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VACCINE AGAINST GROUP B NEISSERIA MENINGITIDIS: PROTECTION TRIAL AND MASS VACCINATION RESULTS IN CUBA

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SUMMARY

The Cuban vaccine, first in the world with proven efficacy against group B-caused disease, is based on outer membrane proteins from B meningococci capable of inducing long-lasting and high-titered bactericidal antibodies in humans. This bactericidal activity has a wide spectrum against all pathogenic group B *Neisseria meningitidis* tested.

A randomized, double-blind controlled trial of the vaccine efficacy was performed during 1987-1989 with 106 000 10-14 years old students from 197 boarding schools in seven provinces.

The efficacy obtained was 83% (χ^2, $p<0.002$; Fischer exact, $p<0.001$). In a second field trial including 133 600 persons from 5 months to 24 years of age in Ciego de Avila province (30 cases/10^5 inhabitants, the highest incidence rate in Cuba) by comparing vaccinated and non-vaccinated population after 2.5 years of observation and careful follow-up, the efficacy and safety was confirmed.

Because of these results and because of the very low reactogenicity of the vaccine, the Ministry of Public Health took the advice of the Scientific Council to vaccinate all children between 3 months and 6 years of age in the most affected provinces. No severe or long lasting reactions to the vaccine were observed after the millions of doses administered. The efficacy of vaccination varied in the provinces between 83% and 94%, among age groups ranging from 3 months and 20 years.

After 3 years of massive application no severe reactions occurred and one of the most severe epidemics has been practically eradicated.

Key words: Evaluation studies; Neisseria meningitidis; Randomized controlled trials; Vaccines

INTRODUCTION

Since 1976, meningococcal disease constitutes a serious health problem in Cuba due to the significant rise in incidence of this disease and its high lethality.

The causal agents at beginning of the outbreak were predominantly group C meningococci and in second place group B meningococci; after the mass vaccination campaign conducted in 1979 using the polysaccharide Merieux A + C, group C caused meningococcal disease was practically eradicated.

In the late 70s and early 80s, a significant incidence increase took place, virtually caused by B meningococci of 4:P1.15 (95%), 15:P1.15 (3%) and 15:P1.16 (1%) serotypes and subtypes.

The peak epidemic year was 1983, although the situation differed from province to province. Higher incidence rates were observed in 1985 in children younger than 6 years (50 cases per 10^5 inhabitants) and among teenagers (20 cases per 10^5). The most affected teenagers were those attending secondary boarding schools in the central provinces of Cuba (57,7 cases per 10^5 students).

B-meningococcal disease has been the most serious infection health hazard of the last two decades in Cuba.

Repeated testing with different vaccine candicates in the United States, Europe, the Soviet Union, and South Africa, revealed many deficiencies among these; a weak, very variable and short-lasting (5-7 months) bactericidal activity; beyond 5-7 months after immunization the concentration of specific antibodies of IgG class, directed against important exposed proteins and other antigens of the bacterial envelope, was very low. In addition, weak or absent anamnestic response to a booster dose after 8 to 12 months of primary immunization was observed as one of the most critical failures.

Thus, taking into consideration the world research state in the B meningococcus vaccine development and our situation with the disease, decision was taken to obtain in short time a good candidate vaccine.

Among different vaccine formulations one was selected for which carefully conducted preclinical and phase I and phase II clinical studies revealed innocuousness, safety and very low reactogenicity, long-lasting bactericidal and specific antibody titers of a broad spectrum against different pathogenic serotypes and subtypes. On the basis of these promising serological efficacy results, this vaccine has been used in a double-blind placebo-vaccine field trials for epidemiological efficacy evaluation.

MATERIALS AND METHODS

Base-line studies

Base-line data on epidemiology of the disease and diagnostic laboratory capability were collected and evaluated during a three-year period in the provinces, before the trial-started (1984-87). Laboratory procedures, case management protocols, and the system of coordinating information from schools, hospitals, provincial, municipal and national epidemiologists, and laboratories have been carefully reviewed, evaluated and when needed established.

Case ascertainment and case definitions were established as well as the isolates and samples transport-conservation-study system.

Vaccine trial design

The trial was a randomized, placebo-controlled, double blind trial of vaccine efficacy. The protection trial was performed among teen-agers attending secondary boarding schools. The target group ranged, from 11 to 16 years of age and was distributed in 197 schools and 7 provinces with the highest incidence rates in this age group (40 cases per 10^5 students at the starting point of the trial).

Sample size was calculated according to the level of efficacy to be detected at 75% or greater, with less than a 0.05 type I error ($\alpha = 0.05$) and a power of 80% ($\beta = 0.2$, taking into account an attack rate of the disease of 40 cases per 10^5 students and a follow-up period of 1-3 year. With this incidence rate at the start of the trial and considering the other statistical factors, 106 251 students for placebo and vaccine groups were calculated to be needed in using schools as randomization units. Each school with approx. 540 students constitutes a homogeneous unit, either completely vaccinated or completely placebo inoculated.

A list of schools was assembled and stratified by province according to incidence of the disease during the last three years, to assure homogeneous distribution of exposure to risk of disease in receiving placebo or vaccine. Afterwards, placebo or vaccine were randomly allocated in each school within every stratus.

