Background: Susceptible health care workers are at risk of acquiring and transmitting vaccine-preventable diseases to or from patients. The objective of this study was to assess antibody levels against diphtheria, tetanus, and varicella in healthcare workers.

Methods: Antibody levels against diphtheria, tetanus, and varicella were measured in health care professionals in 2 neonatal units at the Federal University of São Paulo, Brazil.

Results: Between September and November 2002, 215 of 222 (96.8%) health care workers were studied. Of those, 122 (56.7%) gave oral information regarding their vaccination status against diphtheria and tetanus and only 9 (4.2%) had their vaccination cards. Geometric mean antibody levels against diphtheria, tetanus, and varicella were 0.89 IU/mL (95% CI, 0.73 to 1.08), 0.86 IU/mL (95% CI, 0.68 to 1.07) and 1.10 IU/mL (95% CI, 0.98 to 1.24), respectively. Using internationally accepted definitions, 200 (93.0%) and 182 (84.7%) individuals had full protection against diphtheria and tetanus, respectively. Regarding varicella, 213 (99.1%) individuals were immune and 2 (0.9%) had equivocal immunity against varicella. Of 65 (30.2%) individuals without previous history of the illness, 63 (96.9%) were immune against varicella zoster virus.

Conclusions: Based on serologic screening, most professionals were immune to diphtheria, tetanus, and varicella. Absence of previous history of varicella was an unreliable identifier of susceptibility to varicella zoster virus in the health care workers studied.

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Vaccine-preventable diseases are usually more severe in adults than in children. Therefore, assessing HCWs’ immunity against these diseases and vaccinating susceptible workers provide health and safety benefits for both the workers and the patients for whom they care. It is generally accepted that HCWs should be immunized according to the same immunization schedule for the general population. Specific recommendations regarding immunization of HCWs have been published by the Centers for Disease Control and Prevention. When a self-reported questionnaire cannot provide appropriate vaccination status or immunity, a serologic evaluation should be considered. The present study involved assessing antibody levels against diphtheria, tetanus, and varicella in HCWs from 2 neonatal units in São Paulo, Brazil.

METHODS

A cross-sectional study was performed between September and November 2002 to assess diphtheria, tetanus, and varicella immunity in HCWs from 2 neonatal units at the Federal University of São Paulo, São Paulo, Brazil. The research protocol was approved by the Ethics Committee of the Federal University of São Paulo, and all participants provided informed consent before enrollment in the study.

Demographic information regarding sex, age, career, and previous history of disease or vaccination against...
diphtheria, tetanus, and varicella were collected using a questionnaire. To assess immunity against diphtheria, tetanus, and varicella, 5 mL of blood was collected from each study participant, and serum was separated and stored at −20°C until analysis. Antibodies against diphtheria and tetanus were measured by double-antigen enzyme-linked immunosorbent assay (ELISA), as described previously. For diphtheria antibodies, 0.05 μg/mL of diphtheria toxoid (Butantan Institute, São Paulo, Brazil) diluted in 0.1M carbonate-bicarbonate buffer (pH 9.6) was used to coat 96-well microtiter plates (Thermolab Systems, Helsinki, Finland) overnight at 4°C.

Two-fold serial dilutions of serum samples and of diphtheria reference serum (in-house standard calibrated against National Institute for Biological Standards and Control [NIBSC] reagent 91/534, diphtheria antitoxin human serum) in dilution buffer (0.01 M phosphate-buffered saline [PBS], pH 7.2, and 1% Triton X-100) with 1% bovine serum albumin (BSA) were added to the plate and incubated for 1 hour at 37°C. Then biotin-labeled diphtheria toxoid in dilution buffer was added to the plate and incubated for another 1 hour at 37°C. Streptavidin-alkaline phosphatase conjugate (Zymed Technologies, Invitrogen, Carlsbad, CA) in dilution buffer was incubated for 1 hour at 37°C. p-Nitrophenyl-phosphate (Sigma Aldrich, St Louis, MO) in 1 M diethanolamine and 0.005 M magnesium chloride buffer (pH 9.8) was used as a substrate, and the absorbance was read at 450 nm in an immunoreader ELX-800 (Bio-Tek Instruments, Winooski, VT). Between steps, the plate was washed 5 times in dilution buffer.

For tetanus antibodies, the same method was applied with some modifications: 0.08 μg/mL of tetanus toxoid (Butantan Institute), tetanus reference serum (in-house standard calibrated against NIBSC reagent 76/589, tetanus antitoxin human immunoglobulin), and biotin-labeled tetanus toxoid were used as substitutes for the corresponding diphtheria reagents.

