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Estimation of Vaccine Efficacy in the Presence of Waning: Application to Cholera Vaccines

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The authors present a nonparametric method for estimating vaccine efficacy as a smooth function of time from vaccine trials. Use of the method requires a minimum of assumptions. Estimation is based on the smoothed case hazard rate ratio comparing the vaccinated with the unvaccinated. The estimation procedure allows investigators to assess time-varying changes in vaccine-induced protection, such as those produced by waning and boosting. The authors use the method to reanalyze data from a vaccine trial of two cholera vaccines in rural Bangladesh. This analysis reveals the differential protection and waning effects for the vaccines as a function of biotype and age. *Am J Epidemiol* 1998;147:948–59.

cholera; communicable diseases; epidemiologic methods; statistics; survival analysis; vaccines

Vaccine efficacy (VE) is generally estimated by VE = 1 − RR, where RR is some measure of relative risk in the vaccinated compared with the unvaccinated group. The VE may wane with time, so that the relative risk estimates also change with time. Thus, we would like to estimate the VE at time, t, after vaccination, i.e., $VE(t) = 1 - RR(t)$. For time-to-event (infection or illness) data, the $RR(t)$ is usually measured by a rate ratio. However, this is a difficult task if the vaccine effects do not follow a simplified model, such as exponential decline in protection. Gilks et al. (1) modeled the rate of waning of antibody titer, but many efficacy studies have illness data, not antibody titers, as outcomes. Furthermore, the relation between actual protection and immune markers as surrogates for protection is not well understood. Farrington (2) demonstrated that conclusions about waning of vaccine-induced immunity from field observations depend on assumptions about the distribution of vaccine protection and the choice of efficacy measures, but did not deal with estimation. One approach to detect waning of protection from field observations has been to calculate the vaccine efficacy from relative Poisson rates for successive time periods (3), but this approach does not yield an easily interpretable estimate of the rate of waning of protection. Several methods have been developed for nonparametric estimation of time-varying relative risks (4–8). In this paper, we present a method for nonparametrically estimating $VE(t) = 1 - RR(t)$ from time-to-event data when the protective effects of the vaccine can change over time. If we let $\lambda_{unvac}(t)$ and $\lambda_{vac}(t)$ be the population-level, case hazard rates in the unvaccinated and vaccinated groups, respectively, then $RR(t) = \lambda_{vac}(t)/\lambda_{unvac}(t)$. The method that we present here involves constructing smoothed curves for the estimated $RR(t)$.

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Abbreviations: BS-WC, B subunit killed whole cell; CI, confidence interval; RR, relative risk; VE vaccine efficacy; WC, killed whole cell.

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MATERIALS AND METHODS

Estimating vaccine efficacy

To estimate $RR(t)$ nonparametrically, we use a method based on smoothing scaled residuals from a proportional hazards model (6–9) (details are given in the Appendix). In general, we code vaccine effects with a dichotomous variable $z = 1$ for vaccine and $z = 0$ for placebo, with $\beta(t)$ as the time-varying coefficient for the vaccine effect. Then our goal is to find the smoothed VE estimate $\widehat{VE}(t) = 1 - \widehat{RR}(t) = 1 - e^{\hat{\beta}(t)}$ and its standard error. The procedure consists of four main steps. The first step is to fit an ordinary proportional hazards model to the data using the partial

likelihood function. The second step is to compute the scaled differences between the actual and expected covariate values at each event time. These differences are referred to as Schoenfeld residuals (6, 7). The third step is to scale these residuals and add the coefficient from the ordinary proportional hazards model. Then, the fourth step is to recover the time-varying regression coefficient, $\beta(t)$, by smoothing the sum of the estimated proportional hazards coefficient and the rescaled Schoenfeld residuals over time. Conceptually, we are nonparametrically estimating the instantaneous hazard rate ratio $RR(t) = e^{\beta(t)}$. The method also provides a hypothesis test for departures from the proportional hazards assumption (7), the null hypothesis being that the vaccine effect does not vary with time. The null hypothesis of no time-varying effects, or proportional hazards, can be written as $H_0: \beta(t) = \beta$ for all t.

Cholera vaccine trial in Bangladesh

As an example, we estimate the efficacy of killed whole-cell-only (WC) and B subunit killed whole-cell (BS-WC) oral cholera vaccines over 4½ years of a vaccine trial in rural Matlab, Bangladesh (see Clemens et al. (3) for details). The placebo was a killed *Escherichia coli* strain. The trial was randomized and double-blinded among 89,596 subjects aged 2–15 years (male and female) and greater than 15 years (females only). We restrict our analyses to subjects that received three doses of vaccine or placebo (i.e., the full vaccination regimen) before May 1, 1985. There were 20,837, 20,743, and 20,705 such subjects in the placebo, WC, and BS-WC arms of the trial, respectively. The data have been previously analyzed by Clemens et al. (3) and van Loon et al. (10) using the Poisson rates from successive time periods to assess waning protection.

RESULTS

The events of interest are reported, confirmed cases of cholera illness among the study subjects. Cases were classified into the two major biotypes that circulated during the trial, classic and El Tor cholera. Figure 1 shows a plot of the total number of reported cholera cases for the 4½-year period from May 1, 1985, through November 31, 1989, among subjects that received three doses of vaccine or placebo. Note that cholera incidence was highly seasonal. We computed Kaplan-Meier estimates of the cholera case-survival curves, $S(t)$, for the placebo and two vaccine groups. Figure 2 shows plots of $\ln(-\ln S(t))$ of the Kaplan-Meier estimates of the survival curves for the placebo and two vaccines. The fact that there is good separation between the vaccine and placebo curves indicates that the vaccines give protection. The BS-WC vaccine provides better protection during the first year. The curves slowly approach one another indicating the waning of protective effect, but this is difficult to see with plots based on cumulative incidence.

We used the method described above to estimate smooth plots of the $VE(t)$. The smoothing was carried out with regression splines with four degrees of freedom. In those analyses which were not stratified by age group, we control for age effects by including a term for age group in the model. Figure 3 shows the

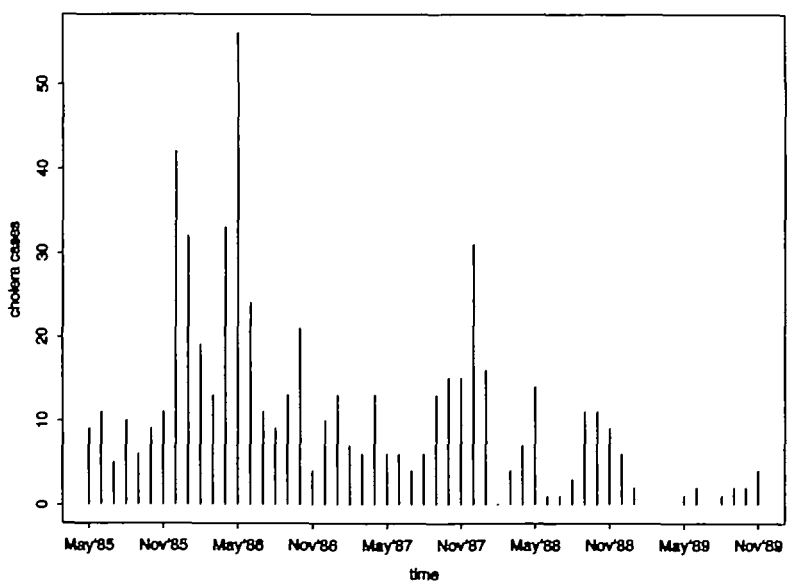

FIGURE 1. Total number of reported cholera cases in the vaccine trial in Matlab, Bangladesh, for the 4½ year period from May 1, 1985, through November 31, 1989. $S(t)$, cholera case-survival curves.

