; 12.2%–66.9%), weighted analysis). In all, only four seronegative mothers (18.1% (95% CI; 0%–37.1%), weighted analysis) had at least one of these opportunities for vaccination and were eligible for TT vaccination according to their TT history, providing a very approximate indication of the extent to which screening and vaccinating mothers with TT at the time of delivery at maternity wards and when their children receive their first DPT injection (with subsequent doses of TT given later, if indicated) could lead to the vaccination and seroconversion of mothers who might otherwise enter their next pregnancy seronegative. Mothers of children who may have been too young to receive the first dose of DPT were included in this analysis, because excluding them would have required limiting the analysis to mothers whose child had an exact date of birth recorded.

Vaccination status and serological status were concordant for 79.7% (177/222) of mothers (Table 4). The sensitivity of vaccination status as a predictor of seropositivity was 80.7% (163/202), while the specificity, predictive value positive, and predictive value negative were 70.0% (14/20), 96.4% (163/169) and 26.4% (14/53), respectively. The low predictive value negative is at least partly explained by underreporting of TT doses: 45.5% of mothers who stated they had received no dose of TT were seropositive (Table 2).The weighted median and mean antitoxin titres were 0.35 IU/ml and 0.93 IU/ml, respectively, and the range was 0.00–9.82 IU/ml (Table 5). In all, 73.9% of mothers were protected by titres at least 10-times higher than the level defining seropositivity ($\geqslant$0.01 IU/ml).

In all, 45 mothers stated they received no TT in their last pregnancy, but had received at least one dose before their last

Table 2. **Tetanus antitoxin seroprevalence and titres in mothers of children younger than one year of age, by category, Central African Republic (CAR), 1996**[a]

TT vaccination history	No. mothers, by category	No. mothers seropositive	Titre (IU/ml)	
			Median	Mean
Considered protected at time of last delivery				
$\geqslant$2 doses in last pregnancy (history of previous doses not obtained)	91	87 (95.6)[b]	0.60	1.48
1 dose in last pregnancy and $\geqslant$1 dose previously	48	48 (100)	0.61	0.92
No dose in last pregnancy, but $\geqslant$2 doses before last pregnancy and mother still in the expected period of protection at time her last delivery[b]	30	28 (93.3)	0.19	0.34
Considered not protected at time of last delivery				
1 dose only (in last pregnancy or earlier)	23	22 (95.7)	0.43	0.81
No dose in last pregnancy, $\geqslant$2 doses before last pregnancy, but mother no longer in expected period of protection at time of last delivery[c]	2	2 (100)	0.26	0.26
No dose in last pregnancy, 2–4 doses before last pregnancy, but date of and years since last dose not remembered	2	2 (100)	0.30	0.30
No dose in last pregnancy or earlier	22	10 (45.5)	0.01	0.42
Not protected by doses recorded on questionnaire, but one or more answers were missing that, if not missing, could have led to the mother's being considered protected	4	3 (75.0)	0.44	0.83

[a] Data taken from the seroprevalence subsample of the CAR MICS, 1996 (n = 222). Percentages are calculated from the number of mothers in each category.
[b] Figures in parentheses are percentages.
[c] See (*3*), for expected periods of protection.

Table 3. **Factors evaluated as risk factors for non-protective TT vaccination status and tetanus antitoxin seronegativity among mothers of children younger than one year of age, Central African Republic (CAR), 1996**[a]

		Non-protective TT vaccination status (weighted %)	Weighted adjusted odds ratio (95% CI)	Seronegative (weighted %)	Weighted adjusted odds ratio (95% CI)
No. pregnancies					
	n				
1	44	36.5	2.3 (1.0–5.3)	20.4	2.9 (1.2–7.4)
⩾2	175	23.2	1.0 (reference)	9.1	1.0 (reference)
Received antenatal care					
No	46	60.0	8.1 (3.6–18.0)	28.5	3.8 (1.2-12.2)
Yes	173	15.2	1.0 (reference)	6.0	1.0 (reference)
Area of residence					
Rural	104	32.0	1.2 (0.5–2.9)	18.7	17.8 (1.9–165.3)
Urban	118	16.6	1.0 (reference)	0.9	1.0 (reference)

[a] The data are taken from the seroprevalence subsample of the CAR MICS, 1996 (results weighted, $n = 222$).