Tetanus and diphtheria antibodies were reported in IU/mL using the curve comparison method to transform optical density in concentration units. Patients were classified according to internationally accepted criteria of protective antibody levels. When tetanus and diphtheria antibody concentrations were < 0.01 IU/mL, the immune status was considered absence of protection; at concentrations of 0.01 to 0.09 IU/mL, immune status was considered basic immunity; and at values of 0.1 IU/mL or higher, immune status was considered full protection.

VZIG antibodies were assessed by in-house indirect ELISA. Briefly, Immulon 2 96-well microtiter plates (Dynex, Chantilly, VA) were coated with VZV (Varilrix, Rixensart; SmithKline Beecham, Belgium) diluted 1:100 in 0.1 M carbonate–bicarbonate buffer (pH 9.6) and incubated overnight at 4°C. Two-fold serial dilutions of serum samples and of varicella reference serum (in-house standard calibrated against NIBSC reagent 90/690, VZV antibody human immunoglobulin) in dilution buffer (0.01 M PBS [pH 7.2] and 0.05% Tween 20) with 1% BSA were added to the plate and incubated for 1 hour at 37°C. Then alkaline phosphatase-conjugated rabbit anti-human IgG, specific for γ-chains (Dako, Glostrup, Denmark), in dilution buffer was incubated for 1 hour at 37°C. p-Nitrophenyl-phosphate-disodium (Sigma Aldrich) in 0.1 M diethanolamine and 0.005 M magnesium chloride buffer (pH 9.8) was used as a substrate. Optical density was read at 405 nm in a Bio-Tek Instruments ELX-800 immunoreader, using 630 nm as a reference filter. Between steps, the plate was washed 5 times in dilution buffer. VZV antibodies are expressed in IU/mL using the curve comparison method to transform optical density into concentration units.

To analyze varicella immunity, levels of antibody activity for VZV antibodies were considered as follows: susceptibility (< 50 mIU/mL), equivocal (50 to 100 mIU/mL), and immunity (> 100 mIU/mL), as published by Ory et al. Statistical analysis

Due to the nonnormal distribution of the data, values were log-transformed before analysis. Statistical analysis was carried out using StataC, version 7.0 for Windows (StataCorp, College Station, TX). Comparison of antibody levels among different professional categories and different age groups (age 20 to 29, 30 to 39, 40 to 49, and 50 to 64 years) was performed by 1-way analysis of variance (ANOVA). Proportions of immune individuals in different professional categories and age groups were compared using the χ² association test or Fisher’s exact test. The differences were considered significant at P ≤ .05.

RESULTS

Between September and November 2002, 222 HCWs were working in the 2 neonatal units at the Federal University of São Paulo, Brazil. Of these, 215 (97%) signed informed consent and were enrolled in the study. The hospital staff comprised 31 neonatologists (14.4%), 134 nurses (62.5%), 24 pediatric residents (11.2%), 20 physiotherapist residents (9.5%), 1 nutritionist, 1 psychologist, and 4 administration staff. Mean age of the study participants was 35.3 years (range, 20.7 to 64.0 years) and 202 were female (94.0%).

Of the 215 participants, 122 (56.7%) gave oral information regarding their previous vaccination status...
against diphtheria and tetanus. Of these, only 9 (4.2%) showed their vaccine card. A history of previous varicella infection was reported by 70% (150/215). The geometric means of antibody levels against diphtheria, tetanus, and varicella measured in the 215 HCWs were 0.89 UI/mL (95% confidence interval [CI] 0.73 to 1.08), 0.86 UI/mL (95% CI 0.68 to 1.07), and 1.10 UI/mL (95% CI 0.98 to 1.24), respectively.

Based on the internationally accepted definitions, 200 of the 215 HCWs (93.0%) were fully protected and 15 (7.0%) had basic protection against diphtheria. Regarding tetanus, 182 of the HCWs (84.7%) were immune and 33 (15.3%) had basic protection. In relation to varicella, 213 of the HCWs (99.1%) were considered immune and 2 (0.9%) had equivocal immune status against this disease. All individuals with previous history of varicella infection had full immunity against the illness. Of 65 HCWs without previous history of infection, only 2 (3.0%; 1 nurse and 1 administrative staff member) had equivocal immunity against varicella.

Comparison by 1-way ANOVA revealed no differences in antibody levels against tetanus, diphtheria, and varicella among different professional categories of HCWs (Table 1). Analysis of mean geometric titers of antibodies stratified by age group showed that HCWs age 50 and older had the lowest geometric mean antibodies against tetanus, those age 20 to 29 had the highest geometric mean antibodies against diphtheria, and those age 20 to 29 and 30 to 39 had the highest geometric mean antibodies against varicella. These differences did not reach statistical significance, however (Table 2).