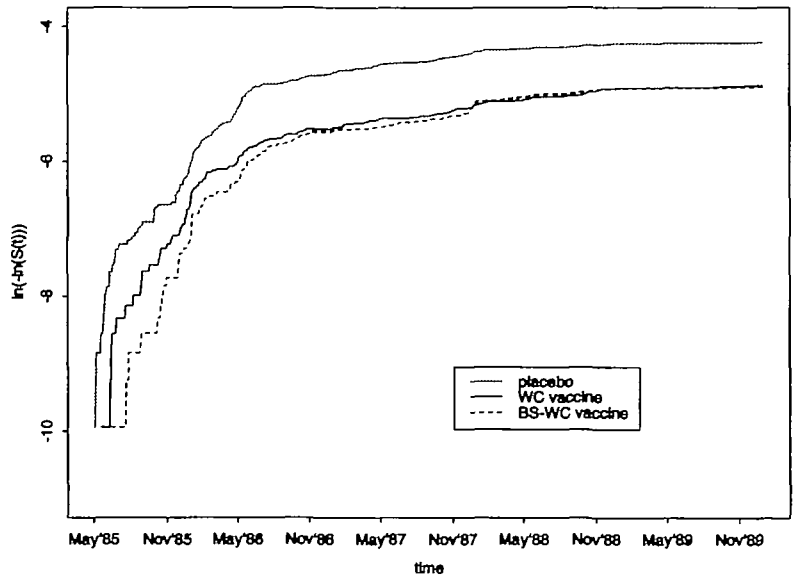

FIGURE 2. Log-minus-log plots of the Kaplan-Meier estimates of the survival curves for the placebo and two vaccines, Matlab, Bangladesh, May 1, 1985, through November 31, 1989. WC, whole killed cell; BS-WC, B subunit whole killed cell.

plot of the VE(t) estimates for WC and BS-WC vaccines for both biotypes, and table 1 gives the efficacy estimates and approximate 95 percent confidence intervals for selected time points throughout the study. The p values for the hypothesis test for departures from the proportional hazards assumption are 0.008 and 0.002 for the estimated model of the WC and BS-WC vaccines, respectively. This indicates that there is significant waning. The WC vaccine gives fairly constant and significant protection, with a VE of about 0.50, for the first 2½ years of the trial, but then protection appears to wane rapidly. After 3 years of the trial (May 1988), the point estimate of the VE is 0.245 and the 95 percent confidence interval covers zero. Protection from WC-BS vaccine starts out higher than for the WC vaccine, i.e., 0.713 versus 0.430, but then gradually wanes at a fairly constant rate, i.e., about 2–3 percent per month.

Figure 4 and tables 2 and 3 give the biotype-specific estimated VE of the two vaccines. There were no classic biotype cholera cases after the third year. On average, both vaccines give better protection against the classic than against the El Tor biotype. The WC vaccine gives fairly constant and significant protection against the classic biotype, with an estimated VE of about 0.65, for the first 2½ years of the trial. For the El Tor biotype, such protection is estimated to be about VE = 0.40 for the first 2½ years of the trial. The initial VE estimates (i.e., at time zero) for the BS-WC vaccine were 0.848 and 0.715 against the classic and El Tor biotypes, respectively. However, protection waned with time.

Figure 5 and tables 4 and 5 give the age-specific estimated VE of the two vaccines. Both vaccines give little protection in the 2- to 5-year age range, but significant protection to those older than age 5 years. Figures 6 and 7, and tables 6-9, give the biotype-specific and age group-specific estimated VE for both vaccines. For the >5 years age range, both vaccines provided significant protection against both biotypes during the first 2 years of the trial. For the 2- to 5-year age range, both vaccines provided some protection against the classic biotype during the first 2 years, but little protection against the El Tor biotype, although the WC vaccine does appear to give some very short-term protection against the El Tor biotype.

The results of our analyses of the cholera vaccine trial data are similar to the results of the previous analyses of the data (3, 10). In both those analyses, the vaccine trial period was partitioned into discrete periods, usually years. Then, the cholera incidence rates, R, in the placebo and vaccine groups were calculated as the number of cases divided by the number of person-days of risk for each period, i.e., R = (number of cases during the period/person-days of risk during the period). The vaccine efficacy for a period was estimated as $1-(R(\text{vaccinated})/R(\text{placebo}))$. The usual statistics were computed for period-specific hypothesis tests and confidence intervals. For example, Clemens et al. (3) estimated the VE for the BS-WC vaccine to be 0.62 for the first year, with 0.50 as the lower boundary of the one-tailed 95 percent confidence interval. We produce a continuous estimate over the first year (table 1) which is 0.713 (95 percent

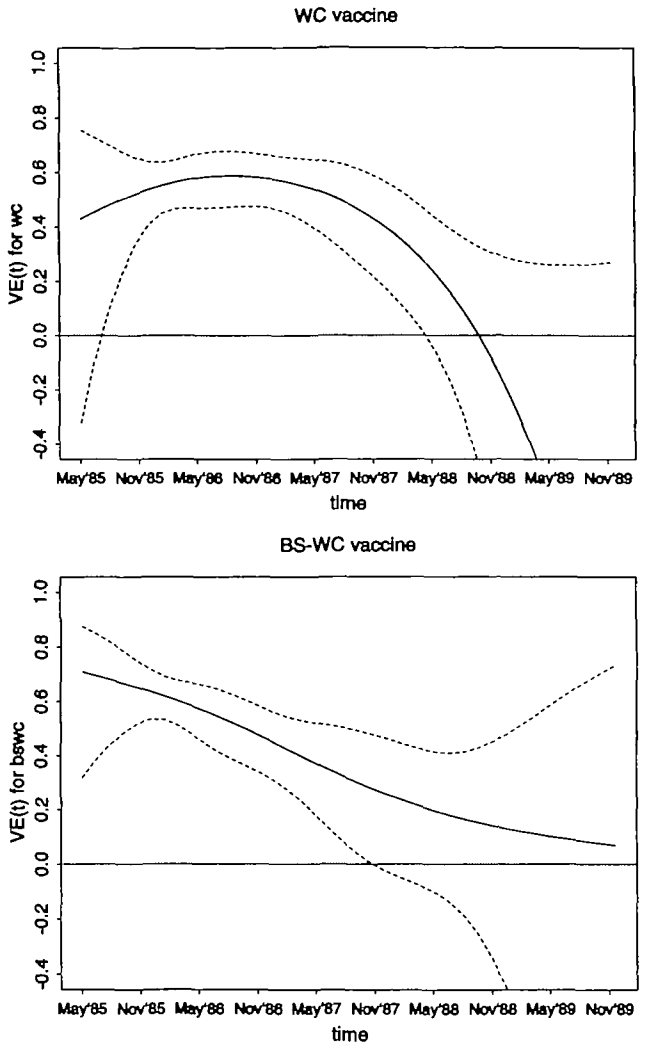

FIGURE 3. Nonparametric smoothed plots of $\widehat{VE}(t)$ versus t, with 95% confidence intervals, for the whole killed cell (WC) and B subunit whole killed cell (BS-WC) vaccines, Matlab, Bangladesh, May 1, 1985, through November 31, 1989. VE, vaccine efficacy; t, time.