Table 4. **Concordance between immunization status and tetanus antitoxin seropositivity among mothers of children younger than one year of age, Central African Republic, 1996**[a]

Protected according to vaccination history	**Seropositive**		
	Yes	**No**	**Total**
Yes	163	6	169
No	39	14	53
Total	**202**	**20**	**222**

[a] Data taken from the seroprevalence subsample of the CAR MICS, 1996 ($n = 222$, analysis weighted).

Table 5. **Tetanus antitoxin titres in mothers of children younger than one year of age, Central African Republic (CAR), 1996**[a]

Median titre	0.35 IU/ml
Mean titre	0.93 IU/ml
Range	0.00–9.82 IU/ml
Distribution of titres (IU/ml)	
titre <0.01	11.3%
0.01 ⩽ titre <0.10	14.8%
0.10 ⩽ titre <1.00	50.7%
titre ⩾ 1.00	23.2%

[a] Data taken from the seroprevalence subsample of the CAR MICS, 1996 ($n = 222$, analysis weighted).

pregnancy, and were asked probing questions about their TT vaccination history after the MICS TT questionnaire was completed. Based on answers to the probing questions, the number of TT doses received before the last pregnancy increased for 18 of these mothers (40.0%) and decreased for one (2.2%). However, only three mothers were reclassified as to whether they were protected at the time of delivery by their vaccination history, and the weighted point estimate for TT coverage among all 222 mothers that included the information obtained with probing questions, 75.7% (95% CI: 68.9%–82.5%), was close to the point estimate based on answers without probing questions (74.4%). We have chosen to present only the results that were obtained without using probing questions.

Potential selection bias in national estimates of seroprevalence was created by excluding mothers whose filter-paper blood specimen could not be matched with a questionnaire and mothers who stated they had received TT since their delivery, or did not know if they had received TT since their delivery. Unweighted seroprevalence (88.9%) among the 27 mothers excluded because their filter paper was not matched with a questionnaire was nearly the same as the weighted seroprevalence among the 222 mothers (88.7%). We also compared TT coverage among the mothers in the seroprevalence analysis (74.4%) with TT coverage among the 37 mothers excluded because they had received TT since their delivery (90.2%, odds ratio (OR) = 3.2, 95% CI; 1.01–10.0), and among the 934 mothers of children under one year of age in the MICS who were not in the seroprevalence analysis and who included the two groups that were excluded (72.9%, OR = 0.9, 95% CI; 0.6–1.4, weighted analysis). When mothers who stated they received TT since their delivery were added to the seroprevalence analysis (for a total of 259 mothers), seroprevalence increased to 89.5% (95% CI; 84.7%–94.4%).

Discussion

Our study was undertaken to evaluate TT coverage from the MICS as an indicator of programme achievement. We compared TT coverage with tetanus antitoxin seroprevalence among 222 mothers of children less than one year of age in the CAR MICS. Although TT coverage among these mothers was relatively high (74.4%), it was nonetheless a conservative indicator of the prevalence of serological protection (88.7%) that the national vaccination programme had achieved. Based on our point estimate of seroprevalence, the World Summit for Children year 2000 goal of protecting 90% of newborns against neonatal tetanus had nearly been reached by mid-decade in the CAR, a country facing major public health challenges and resource constraints.

The accuracy of TT coverage estimates may vary between countries according to the proportion of TT doses given several years in the past (including those given as part of DPT vaccinations in infancy), whether TT is given outside of antenatal visits, and the availability of cards (if information

from cards is used to determine TT vaccination status). Thus, it is important to determine if the relationship between TT coverage (measured by survey and using the MICS TT questionnaire) and tetanus seroprevalence that we found for the CAR also holds for other countries. This relationship was studied in Namibia and Togo in 2000 (E. Holt, personal communication); patterns from these and the present study should be taken into account when drawing conclusions about achievement of the year 2000 World Summit for Children TT protection goal. Since factors affecting the relationship between tetanus antitoxin seroprevalence and TT coverage measured by survey may change over time, tetanus serology is likely to remain a valuable addition to surveys that measure TT coverage.