Levels of immunity against diphtheria according to professional categories and age groups are shown in Table 3. All professional categories presented similar percentages of individuals with full protection. In terms of age groups, HCWs age 50 to 49 years had a smaller proportion of individuals with full immunity compared with the 20 to 29 and 50 to 64 age groups; however, this difference did not reach statistical significance (P = .052).

For tetanus antibodies, no differences in the percentage of HCWs with full immunity according to age (P = .677) or professional category (P = .066) were found (Table 4).

**DISCUSSION**

Infants in neonatal intensive care units may be at greater risk for vaccine-preventable diseases after exposure compared with healthy term neonates. They have a decreased immune response, as well a lower antibody level acquired through the placenta; this is especially attenuated in those born before 32 weeks of gestation.1,2 In addition, during their hospital stay, critically ill newborns are submitted to multiple blood samplings for diagnostic testing, which remove antibody-containing plasma. Blood replacement with packed red blood cells, which contain only small amounts of plasma, might decrease the levels of these antibodies even more.16 For these reasons, the control of vaccine-preventable diseases is important to improve the quality of neonatal care. However, even in developed countries, this control is usually
achieved by vaccination of HCWs and serologic screening for only specific diseases, such as varicella.

In the present study, although HCWs were supposed to be immunized with the same vaccination schedule applied to the general population, most of them were not aware of their immunization schedule and had not kept their vaccination cards. This made evaluation of immunity against diphtheria and tetanus more difficult, because we had to rely on serology results. Based on serology analysis, we found that most of the HCWs of the Neonatal Units from the Federal University of São Paulo were immune to diphtheria and tetanus, despite the paucity of vaccination records for these diseases.

Regarding varicella, 213 of the 215 HCWs (99.1%) were immune against varicella, and 2 (0.9%) had equivocal immunity. The history of previous varicella infection was found to be reliable, with sensitivity and positive predictive value of 100%, meaning that 100% of the HCWs had protective levels of antibodies, similar to the rates recently reported by others. However, a negative history of previous illness had poor sensitivity to detect susceptible individuals. Of the 65 HCWs with a negative history of previous varicella infection, 63 (96.9%) were immune against varicella. In a study of 356 nurses in training in Scotland, Waclawski and Stewart concluded that the absence of past history of chickenpox or shingles is an unreliable identifier of susceptibility to VZV in HCWs. These authors found that the positive predictive value of a history of VZV infection for seropositivity was 98% (286/292) and the negative predictive value was 14% (9/64). Screening using past clinical history compatible with VZV infection would have missed 40% of those possibly susceptible to VZV on the basis of the ELISA IgG test. Similar results were reported by Stover et al., who found that 26 of 27 HCWs with uncertain or negative history of varicella infection had detectable antibodies against VZV.

In the present study, the finding of 65 (30%) HCWs with no previous history of varicella and 2 (0.9%) not fully protected HCWs working in the 2 neonatal units is cause for concern. These results reinforce the need to investigate HCWs’ immunity against varicella before they are admitted to a neonatal unit, considering that management of exposure to varicella in a neonatal intensive care unit can be costly and lead to extra work.

The most immediate need is to establish who should receive VZIG that, as passive immunization, should be carried out as soon as possible to attain maximum benefit. It is recommended that no more than 96 hours elapse from the time of exposure to the administration of VZIG. All preterm infants born before 28 weeks of gestation, regardless of whether or not the mother had varicella infection before gestation, as well as those of any gestational age whose mothers did not have varicella should receive specific immunoglobulin against varicella. In addition, a cohort of susceptible patients who cannot be discharged and susceptible staff also should be followed. For preterm infants, a 28-day quarantine period is needed if VZIG is administered. For adults, a serologic antibody screening in those with a negative or uncertain history of previous varicella infection, followed by vaccination of susceptible individuals would be more cost-effective.

Based on the internationally accepted definitions, none of the HCWs was susceptible to diphtheria or tetanus; however, 7.0% were not fully protected against diphtheria, and 15.3% had only basic protection against tetanus. The prevalence of immunity against
diphtheria observed in the present study was higher than that found by other researchers. John et al., using the same criteria, found that of 100 hospital staff members in a German study, only 26% were fully protected, 48% had basic protection, and 26% were susceptible to diphtheria. Heath et al. found that among 51 healthy volunteers aged 18 to 59 years at a teaching hospital in Australia, 57% had serologic evidence of full protection, 31% had basic protection, and 12% were susceptible to diphtheria, whereas 90% were immune to tetanus. The higher level of immunity against diphtheria and tetanus found in the present study suggests immunity conferred through vaccination. Actually, a reduction in the prevalence of both diseases in all groups has been noted in Brazil in recent years.