confidence interval (CI) 0.320–0.879) at day zero, 0.650 (95 percent CI 0.523–0.743) at 6 months, and 0.572 (95 percent CI 0.457–0.662) at 1 year. Thus, our estimate at the middle of the first year is very close to that of Clemens et al. for the first year. However, our continuous estimate shows the persistent waning over the first year, an observation which is lost when the data from the first year are aggregated as in Clemens et al. Continuing the comparison, for the BS-WC vaccine, Clemens et al. estimated the VE for the BS-WC vaccine to be 0.17 for the third year, with a −0.15 lower boundary of the one-tailed 95 percent confidence interval. Our estimate for the middle of the third year is 0.280 (95 percent CI 0.006–0.478), with an estimate of 0.202 (95 percent CI −0.089 to 0.416) at the end of the third year. Thus, the Clemens et al. estimate is a bit too low. This discrepancy is largely due to an aberrant large spike of cases in the BS-WC vaccine arm in December 1987, which the nonparametric method presented here smooths out.

Although both vaccines provide protection that wanes with time, figures 3–7 reveal that the waning pattern varies as a function of type of vaccine, infecting cholera subtype, and age group. Overall, the WC vaccine provides fairly constant protection for the first 2 years, followed by a relatively fast decrease in protection. In contrast, the BS-WC vaccine provides better initial protection than the WC vaccine, followed by steady waning over the entire 4½ years of observation. Although both vaccines protected people in the >5 year age group better than those in the 2- to 5-year age group, figures 6 and 7 reveal considerable interaction. For the 2- to 5-year age group, there appears to be early waning (most pronounced for the El Tor biotype) for the BS-WC vaccine, but not for the WC vaccine. In addition, protection for the first 2 years is nearly identical for the BS-WC and WC vaccine against the classic biotype. In contrast, the WC vaccine gives no protection at all against the El Tor biotype, while the BS-WC vaccine gives only some possible very early protection against the El Tor biotype. For the >5 year age group, the WC vaccine appears to give relatively sustained protection against the classic biotype, and the BS-WC vaccine seems to confer relatively sustained protection against the El Tor biotype. The WC vaccine seems to provide only 2-year protection against the El Tor biotype, and the same seems to be true of the BS-WC vaccine against the classic biotype.

DISCUSSION

We apply a method for nonparametrically estimating VE(t) as a smoothed, continuous function of time since vaccination. Although VE is only estimated at event times, smooth plots of this function help the investigator visualize how the protective effects of the vaccine vary over time. When the VE varies with time since vaccination, there is no simple test for whether the vaccine gives statistically significant protection. There may be significant protection at one time period, but not for another. However, the point-wise 95 percent confidence intervals on the VE(t) provide rough estimates of when the vaccine is providing statistically significant protection. Although we have applied the technique to the problem of detecting and estimating the waning effects of vaccine-induced protection, we could assess any time-dependent vaccine effect (8). For example, in some trials, where vaccination occurs with multiple doses over a long period of time, pro-

TABLE 1. Estimated VE(t)*, with 95% confidence intervals (CI), for the WC* and BS-WC* vaccines, Matlab, Bangladesh, May 1, 1985, through November 31, 1989

Date	Day	WC vaccine		BS-WC vaccine	
		VE(day)	95% CI	VE(day)	95% CI
May 1985	0	0.430	−0.342 to 0.758	0.713	0.320 to 0.879
November 1985	183	0.525	0.356 to 0.650	0.650	0.523 to 0.743
May 1986	365	0.579	0.467 to 0.667	0.572	0.457 to 0.662
November 1986	548	0.583	0.478 to 0.667	0.476	0.344 to 0.582
May 1987	730	0.538	0.394 to 0.648	0.374	0.176 to 0.524
November 1987	913	0.433	0.220 to 0.588	0.280	0.006 to 0.478
May 1988	1,095	0.245	−0.028 to 0.445	0.202	−0.089 to 0.416
November 1988	1,278	−0.073	−0.664 to 0.308	0.141	−0.338 to 0.448
May 1989	1,460	−0.590	−2.400 to 0.257	0.092	−0.955 to 0.578

* VE, vaccine efficacy; t, time; WC, whole cell; BS-WC, B-subunit whole cell.

