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Serologic status and measles attack rates among vaccinated and unvaccinated children in rural Senegal

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During a measles vaccine trial in a rural area of Senegal, antibody status was examined within 10 days of exposure for 228 previously vaccinated and 313 unvaccinated children more than 12 months old who were exposed to measles at home. Thirty-six percent of the children developed clinical measles, the clinical diagnosis being confirmed for 135 of the 137 children from whom 2 blood samples were collected. Vaccine efficacy was 90% (95% confidence interval, 83 to 94%). The hemagglutinin-inhibiting antibodies (HI) or plaque neutralizing antibodies (PN) assays were equally efficient in predicting susceptibility and protection against measles. Vaccinated children who had no detectable HI or PN antibodies at exposure had significant protection against measles compared with seronegative unvaccinated children (HI vaccine efficacy, 49% (95% confidence interval, 21 to 68%); PN vaccine efficacy, 43% (95% confidence interval, 12 to 62%)). The attack rate was high for children with a titer of 40 to 125 mIU) 67% (4 of 6) of those with a positive hemagglutinin-inhibiting antibody test and 36% (13 of 36) of those with a positive PN test developed measles. Attack rates among children with HI or PN titers above 125 mIU were 2% (6 of 295) and 3% (7 of 258), respectively. Because titers of ≤120 mIU have been found to offer little protection in another study, this antibody level may be the best screening value for assessing susceptibility and protection against measles. However, it should be noted that many seronegative vaccinated children are protected against measles infection.

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INTRODUCTION

The presence or absence of measles antibodies has been assumed to correlate with protection and susceptibility to natural measles infection.[1] Measles immunization policies for developing countries have been based largely on immunogenicity studies.[2, 3] Given the age-specific incidence pattern observed in a rural area

of Kenya and applying the assumptions that seroconversion is equivalent to protection and nonseroconversion is equivalent to full susceptibility, the number of measles cases prevented by vaccination at different ages was projected in order to reach the conclusion that 9 months represented the optimal age for measles immunization.[2] However, there are now a number of reports[4–8] of individuals who had measles antibodies after immunization and later developed measles infection. A study from the United States of 80 blood donors, of whom 8 developed measles during an outbreak in a university college, reported that individuals with low measles antibody titers (≤120 mIU) were susceptible to infection.[4] On the other hand although virtually everybody contracts measles in virgin soil populations,[9] observations from immunized populations[10, 11] suggest that undetectable antibodies may not necessarily imply that the individual is fully susceptible to disease. Presumably they have low undetectable antibodies and/or cellular immunity.

During a trial of high titer measles vaccine in rural Senegal,[6] we collected blood samples shortly after exposure from children living in the same compound as a measles case. Thus it was possible to assess susceptibility among vaccinated children with no antibodies to measles, as well as protection among children who had antibodies by either hemagglutinin-inhibiting (HI) or plaque neutralization (PN) assays.

surveillance for acute cases of measles was initiated in the area. The present analysis includes children who were exposed to or developed measles between July, 1987, and December, 1990. Vaccine efficacy has previously been analyzed for the subgroup of children who participated in the trial of high titer measles vaccine.[6]

When a field assistant was informed about a case of suspected measles, the project physician was called to examine the child. During the visit information was sought on how the first case had contracted measles and contacts were traced. Hence new measles cases were found both by routine surveillance and through active follow-up of contacts by the physician. In order to assess the completeness of the surveillance, mothers were asked in the census whether their children had had measles. Fewer than 5% additional cases were found this way, and virtually all of these cases had occurred outside the study area.

At the first visit in a compound a census was made of all children less than 15 years of age. When parents consented a blood sample was taken by finger prick from cases and exposed children who had no previous history of measles infection. Convalescent samples from children who developed measles were collected 4 to 5 weeks after the beginning of symptoms but not from exposed children with no signs of measles infection.

Clinical examination and case definition. Com-

exposed in the first generation without contracting measles, only to become infected in a later generation after a sibling had developed measles in the same hut.[15] In the present analysis we used only the closest exposure for each child.

Measles vaccination in the study area and immunization status. There were measles vaccination campaigns from 1979 to 1983 in Niakhar and again in 1986 to 1987 during the accelerated phase of the Expanded Programme on Immunization. However, until 1987 coverage was low.[15] From July, 1987, *Bacillus Calmette-Guérin,* diphtheria, pertussis, tetanus, polio, measles and yellow fever vaccines were offered systematically in immunization sessions organized once a month at each of the three health centers as part of trials of measles and pertussis vaccines.[6, 16, 17] The coverage among 1- to 2-year-old children born in the study area increased from 36% in 1986 to 82% in 1990.