Most of the HCWs in the present study as immune against diphtheria and tetanus, although 7% to 15% had only basic protection, demonstrating the need for booster vaccinations with tetanus and diphtheria toxoids every 10 years. This measure has become a standard of care worldwide.

However, pertussis has become more prevalent in the United States over the last 20 years, particularly among adolescents and adults. Childhood vaccination against pertussis provides only 5 to 10 years of immunity, and antibiotics do little to affect symptoms once coughing begins. An increased incidence of disease in older people, such as HCWs, may be of public health significance, because these people could serve as reservoirs for Bordetella pertussis infections in very young infants without protective antibody levels, in whom pertussis can be severe and even life-threatening. Antimicrobial therapy, although effective in eradicating the organism from the respiratory tract, does not alter the progression of disease unless given early, during the catarrhal phase, when pertussis is rarely suspected. Therefore, the control of this disease must be based on preventive vaccination of HCWs, because newborn infants might have not acquired adequate antibody levels through the placenta. Consequently, the current recommendations are for all adults age 19 to 64 years to receive booster doses of pertussis.

Pichichero et al. assessed the immunogenicity and reactogenicity of a tetanus-diphtheria 5-component (pertussis toxoid, filamentous hemagglutinin, pertactin, and fimbriae types 2 and 3) acellular pertussis vaccine (Tdap) in 4480 adolescents and adults age 11 to 64 years and found comparable safety and reactogenicity between Tdap and Td (tetanus and diphtheria toxoids). For both Tdap and Td, more than 94% of the subjects had protective antibody concentrations of at least 0.1 IU/mL for diphtheria and nearly 100% had the same for tetanus. Geometric mean antibody titers to pertussis toxoid, filamentous hemagglutinin, pertactin, and fimbriae types 2 and 3 exceeded (by 2.1 to 5.4 times) the levels in infants who had received 3 doses of the analogous DTap vaccine.

According to Celikbas et al., the serologic screening of all HCWs, followed by vaccination of susceptible individuals against varicella, is cost-effective. However, the most cost-effective assessment usually recommended is determination of the serologic status of individuals with no previous history of illness. Our findings also support this policy; all HCWs with a positive history of previous varicella were immune, and among those 65 HCWs with no previous history, only 2 (0.5%) had equivocal immunity against varicella.

In the present investigation, the geometric mean antibody levels against diphtheria, tetanus, and varicella were similar among different professional categories. These results suggest that all professional categories have the same probability of being susceptible to vaccine-preventable diseases.

Antibody levels according to age group were analyzed because antibodies produced by routine childhood immunization against diphtheria and tetanus provide immunity for about 10 years, after which booster vaccines are necessary to maintain protection against both diseases. Heath et al. found that only 52% of adults over age 49 years had protective tetanus antibody levels. In the present study, no statistical differences were noted among the different age strata. The reported percentage of HCWs susceptible to varicella varies from 1.1% to 8.8%. In our study, the high prevalence of immunity likely was due to natural infection, because varicella vaccine is not available in the routine Brazilian calendar.

The limitations of our study were a predominance of women (94%) and a small number of professionals from the administrative area (6) compared with other categories of HCWs. In our investigation, we intended to include all of the HCWs in the 2 neonatal units, but were able to include 215 of 222 (97%). Women comprise the great majority of neonatal unit staff, at least in Brazil. Whether or not women have better immune status than men remains controversial. In developed countries, men may be better protected, probably because of additional immunizations given during military service or professional activities. In India, where tetanus toxoid is routinely given to women to prevent neonatal tetanus, the immunity status of women is generally better than that of men. In addition, for a study of vaccine-preventable diseases, it is important to include all people circulating in the neonatal unit. Interestingly, in the present study, there were 2 HCWs (1 nurse and 1 administrative staff member) with equivocal immunity against varicella. This suggests the importance of testing immunity in all workers in the neonatal unit, regardless of their function.
Our results demonstrate that most of the HCWs in the 2 neonatal units surveyed were immune to diphtheria, tetanus, and varicella. Nonetheless, all HCWs should be advised to receive booster doses of diphtheria, tetanus, and acellular pertussis. In the case of varicella, those HCWs without a clear history of previous infection should be assessed for antibody levels and vaccinated if susceptible.

References