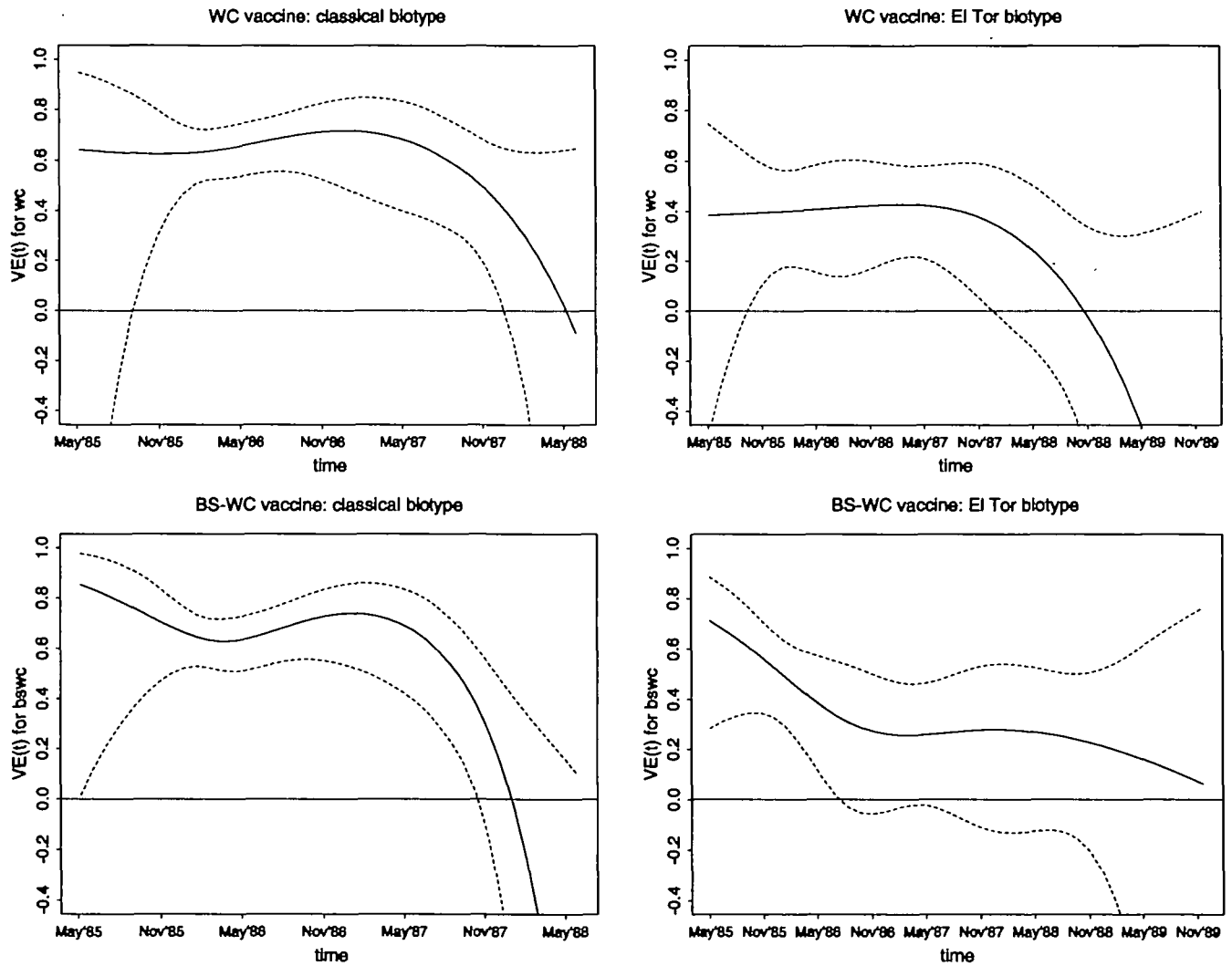

FIGURE 4. Nonparametric smoothed plots of $\widehat{VE}(t)$ versus t, with 95% confidence intervals, for the whole killed cell (WC) and B subunit whole killed cell (BS-WC) vaccines, stratified by biotype, Matlab, Bangladesh, May 1, 1985, through November 31, 1989. There were no classic cholera biotype cases after the third year. VE, vaccine efficacy; t, time.

TABLE 2. Estimated VE(t)*, with 95% confidence intervals (CI), for the WC* and BS-WC* vaccines, classic biotype, Matlab, Bangladesh, May 1, 1985, through November 31, 1989

Date	Day	WC vaccine		BS-WC vaccine	
		VE(day)	95% CI	VE(day)	95% CI
May 1985	0	0.643	−1.631 to 0.951	0.848	0.070 to 0.978
November 1985	183	0.626	0.315 to 0.795	0.707	0.471 to 0.838
May 1986	365	0.656	0.536 to 0.745	0.636	0.513 to 0.728
November 1986	548	0.711	0.532 to 0.821	0.713	0.540 to 0.821
May 1987	730	0.686	0.406 to 0.834	0.679	0.402 to 0.828
November 1987	913	0.509	0.211 to 0.694	0.331	0.063 to 0.579
May 1988†	1,095	−0.006	−1.799 to 0.638	−1.216	−5.029 to 0.185

* VE, vaccine efficacy; t, time; WC, whole cell; BS-WC, B-subunit whole cell.
† There were no cholera classic cases after the third year.

TABLE 3. Estimated VE(t)*, with 95% confidence intervals (CI), for the WC* and BS-WC* vaccines, El Tor biotype, Matlab, Bangladesh, May 1, 1985, through November 31, 1989

Date	Day	WC vaccine		BS-WC vaccine	
		VE(day)	95% CI	VE(day)	95% CI
May 1985	0	0.387	−0.504 to 0.750	0.715	0.283 to 0.887
November 1985	183	0.393	0.103 to 0.590	0.561	0.343 to 0.706
May 1986	365	0.404	0.148 to 0.583	0.383	0.109 to 0.573
November 1986	548	0.420	0.168 to 0.596	0.273	−0.055 to 0.499
May 1987	730	0.424	0.211 to 0.579	0.261	−0.021 to 0.465
November 1987	913	0.378	0.055 to 0.591	0.279	−0.109 to 0.532
May 1988	1,095	0.245	−0.145 to 0.501	0.269	−0.121 to 0.523
November 1988	1,278	−0.016	−0.559 to 0.338	0.227	−0.200 to 0.502
May 1989	1,460	−0.460	−2.074 to 0.306	0.160	−0.807 to 0.610

* VE, vaccine efficacy; t, time; WC, whole cell; BS-WC, B-subunit whole cell.

tection may at first increase and then decrease with time.

The results of this method must be interpreted carefully. Smoothed values at the beginning and end of the observation period are uncertain, with large confidence intervals. This is a typical effect of smoothing which is exacerbated when the number of events decreases near the end of the observation period. For example, in the cholera vaccine trial data analyzed in this paper, overall cholera incidence began to drop during the last year of the trial (see figure 1). Thus, the VE estimates during the last year are unreliable. Nonetheless, the estimated efficacy clearly appears to wane (e.g., see figure 3). The approach for estimating VE(t) based on the scaled Schoenfeld residuals (6, 7) provides a graphic interpretation of time-varying effects of VE as well as a test for departure from the proportional hazards assumption. Plots of $\ln(-\ln S(t))$ are frequently used to assess graphically whether the proportional hazards assumption holds for time-to-event data. Since these are cumulative hazard function plots, they can fail to give a clear picture of time-varying effects that occur later in the study after a substantial number of events have occurred. Figure 2 provides a good illustration of this problem. The placebo and vaccine curves should be roughly parallel for all time if there were no time-varying effects. The early waning of protection of the BS-WC vaccine is readily apparent, especially with respect to the WC vaccine. The placebo and BS-WC are clearly further apart around November 1985 than they are after November 1986. In addition, the protective effects of the two vaccines appear to be converging after November 1986. It is much harder to see the later waning of both vaccines after the first 3 years. Close inspection reveals that the vaccine and placebo curves are closer together around May 1989 than they are around November 1985. In contrast, the nonparametrically estimated smoothed VE(t) (figure 3) gives a much clearer picture of the time-varying effects. If the proportional hazards assumption were valid, then these curves should be roughly straight lines, with zero slope. We refer to such protection as leaky or as partial protection (11–13). The curves in figure 3 clearly have negative slope. In addition, the null hypothesis of a constant effect over time was rejected for both vaccines. This test, however, can be underpowered for small numbers of events (7).