From 1983 to 1986 information on vaccines were gathered through the annual censuses and health centre immunization registers. Documentation provided during the previous campaign may have been incomplete or lost subsequently. Hence some individuals were probably immunized without this being recorded by the project. For the children born after February, 1987, the project provided nearly all measles immunizations and the quality of the information on immunization status is likely to be much better than for the children born earlier.[6] The analysis was therefore adjusted for the period in which the children were born. Children who had measles within 2 weeks of vaccination were classified as unvaccinated.

Analysis of blood samples and seroconversion. Blood samples were analysed for measles HI antibodies at the MRC Laboratories, The Gambia.[18] The sensitivity of this test was 31.25 mIU. Because the test started with a 1:2 dilution, the minimum detectable titer was 62.5 mIU. Neutralization antibodies were measured by a standard plaque reduction neutralization assay (PN).[19] This assay, which used an inoculum of 30 to 50 plaque-forming units of low passage Edmonston measles virus (courtesy of Dr. P. Albrecht), had a sensitivity of 4 mIU. With a starting serum dilution of 1:10, the minimum detectable titer of antibody was 40 mIU/ml. Three percent of the samples (24 of 758) with grossly discrepant values between the 2 assays were retested in both tests, in case samples had been misread or misnumbered, and the results of the second test were regarded as valid.

Protection in relation to antibody titer has been analyzed for children from whom a blood sample was collected within 10 days of the onset of symptoms in the index case in the compound.[6] Seroconversion was analyzed for children from whom the first sample was collected immediately before infection or within 3 days of the onset of symptoms, with the second sample obtained 17 to 57 days after the onset of disease. A 4-fold increase in titer in conjunction with clinical features of measles was considered proof of acute infection.

Statistics. The protective efficacy against measles infection was examined by comparing secondary attack rates (SAR) for vaccinated and unvaccinated children

$$\text{Vaccine efficacy (\%)} = (1 - (\text{SAR for vaccinated}/\text{SAR for unvaccinated})) \times 100$$

Relative risks and Greenland-Robins approximate 95% confidence intervals have been calculated using the EPIINFO program.

RESULTS

Study population and seropositivity. Among the 1218 resident children younger than 15 years of age exposed to measles in the compound, 215 had had measles. Of the 1013 children with no history of previous measles infection, 255 (25%) were absent or refused and 758 (75%) had a blood test taken, 588 (78%) within 10 days and 170 (22%) more than 10 after exposure. Among the 588 children who provided a blood sample within 10 days, 245 (42%) of the children were vaccinated and 343 (58%) were unvaccinated. There was no difference in the level of HI antibodies among those examined within the first 10 or even within the first 20 days of exposure (data available at request). In order to avoid confounding by maternal antibodies, the main analysis was confined to the 541 (92%) of the 588 children who were more than 1 year old (>365 days) when the first blood sample was taken.

Among immunized children 88% had detectable HI antibodies to measles (Table 1). The proportion of seropositive children decreased with time since immunization ($P = 0.001$) (data available at request). Of 313 unvaccinated children with no history of measles-like rashes 32% had detectable HI antibodies to measles. The seropositive unimmunized children (Table 1) were significantly older than the seronegative unimmunized ($P < 0.001$, Kruskal-Wallis, chi square = 20.8) and the immunized children ($P < 0.001$, Kruskal-Wallis, chi square = 104.2).

Serologic responses in relation to clinical measles. One hundred ninety-five children developed clinical measles; the first sample was taken from them on an average of 8 days before the onset of symptoms (range, 35 days before symptoms to the first day of symptoms). For 137 children a second blood sample was taken more than 2 weeks after onset of symptoms (range, 17 to 57 days); 135 children (99%) had a 4-fold or greater increase in titer. One child had no detectable antibody in either sample, and in one child the initial titer fell from 8000 mIU to 2000 mIU. Thus the

TABLE 1. Secondary attack rates according to immunization status, HI seropositivity test and intensity of exposure: Niakhar, 1987 to 1990

Note: When the data were analyzed in relation to PN antibodies, the attack rates were 48% (11 of 23) for seronegative vaccinated, 1% (2 of 192) for seropositive vaccinated, 82% (149 of 181) for seronegative unvaccinated and a higher rate of 18% (18 of 102) for nonvaccinated seropositive children.

Type of Exposure	Secondary Attack Rate (%)			
	Vaccinated against measles		Nonvaccinated	
	Seronegative	Seropositive	Seronegative	Seropositive
Median age (months)	39	46	86	115
Compound	29 (2/7)*	0 (0/51)	64 (30/47)	6 (1/17)
Household	80 (4/5)	2 (1/42)	84 (49/58)	19 (5/27)
Hut	33 (5/15)	1 (1/108)	88 (95/108)	4 (2/56)
Total	41 (11/27)	1 (2/201)	82 (174/213)	8 (8/100)

* Numbers in parentheses, number of cases/number exposed.

predictive value of a clinical diagnosis was 99% according to the criteria set for this study.