In our survey, tetanus antitoxin titres were usually well above the threshold level defining serological protection and seropositivity ($\geqslant$0.01 IU/ml). In all, 73.9% of mothers had a titre at least 10-times higher than the threshold level. Two characteristics of the TT vaccination histories help explain the relationship between these histories and the generally high antitoxin titres. First, tetanus antitoxin seroprevalence among the 22 mothers who reported they had never received a TT vaccination indicates there was underreporting of doses. Second, almost two-thirds of mothers considered protected by their vaccination history received their last dose of TT when they were pregnant with their 0–11 month-old child. Therefore, they would often have been in the high end of the antibody response curve (*9*).

In the CAR MICS, information from records kept by the mother were not used in completing the TT questionnaire. The *End-Decade Multiple Indicator Survey Manual* states clearly that such information should be used ("if a card is present, use it to assist with answers to the following questions") (*13*). The use of written records, as recommended for the year 2000 MICS, has the potential of obtaining more accurate TT histories, but also of complicating the interpretation of TT coverage results. When TT doses in written records are not clearly the same as those described by mothers, interviewers use their own (unrecorded) judgment in sorting out what mothers most likely received, and may differ in deciding whether doses described by mothers were in addition to those confirmed by written records or the same. Also, TT coverage estimates may differ not only because of real differences in vaccination status but also in how frequently mothers have written records of TT vaccinations and how complete they are. The interpretation of TT coverage using the MICS questionnaire will be relatively straightforward if subsequent studies show little variability in the relationship between coverage and seroprevalence; if variability is substantial, the combining of information from cards and mothers' verbal histories might be reviewed as a potential cause.

When mothers said they received no dose of TT in their last pregnancy but at least one dose previously, probing questions about the number of such doses after the MICS questions were asked. TT coverage, using the probing questions was nearly the same as when they were not used. Future surveys might examine the need for further probing questions by adding them for mothers who answer "no" when asked if they received a dose before their last pregnancy.

Having been pregnant only once, residence in a rural area, and having received no antenatal care during the last pregnancy were independent risk factors for seronegativity. Of these, residence in a rural area was the strongest (weighted adjusted OR 17.8 (95% CI; 1.9–165.3)). Only 0.9% (95% CI; 0.0%–2.5%) of urban mothers were seronegative (a single mother in the survey sample). Efforts to increase seroprevalence should be guided by these findings.

WHO recommends reducing missed opportunities for TT vaccination, including at visits mothers make to health facilities to have their children vaccinated (*14, 15*). Visits to health facilities for delivery of a child are also opportunities to vaccinate the mother. We looked at the potential impact of vaccinating mothers at these visits by restricting our analysis to seronegative mothers, and found that 39.5% (weighted analysis) of them gave birth at a health facility or stated their child received the first dose of DPT, although at these visits only 16.5% were eligible to be vaccinated based on their TT vaccination history. We urge that a similar analysis be done in future seroprevalence surveys; our results were imprecise because of the small number of seronegative women, and should be considered as minimum estimates of potential impact, since they included mothers of all children, not just mothers of children old enough to have received their first DPT injection. Even mothers not yet eligible for their next dose of TT can benefit from systematic TT screening at these contacts, since they can be counseled on when to return for their next dose.

The relationship between our TT coverage and tetanus antitoxin seroprevalence estimates may have been affected by selection bias. In all, 9.4% of blood spots could not be matched with a survey questionnaire, due to the theft of questionnaires before the primary identification numbers were entered in the database; and 14.3% of mothers whose blood spots were matched to a questionnaire were excluded from the analysis because they stated they had received TT since their delivery, or were unable to say if they had received TT since their delivery. It is reassuring that the TT coverage of mothers in the seroprevalence analysis (74.4%) was close to the TT coverage (72.9%) among the remaining 934 mothers of children less than one year of age in the MICS (who included both groups of excluded mothers). The household lists and maps used for sampling had been assembled two years earlier for the Demographic and Health Survey. Mothers of children less than one year of age who lived in dwellings constructed after this date were excluded from our survey sample: another source of potential selection bias.