Different underlying biologic mechanisms, besides waning of protection, can produce time-varying estimates of VE(t). If the immune protection induced by the vaccine is boosted by exposure to natural infection,

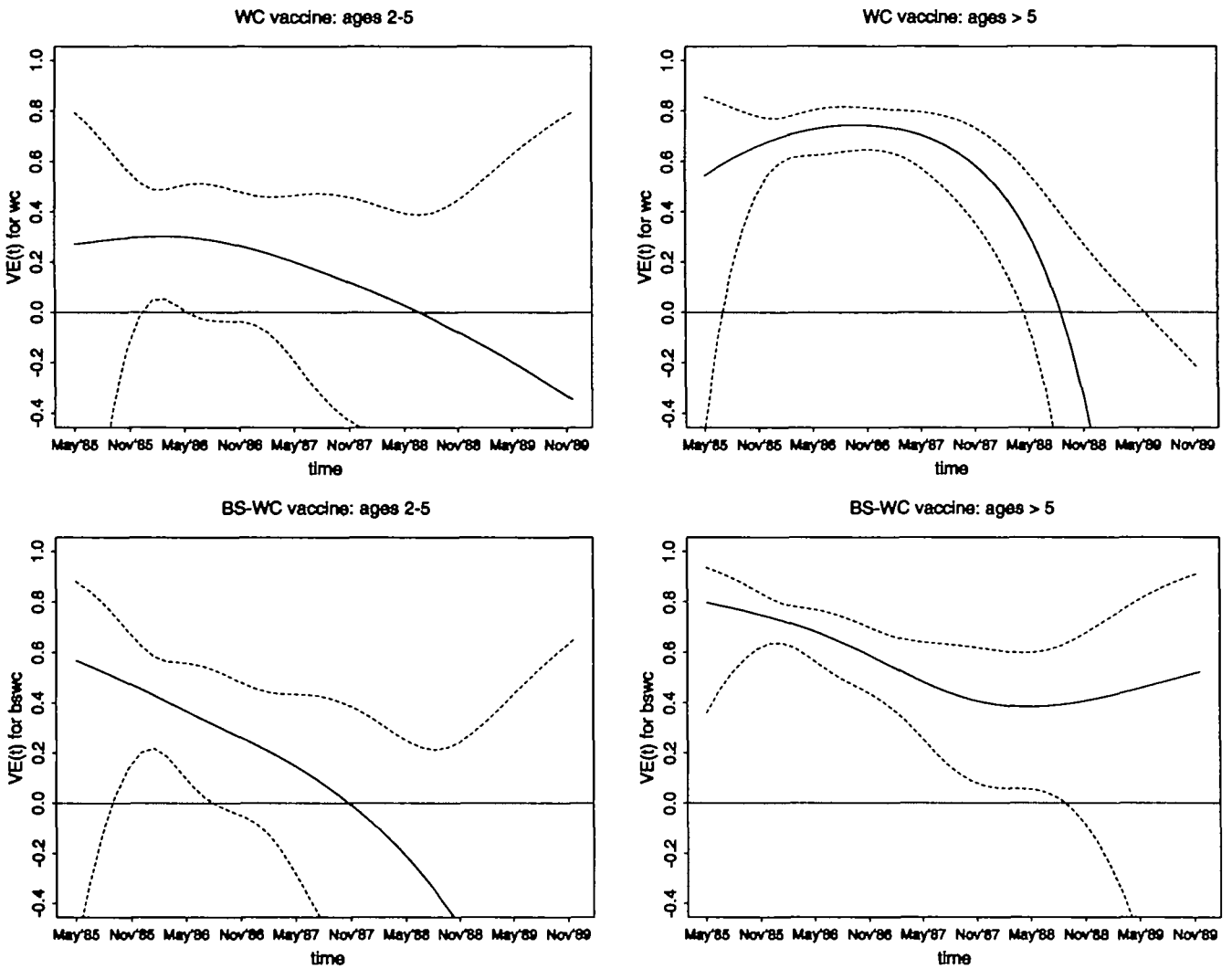

FIGURE 5. Nonparametric smoothed plots of $\widehat{VE}(t)$ versus t, with 95% confidence intervals, for the whole killed cell (WC) and B subunit whole killed cell (BS-WC) vaccines, stratified by age group, Matlab, Bangladesh, May 1, 1985, through November 31, 1989. VE, vaccine efficacy; t, time.

TABLE 4. Estimated VE(t)*, with 95% confidence intervals (CI), for the WC* and BS-WC* vaccines, ages 2- to 5-years, Matlab, Bangladesh, May 1, 1985, through November 31, 1989

Date	Day	WC vaccine		BS-WC vaccine	
		VE(day)	95% CI	VE(day)	95% CI
May 1985	0	0.273	−1.642 to 0.800	0.578	−0.573 to 0.887
November 1985	183	0.298	−0.112 to 0.557	0.474	0.160 to 0.671
May 1986	365	0.300	0.001 to 0.509	0.364	0.087 to 0.557
November 1986	548	0.265	−0.031 to 0.475	0.261	−0.042 to 0.476
May 1987	730	0.201	−0.203 to 0.470	0.152	−0.288 to 0.441
November 1987	913	0.122	−0.421 to 0.457	0.001	−0.630 to 0.388
May 1988	1,095	0.030	−0.544 to 0.390	−0.206	−0.936 to 0.249
November 1988	1,278	−0.077	−1.085 to 0.444	−0.487	−1.915 to 0.241
May 1989	1,460	−0.198	−2.764 to 0.619	−0.857	−4.962 to 0.422

* VE, vaccine efficacy; t, time; WC, whole cell; BS-WC, B-subunit whole cell.

TABLE 5. Estimated VE(t)*, with 95% confidence intervals (CI), for the WC* and BS-WC* vaccines, ages >5 years, Matlab, Bangladesh, May 1, 1985, through November 31, 1989

Date	Day	WC vaccine		BS-WC vaccine	
		VE(day)	95% CI	VE(day)	95% CI
May 1985	0	0.544	−0.481 to 0.859	0.800	0.354 to 0.938
November 1985	183	0.660	0.483 to 0.776	0.750	0.620 to 0.835
May 1986	365	0.725	0.620 to 0.801	0.682	0.561 to 0.769
November 1986	548	0.742	0.649 to 0.810	0.586	0.438 to 0.696
May 1987	730	0.707	0.574 to 0.798	0.483	0.250 to 0.644
November 1987	913	0.590	0.363 to 0.735	0.412	0.090 to 0.620
May 1988	1,095	0.314	−0.049 to 0.551	0.388	0.067 to 0.599
November 1988	1,278	−0.319	−1.410 to 0.278	0.406	−0.083 to 0.674
May 1989	1,460	−1.768	−6.866 to 0.026	0.448	−0.559 to 0.805

* VE, vaccine efficacy; t, time; WC, whole cell; BS-WC, B-subunit whole cell.