Vaccine efficacy. The secondary attack rate (Table 1) was higher if exposure occurred in the household (relative risk, 1.49 (95% confidence interval, 1.15 to 1.92)) or in the same hut (relative risk, 1.35 (95% confidence interval, 1.08 to 1.69)) than from someone living in the compound but in a different household, whereas there was no difference between the attack rates in the household or hut. There was no difference in attack rate by age or sex. Hence we have adjusted for intensity of exposure in the analysis of vaccine efficacy. Analyzed without regard to the serologic status, vaccine efficacy was 90% (95% confidence interval, 83 to 94%) for vaccinated children when compared with children with no history of measles infection or of measles immunization.

Secondary attack rates among seronegative and seropositive children. If the analysis of attack rates is limited to all HI-seronegative children (Table 1), the vaccinated seronegative children had a protective efficacy of 49% (95% confidence interval, 21 to 68%) compared with seronegative unvaccinated children. The tendency remained the same (vaccine efficacy, 50% (95% confidence interval, 8 to 73%)) if the analysis was limited to the children who had not been enrolled in the high titer trial.[3] The protective efficacy among vaccinated children who were seronegative in the PN antibody assay was 43% (95% confidence interval, 12 to 62%) (data available on request). Seropositivity and attack rates were essentially similar when the analysis was limited to samples collected within 3 days of exposure (Table 2).

Among the HI-seronegative vaccinated children (Table 1), six had received a high titer measles vaccine at 5 months and had been tested at 10 months of age. Three children who developed measles had titers of 63, 63 and 500 mIU at 10 months of age. The three seronegative children who did not develop measles had titers of <63, 125 and 250 mIU at 10 months of age.

Vaccinated children who were seropositive in either the HI (Table 1) or PN test had significantly lower attack rates than the seropositive unvaccinated children. Twenty-three children who had a positive blood sample at exposure developed measles: in 7 both HI and PN antibodies were detected; in 3 only HI antibodies were detected; and 13 were positive only in the PN assay. The first clinical symptoms occurred from 0 to 28 days after the sample had been collected (data available at request).

Protective HI or PN antibodies. All samples from children exposed within 10 days were assayed by the more sensitive PN test. After logarithmic transformation PN and HI results were strongly correlated (r = 0.85 (95% confidence interval, 0.83 to 0.88)). In Table 3 we present the attack rate among immunized and nonimmunized children according to the antibody titer measured by the two tests. The attack rates were high for children with titers below 125 mIU in either test. Among children with higher antibody titers, 2 to 3%

TABLE 2. Secondary attack rates according to immunization status, seropositivity in the plaque neutralization test and intensity of exposure (only children exposed within 3 days of the first case in the compound): Niakhar, 1987 to 1990

Type of Exposure	Secondary Attack Rate (%)			
	Vaccinated against measles		Nonvaccinated	
	Seronegative	Seropositive	Seronegative	Seropositive
Compound		0 (0/13)	70 (7/10)	0 (0/3)
Household	100 (2/2)*	0 (0/10)	90 (9/10)	27 (3/11)
Hut	0 (0/3)	4 (1/23)	94 (16/17)	20 (2/10)
Total	40 (2/5)	2 (1/46)	86 (32/37)	21 (5/24)

* Numbers in parentheses, number of cases/number exposed.

TABLE 3. Attack rates among children exposed in the compound according to HI and PN antibody titers (mIU) within 10 days of exposure: Niakhar, 1987 to 1990

HI Titer (mIU)	%	PN Titer (mIU)	%
Unimmunized children			
<63	82 (174/213)*	<40	82 (149/181)
63–125	100 (4/4)	40–125	57 (13/23)
		126–249	11 (1/9)
250–1000	0 (0/33)	250–1000	5 (2/44)
>1000	6 (4/63)	>1000	8 (2/26)
Total	58 (182/313)		59 (167/283)
Immunized children			
<63	41 (11/27)	<40	48 (11/23)
63–125	0 (0/2)	40–125	0 (0/13)
		126–249	5 (1/21)
250–1000	2 (1/63)	250–1000	1 (1/99)
>1000	1 (1/136)	>1000	0 (0/59)
Total	6 (13/228)		6 (13/215)
All children			
<63	77 (185/240)	<40	75 (160/204)
63–125	67 (4/6)	40–125	36 (13/36)
		126–249	7 (2/30)
250–1000	1 (1/96)	250–1000	2 (3/143)
>1000	3 (5/199)	>1000	2 (2/85)
Total	36 (195/541)		36 (180/498)

* Numbers in parentheses, number of cases/number exposed.

developed measles, and there was no difference in the

same if we did not remove the discrepant values and use the first antibody results; for example sensitivity and specificity of an HI antibody value of <125 mIU

tively.