The vaccination of mothers after delivery (for example, at visits when their infants are vaccinated) is a promising means of increasing the proportion of next-born children who are protected against neonatal tetanus, but complicates efforts to estimate serologic protection at birth through household surveys. Excluding mothers vaccinated since delivery makes the seroprevalence sample less representative, while including them means that titres are used in the analysis that were raised by a TT dose since birth. In our study, the exclusion of these mothers made little difference, since seroprevalence, when the mothers were included, increased only slightly (to 89.5%). However, TT vaccination after delivery may pose a more serious difficulty for estimating serological protection at birth in countries where this practice is more frequent.

TT questionnaires were completed only for mothers of surviving children younger than one year of age. The exclusion of mothers of non-surviving children, which simplifies the identification of mothers to be interviewed, is in accordance with WHO guidelines for vaccination coverage surveys (*16*). However, the concept of TT coverage applies to all live births. Therefore, the TT questionnaire for the year 2000 MICS was

completed for mothers of surviving children, as well as for mothers of children who died (*13*).

TT coverage levels in the CAR by the "TT2+" method (the reported number of second, third, fourth and fifth doses of TT administered to pregnant women during a calendar year, divided by the estimated number of newborns during the year), were 10%, 15% and 15%, for 1994, 1995 and 1996, respectively (*17*). Our study shows how misleading such estimates can be. TT2+ coverage estimates can be low because health workers underreport the number of TT doses administered. However, even when vaccination reports are complete and accurate, TT2+ coverage estimates can be low because mothers who do not receive TT during antenatal care, but who are nonetheless protected according to their TT vaccination history, are not included in the numerator, and the resulting error can be expected to increase with time (*18*). The "Protection-at-Birth" method of monitoring protection against neonatal tetanus, in which the TT vaccination status of mothers is determined when their children receive DPT1, is also based on routine reporting from health facilities, but avoids the methodological problems of the TT2+ method, and is recommended by WHO (*19*).

A competition ELISA and a toxin-binding inhibition test have been available for several years that avoid the overestimation of tetanus antitoxin titres in low-titre sera, a drawback of previous in vitro tests, especially for population surveys (*20, 21*). However, both tests are more complicated than routine, solid-phase, indirect ELISAs, because they require additional dilution series or incubation steps. The recently-developed, double-antigen ELISA used in our survey has the advantages of correlating well with in vivo testing, even with low-titre sera, and of being no more complicated to perform than the indirect ELISA method (*8*) .

In the CAR, TT coverage measured by the MICS was a conservative indicator of the prevalence of serological protection against neonatal tetanus. If TT coverage as measured by the MICS similarly underestimates serological protection in other countries, it may not document fully the extent to which the year 2000 goal of protecting at least 90% of newborns against neonatal tetanus was achieved. Based on our point estimate of seroprevalence, this goal was almost reached in the CAR by mid-decade. We found that tetanus seroprevalence surveys are a practical and useful tool when the accuracy of TT coverage estimates as a measure of programme achievement is uncertain. ■

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In Memoriam: Dr Iver Heron, a co-investigator in our study and highly esteemed colleague, succumbed to a chronic illness on 30 October, 1998. His untimely death cut short a career that included major contributions to understanding the serology of bacterial infections and toxins. The competition ELISA and double-antigen ELISA described in our paper were developed under his guidance. We would like to recognize the debt that current advances in tetanus serology owe to his work, and his support of efforts to address health priorities in developing countries.

Conflicts of interest: none declared.

Résumé

La couverture par l'anatoxine tétanique est un indicateur de protection sérologique contre le tétanos néonatal

Objectif Une enquête de type MICS (Multiple-Indicator Cluster Survey), enquête en grappe portant sur de nombreux indicateurs, a été réalisée au milieu de la décennie dans plus de 60 pays en développement pour mesurer la progression vers les objectifs fixés en 2000 au Sommet mondial pour les enfants. Parmi ces objectifs figurait la protection d'au moins 90 % des enfants contre le tétanos néonatal par la vaccination de leur mère, mesurée d'après la couverture par l'anatoxine tétanique (AT). En République centrafricaine, des tests sérologiques ont été ajoutés à l'enquête MICS pour mieux comprendre les relations qui unissent les estimations de la couverture par l'anatoxine tétanique obtenues dans l'enquête et la prévalence de la protection sérologique.