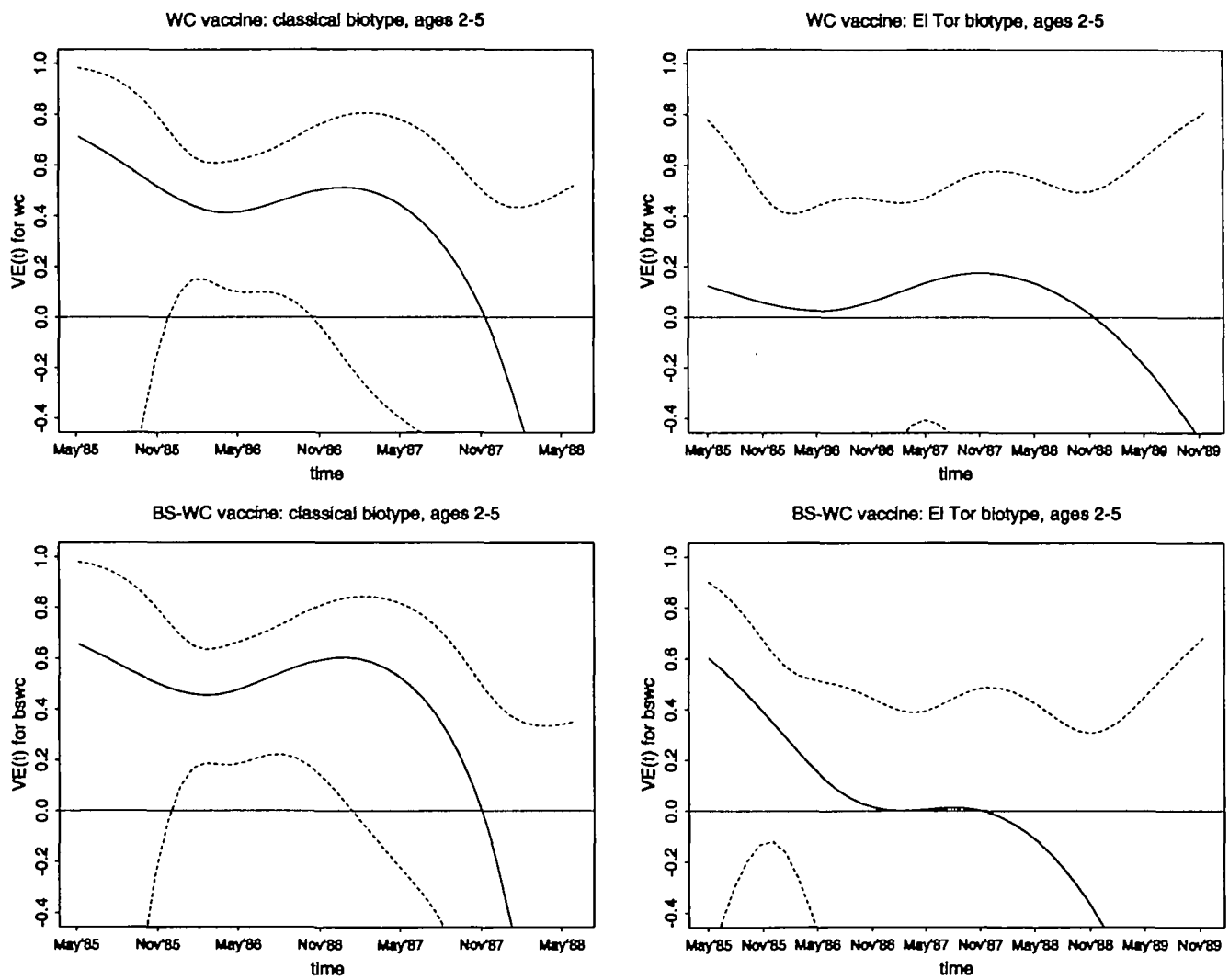

FIGURE 6. Nonparametric smoothed plots of $\widehat{VE}(t)$ versus t, with 95% confidence intervals, for the whole killed cell (WC) and B subunit whole killed cell (BS-WC) vaccines, 2- to 5-years age group, stratified by biotype, Matlab, Bangladesh, May 1, 1985, through November 31, 1989. VE, vaccine efficacy; t, time.

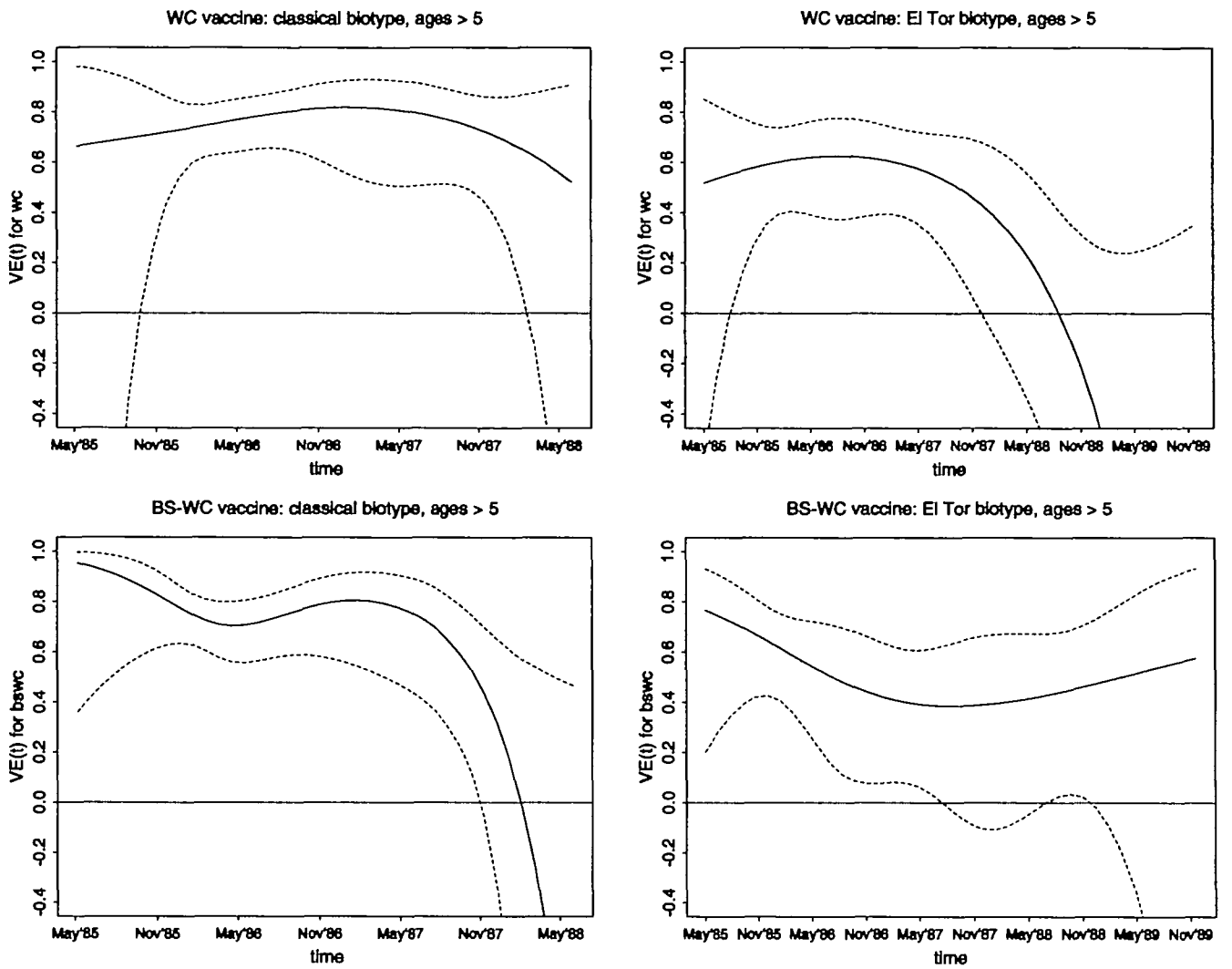

FIGURE 7. Nonparametric smoothed plots of $\widehat{VE}(t)$ versus t, with 95% confidence intervals, for the whole killed cell (WC) and B subunit whole killed cell (BS-WC) vaccines, >5 years age group, stratified by biotype, Matlab, Bangladesh, May 1, 1985, through November 31, 1989. VE, vaccine efficacy; t, time.