DISCUSSION

Measles is currently estimated to kill 1.4 million children/year in developing countries. Many of these deaths occur in children who develop measles before the current age at immunization.[7] Hence changes in vaccine strategy or type of vaccine which permit a reduction in the age of immunization are desirable, because they may contribute to the reduction of measles-associated mortality. The World Health Organization recommendations regarding type and age at measles immunization have been based mainly on seroconversion studies.[2, 3] There is some indication that antibody titers fall to low or undetectable levels in populations with little reexposure. This problem may be larger in areas where immunization was introduced early and the initial antibody response was lower. Hence it seems possible that with increasing coverage in many developing countries, it may be necessary to

antibody response or sufficient cellular immunity to provide protection against clinical infection even though they had undetectable antibodies. Some seronegative unvaccinated children exposed in the hut or in the household did not develop clinical measles, but because these children were not bled a second time, it was not possible to ascertain whether they had subclinical infection. From the present study up to 50% of immunized children who are seronegative may have sufficient immunity to prevent clinical disease and this estimate is not changed by using a more sensitive assay.

Measles antigen is presented to both T and B cells in the normal child, and a strong correlation between the presence of antibodies and cell-mediated immunity makes the separate contribution of each difficult to determine. However, children with congenital agammaglobulinemia contract measles and pursue a typical clinical course with rash and subsequent immunity despite the absence of detectable measles antibodies postinfection[20] and it has been suggested that antibody production in measles is of minimal or no importance.[21] On the other hand immunoglobulin prevents infection in susceptible contacts when given within a few days of exposure, and humoral immunity may therefore play an important role in the prevention of systemic infection. Hence protection can be provided by both humoral and cellular immunity. Thus lack of antibodies may not indicate susceptibility to disease as demonstrated by our observations and observations in agammaglobulinemic children.

The presence of measles antibody is usually believed to protect against acquisition of disease.[1, 11] However, there have been some reports from developing countries of children with antibodies developing measles,[7, 8] and the degree of protection may depend on the amount of antibody and the intensity of exposure. For example in virgin soil epidemics, protective doses of immunoglobulin have been found not to protect but only to modify measles infection.[9] One study of blood donors from the United States indicated that 8 of 9 individuals with PN-positive titers ≤120 mIU developed clinical disease when exposed to measles whereas several donors with higher titers had significant increases in titers but did not develop the full clinical picture, although some reported symptoms.[4] Our data from Senegal indicated that children with antibody titers ≤125 mIU in either the HI or PN test had only limited protection against measles, whereas children with titers above 125 mIU in either test had low attack rates (Table 3). Hence a level of 125 mIU of measles antibodies may be the best screening value for determining likely protection against measles. It should be noted that some children with higher titers developed clinical measles. Although the PN test was able to distinguish somewhat lower amounts of antibody than the HI test and neutralizing antibodies are believed to be more relevant to protection, the PN test did not provide a better assessment of protection and susceptibility than the HI test.

A surprising and unexplained observation was that most of the children who had antibodies but developed measles were found in the unvaccinated group and not in the immunized group. The seropositive "unimmunized" children were considerably older than other groups (Table 1) and they may have had antibodies because of undocumented measles immunizations either outside the area or in the previous less well-documented campaigns or to previous measles infection. The lower antibody titers and higher attack rates in this group may be related to a longer time since the suspected immunization. It is also possible that some of the children in this group had experienced unrecognized measles infection.

Our study as well as those of others[4] suggest that the assumptions of a clear correlation between seropositivity and protection or seronegativity and susceptibility are not inviolate. Even if standard assays are substituted with the more sensitive PN assay, the absence of detectable antibody is not equivalent to susceptibility; also some of the immunized children with antibodies may develop measles. Raising the cutoff to an antibody titer of 125 mIU would improve the prediction of susceptibility. Because there were similar findings in an American study,[4] titers ≤125 mIU should not be considered protective in studies of vaccine-related antibody responses or in monitoring antibody titers in different populations.

Serologic data provide some guidance to the protective efficacy of various immunization strategies. However, vaccination policies affecting millions of children should not be based exclusively on projections from serologic studies but on specific studies comparing the impact of alternative policies on morbidity and mortality.[17, 22]

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