Méthodes Dans l'enquête MICS réalisée en République centrafricaine, les mères des enfants de moins d'un an ont été interrogées sur les antécédents de vaccination par l'anatoxine tétanique en utilisant le questionnaire de l'enquête MICS. Un sous-échantillon de mères a été examiné à la recherche des antitoxines antitétaniques au moyen d'un test ELISA (*enzyme-linked immunoadsorbent assay* : titrage au moyen d'un immunoadsorbant lié à une enzyme) utilisant deux antigènes. La séropositivité était définie par un titre $\geqslant$ 0,01 UI/ml et la couverture par l'anatoxine tétanique par la proportion de mères protégées au moment de l'accouchement documentée par leurs antécédents de vaccination par l'anatoxine tétanique.

Résultats Parmi les 222 mères du sous-échantillon, la couverture par AT, après pondération, était de 74,4 % (IC 95 % : 67,0 %-81,7 %) et la séroprévalence des antitoxines antitétaniques était de 88,7 % (IC 95 % : 83,2 %-94,2 %). Le titre médian pondéré en antitoxines était de 0,35 UI/ml.

Conclusion En République centrafricaine, la couverture par AT est inférieure à la prévalence de la séroprotection contre le tétanos néonatal. Si cette relation reste valable pour d'autres pays, les estimations de la couverture par AT à partir des enquêtes MICS pourraient sous-estimer le degré de réalisation de l'objectif fixé en 2000 concernant la protection contre le tétanos néonatal. De plus, nous avons mis en évidence une protection sérologique élevée dans un pays qui doit faire face à des difficultés majeures au plan de la santé publique et à une grave pénurie de ressources.

Resumen

La cobertura con anatoxina tetánica como indicador de la protección serológica contra el tétanos neonatal

Objetivo A mediados del decenio se llevó a cabo una encuesta a base de indicadores múltiples (MICS) en más de 60 países en desarrollo para medir los progresos realizados hacia las metas fijadas para el año 2000 en la Cumbre Mundial en favor de la Infancia. Entre esas metas figura la protección de al menos el 90% de los niños contra el tétanos neonatal mediante la inmunización de sus madres, estimada en función de la cobertura con anatoxina tetánica (TT). En la República Centroafricana (RCA), además de la MICS se hicieron pruebas serológicas para comprender mejor la relación entre las estimaciones encuestales de la cobertura con TT y la prevalencia de protección serológica.

Métodos En la MICS de la RCA, las madres de niños menores de un año aportaron verbalmente datos sobre las vacunas de TT que habían recibido, respondiendo al cuestionario de la MICS sobre la TT. En una submuestra de madres se determinaron los niveles de antitoxina tetánica mediante una prueba de inmunosorción enzimática (ELISA) con dos antígenos. Se definieron como seropositivas las mujeres con títulos $\geqslant$0,01 UI/ml, y la cobertura antitetánica se definió como la proporción de madres protegidas en el parto a juzgar por sus antecedentes de vacunación con TT.

Resultados Entre las 222 madres de la submuestra, la cobertura ponderada con TT fue del 74,4% (IC95% = 67,0% – 81,7%), y la seroprevalencia de antitoxina tetánica del 88,7% (IC95% = 83,2% – 94,2%). La mediana ponderada del título de antitoxina tetánica fue de 0,35 UI/ml.

Conclusión En la RCA la cobertura con antitoxina tetánica era menor que la prevalencia de protección serológica contra el tétanos neonatal. Si en otros países se da esa misma relación, las estimaciones de la cobertura con TT obtenidas mediante las MICS podrían subestimar el grado de logro de la meta de 2000 de proteger a los niños contra el tétanos neonatal. Hemos demostrado también que se ha logrado un alto nivel de protección serológica en un país enfrentado a grandes retos de salud pública y limitaciones de recursos.

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