TABLE 6. Estimated VE(t)*, with 95% confidence intervals (CI), for the WC* and BS-WC* vaccines, classic biotype, ages 2- to 5-years, Matlab, Bangladesh, May 1, 1985, through November 31, 1989

Date	Day	WC vaccine		BS-WC vaccine	
		VE(day)	95% CI	VE(day)	95% CI
May 1985	0	0.724	−3.841 to 0.984	0.642	−5.792 to 0.981
November 1985	183	0.520	−0.143 to 0.798	0.503	−0.210 to 0.796
May 1986	365	0.412	0.097 to 0.617	0.478	0.189 to 0.664
November 1986	548	0.501	0.004 to 0.750	0.573	0.132 to 0.790
May 1987	730	0.458	−0.350 to 0.783	0.508	−0.257 to 0.808
November 1987	913	0.069	−0.840 to 0.528	0.032	−0.947 to 0.519
May 1988†	1,095	−1.261	−8.826 to 0.450	−1.838	−11.830 to 0.372

* VE, vaccine efficacy; t, time; WC, whole cell; BS-WC, B-subunit whole cell.
† There were no cholera classic cases after the third year.

TABLE 7. Estimated VE(t)*, with 95% confidence intervals (CI), for the WC* and BS-WC* vaccines, classic biotype, ages >5 years, Matlab, Bangladesh, May 1, 1985, through November 31, 1989

Date	Day	WC vaccine		BS-WC vaccine	
		VE(day)	95% CI	VE(day)	95% CI
May 1985	0	0.655	−5.407 to 0.981	0.952	0.306 to 0.997
November 1985	183	0.712	0.304 to 0.881	0.827	0.613 to 0.923
May 1986	365	0.769	0.642 to 0.855	0.705	0.560 to 0.802
November 1986	548	0.811	0.617 to 0.907	0.776	0.575 to 0.882
May 1987	730	0.806	0.507 to 0.923	0.766	0.454 to 0.900
November 1987	913	0.735	0.470 to 0.868	0.487	0.036 to 0.728
May 1988†	1,095	0.554	−0.994 to 0.900	−0.956	−6.652 to 0.500

* VE, vaccine efficacy; t, time; WC, whole cell; BS-WC, B-subunit whole cell.
† There were no cholera classical cases after the third year.

TABLE 8. Estimated VE(t)*, with 95% confidence intervals (CI), for the WC* and BS-WC* vaccines, El Tor biotype, ages 2- to 5-years, Matlab, Bangladesh, May 1, 1985, through November 31, 1989

Date	Day	WC vaccine		BS-WC vaccine	
		VE(day)	95% CI	VE(day)	95% CI
May 1985	0	0.130	−2.512 to 0.784	0.607	−0.617 to 0.904
November 1985	183	0.059	−0.729 to 0.488	0.392	−0.126 to 0.671
May 1986	365	0.024	−0.701 to 0.440	0.151	−0.491 to 0.516
November 1986	548	0.063	−0.645 to 0.466	0.016	−0.738 to 0.443
May 1987	730	0.138	−0.404 to 0.471	0.010	−0.622 to 0.396
November 1987	913	0.178	−0.577 to 0.572	0.005	−0.925 to 0.486
May 1988	1,095	0.138	−0.645 to 0.548	−0.110	−1.136 to 0.423
November 1988	1,278	0.016	−0.914 to 0.494	−0.366	−1.679 to 0.304
May 1989	1,460	−0.186	−2.775 to 0.627	−0.791	−4.782 to 0.446

* VE, vaccine efficacy; t, time; WC, whole cell; BS-WC, B-subunit whole cell.

TABLE 9. Estimated VE(t)*, with 95% confidence intervals (CI), for the WC* and BS-WC* vaccines, El Tor biotype, ages >5 years, Matlab, Bangladesh, May 1, 1985, through November 31, 1989

Date	Day	WC vaccine		BS-WC vaccine	
		VE(day)	95% CI	VE(day)	95% CI
May 1985	0	0.520	−0.576 to 0.854	0.768	0.196 to 0.933
November 1985	183	0.581	0.297 to 0.751	0.665	0.424 to 0.805
May 1986	365	0.618	0.387 to 0.762	0.544	0.251 to 0.722
November 1986	548	0.619	0.385 to 0.764	0.443	0.080 to 0.663
May 1987	730	0.576	0.357 to 0.720	0.393	0.062 to 0.607
November 1987	913	0.465	0.067 to 0.693	0.389	−0.092 to 0.659
May 1988	1,095	0.235	−0.326 to 0.559	0.416	−0.040 to 0.672
November 1988	1,278	−0.209	−1.131 to 0.315	0.461	0.025 to 0.702
May 1989	1,460	−1.038	−4.465 to 0.240	0.515	−0.362 to 0.827

* VE, vaccine efficacy; t, time after vaccination; WC, whole cell; BS-WC, B-subunit whole cell.

then the biologic protection at the individual level will increase, and so will the population estimate of VE(t) (14). The methods described here are appropriate to detect increasing protection as well.

Unmeasured heterogeneity of protective effects of the vaccine will also produce time-varying population estimates of VE(t), even when at the individual level the protection induced by the vaccine does not change with time. The most susceptible individuals will tend to become infected first, enriching the population with vaccinated people who have higher protection. The population-level estimate of VE(t) will increase with time even though the individual-level protection is not varying with time. In this case, frailty models can be used to estimate the appropriate efficacy measures of interest (12, 15). Interpretation of the potential reasons for time-varying estimates of VE(t) is still an open problem and will require use of further information on the underlying biology.

Our analysis provides some insights about the time-varying effects of the vaccines employed in this trial that go beyond those from previous analyses. The cholera toxin B subunit theoretically would provide added short-term protection to a WC vaccine. This

hypothesis appears to be confirmed by the early added protection, over the WC vaccine, reflected by the higher early $\widehat{VE}(t)$ for the BS-WC vaccine. Our finding that both vaccines protect better against the classic than the El Tor biotypes could be due to both vaccines containing classic and El Tor cells in a 3-to-1 ratio. We also show that both vaccines protected people in the >5 year age group better than those in the 2- to 5-year age group. The reasons for this are unclear. Protection with killed WC cholera vaccine, similar to the one used in Bangladesh, protected those in the 1- to 5-year age group as well as those people >5 years of age in a recent unblinded year-long trial in Vietnam (16).

The method based on the smoothed, scaled Schoenfeld residuals (6, 7) that we have adapted to construct nonparametric estimates for VE(t) as a smooth continuous function of time provides reliable estimation and analysis of field data. These estimates are virtually assumption-free and provide VE(t) estimation subject to the caveats discussed above. In addition, the method provides a hypothesis test for the presence of time-varying effects (7). Other statistical comparisons can be carried out via the approximate 95 percent confidence intervals. The nonparametrically estimated VE(t) curves can also reveal possible parametric forms for VE(t). Once the time-varying nature of the vaccine-induced protection is revealed, researchers may decide to take the second step of parametrically modeling the VE effects. This can increase the efficiency of the VE estimators, increase the power of statistical tests, and lead to a good physical interpretation of vaccine effects. In many cases, the model fitting can be carried out by using the Poisson form of a general linear model (17) or a time-varying Cox model (8, 9). If there is unmeasured heterogeneity for the vaccine effects, then specially constructed parametric frailty models (12, 15) can be used to estimate VE. Thus, we present a complete framework for estimating VE as a function of time since vaccination.

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APPENDIX

The following development is largely from Schoenfeld (6) and Grambsch and Therneau (7). The Schoenfeld residuals are formulated in the counting process framework, where $N_i(t)$ counts the number of events experienced by each individual by time t for $i = 1, \ldots, n$. The intensity function for time invariant relative risk is given by

$$Y_i(t) \exp\{\boldsymbol{\beta}' \mathbf{Z}_i(t)\} d\Lambda_0(t),$$

where $Y_i(t) = 1$ if individual i is at risk at time t and zero otherwise, and $d\Lambda_0(t)$ is the baseline hazard. Also, $\mathbf{Z}_i$ is a $p \times 1$ vector of covariates for subject i and $\boldsymbol{\beta}$ is a $p \times 1$ vector measuring the effects on survival.

The Schoenfeld residuals (6) can be written as a $p \times d$ matrix $\mathbf{R}$, where p is the number of covariates, indexed by $j = 1, \ldots, p$ and d is the number of unique failure times,

indexed by $k = 1, \ldots, d$. If the conditional weighted mean of covariate j at time k is defined as

$$\bar{Z}_j(k) = \frac{\sum_i Y_i(k) \exp\{\boldsymbol{\beta}' \mathbf{Z}_i(k)\} Z_{ij}(k)}{\sum_i Y_i(k) \exp\{\boldsymbol{\beta}' \mathbf{Z}_i(k)\}},$$

then the Schoenfeld residual for the jth variable at time k is

$$r_j(k) = Z_j(k) - \bar{Z}_j(k),$$

where $Z_j(k)$ is the value of covariate j for the individual who experienced an event at time k.

The true time-varying coefficient $\beta_j(t)$ can be written as

$$\beta_j(t) = \beta_j + \theta_j(t),$$

or the sum of the coefficient from the time-variant Cox model β_j plus a time-dependent component $\theta_j(t)$. It can be shown that $\theta_j(t)$ is captured in the Schoenfeld residuals (6, 7). If $\mathbf{r}(k,\boldsymbol{\beta})$ is the vector of Schoenfeld residuals at time k, we can write

$$\mathbf{r}(k,\boldsymbol{\beta}) = \mathbf{Z}(k) - \bar{\mathbf{Z}}(k,\boldsymbol{\beta}) = [\mathbf{Z}(k) - \bar{\mathbf{Z}}(k,\boldsymbol{\beta}(k))] + [\bar{\mathbf{Z}}(k,\boldsymbol{\beta}(k)) - \bar{\mathbf{Z}}(k,\boldsymbol{\beta})],$$

where $\boldsymbol{\beta}(k)$ is the vector of true time-varying coefficients evaluated at k, so that the first term is a mean 0 random vector. Expanding $\bar{\mathbf{Z}}(k,\boldsymbol{\beta}(k))$ in a one-term Taylor expansion about $\boldsymbol{\beta}$ gives

$$\bar{\mathbf{Z}}(k,\boldsymbol{\beta}(k)) = \bar{\mathbf{Z}}(k,\boldsymbol{\beta}) + [\boldsymbol{\beta}(k) - \boldsymbol{\beta}]\frac{d\bar{\mathbf{Z}}(k,\boldsymbol{\beta})}{d\boldsymbol{\beta}} = \bar{\mathbf{Z}}(k,\boldsymbol{\beta}) + \boldsymbol{\theta}(k)\mathbf{V}(k,\boldsymbol{\beta}),$$

where $\mathbf{V}(k,\boldsymbol{\beta})$ is the covariance matrix for the Cox model parameter estimates. Taking the expectation of the Schoenfeld residuals gives

$$E[\mathbf{r}(k,\boldsymbol{\beta})] \approx \mathbf{V}(k,\boldsymbol{\beta})\boldsymbol{\theta}(k).$$

Thus, a plot with time on the horizontal axis and the jth element of $\hat{\boldsymbol{\beta}} + \hat{\mathbf{V}}^{-1}(k, \boldsymbol{\beta})\hat{\mathbf{r}}(k, \boldsymbol{\beta})$ on the vertical axis demonstrates the functional form of $\beta_j(t) = \ln(\mathrm{RR}_j(t))$, where the inverse of the estimated covariance matrix $\hat{\mathbf{V}}(k, \boldsymbol{\beta})$ is a scaling factor.

To test whether the vaccine effect is constant over the course of the study, we use the test for a significant linear association between the scaled Schoenfeld residuals and time, as suggested by Grambsch and Therneau (7). The test has a standard linear models form and incorporates the uncertainty associated with the Cox model estimate. Approximate 95 percent confidence bands for the smooth VE(t) estimates are also computed using ordinary linear models methods. Let $s_j(k)$ denote the jth element of $\hat{\boldsymbol{\beta}} + \hat{\mathbf{V}}^{-1}(k, \boldsymbol{\beta})\hat{\mathbf{r}}(k, \boldsymbol{\beta})$, and $\mathbf{H}$ represent the linear smoother operator matrix. Then the smooth estimate of $\beta_j(t)$ is asymptotically normal with mean 0 and variance $\mathbf{H}\boldsymbol{\Sigma}_j\mathbf{H}'$, where $\boldsymbol{\Sigma}_j$ is the variance matrix of $s_j(k)$. Pointwise standard error bands are constructed using these estimates (18). To obtain confidence bands on the scale of the VE(t) estimate, we apply the monotonic transformation $\mathrm{VE}(t) = 1 - \exp\{\beta(t)\}$ to the 95 percent piecewise confidence interval for $\beta(t)$ described above.

Analysis and plots were done using modifications of the Splus functions **coxph( )** and **cox.zph( )** (18). Please contact the authors for further details.