To assure blinding, lot identification was performed with randomly generated numbers having a secret master code list. Ten different numbers for placebo and ten for vaccine were used.

The vaccine

The vaccine components are purified proteins from the outer-membrane of group B meningococci enriched with proteins from the high molecular weight proteins complex (65-95 KD) having the greatest capacity of inducing long-lasting bactericidal antibodies. Some of the purified proteins, together with a good controlled proportion of phospholipide and lipopolysaccharide (LPS) form a proteoliposom, and the added amounts of High Molecular + Protein-Complex is non covalently conjugated or attached to the proteoliposome surface. In addition, the whole protein complex is conjugated non covalently, 1:1 proportion with C polysaccharide and finally the whole complex is adsorbed to aluminium hydroxide gel with a controlled particle size.

Vaccine formulation

Each dose contains:

Outer membrane proteins from B meningococci	50 mcg
Purified polysaccharide form C meningococci	50 mcg
Aluminium hydroxide gel	2 mg
Sodium thiomersalate	0.01 mg
PBS csp	0.5 ml
LPS	1%

The commercial name of this preparation is VA-MENGOC-BC^R, a trade mark of Institute Finlay, Habana, Cuba.

Control vaccine (Placebo)

The control vaccine was identical in appearance to the tested vaccine and consisted of a saline placebo containing the adjuvant aluminium hydroxide.

Vaccination, sampling and evaluation

A two 0.5 ml deltoid intramuscular dose with 6 to 8 weeks interval was the vaccination schedule established.

In each province, 300 students randomly chosen were used for immunogenicity and carrier state studies. The samples for these studies were taken immediately before applying the first dose and 4 weeks after the second one. Additional blood and exudate samples were taken every six months after vaccination. ELISA and bactericidal studies were performed as described elsewhere (1).

Only cases with the following criteria were used for efficacy evaluation: confirmed cases are only those with clinical illness from which a B meningococcus was isolated by culture or those with clinical illness with Gram-negative diplococci directly observed and where a latex or ELISA confirmed B antigen was found.

The standard forms for data collection, previously field-tested, were submitted to the Ministry of Public Health and stored in the Efficacy Trial Data Base Computer System.

SAS-PC version 6.03 and EPI-INFO were used for data analysis and study monitoring, relative risk calculation for vaccine and placebo groups with schools as randomization units, individual or cluster approaches. The comparisons between placebo and vaccinees regarding ELISA and bactericidal titer distributions were performed with the help of a computing program having common non-parametric tests, Wilcoxon Rank-Sum Test, t-Test, percentile distribution and binomial distribution analysis of probabilities and the statistical analysis significance tests-Chi-square and Fischer Exact.

RESULTS AND DISCUSSION

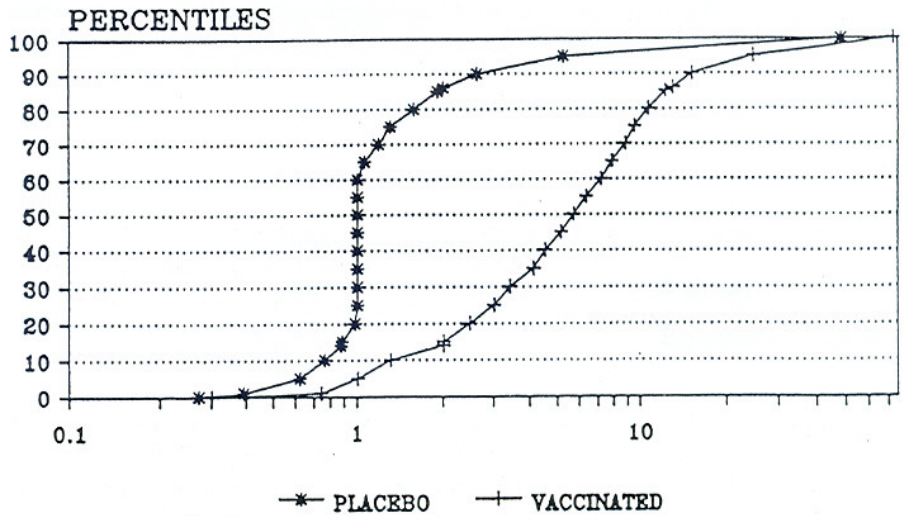

Figure 1
Cumulative distribution of the ratio ELISA2/ELISA1 four weeks after vaccination

The study of the meningococcus carrier state shows no significant difference between placebo-injected controls and vaccinees when the sample was taken four weeks after vaccination. Six months after vaccination, a significant lowering in carrier state in vaccinees was observed, but more careful assessment of variations and mechanisms must be performed in the future for conclusive statements.

In the vaccine group, an 88% of seroconversion (E2/E1) was detected using the ELISA method; in percentile 50 (median of the group), a six-fold increase was observed. On the other hand, in the placebo group, percentile 80 shows just one as E2/E1 ratio and only 14% of this group shows two-fold increase (correlating quite well with carrier state). Comparing the cumulative distribution of the E2/E1 ratio in placebo and vaccine four weeks after vaccination, a significant difference with $p < 0.001$ is seen. (Fig. 1).

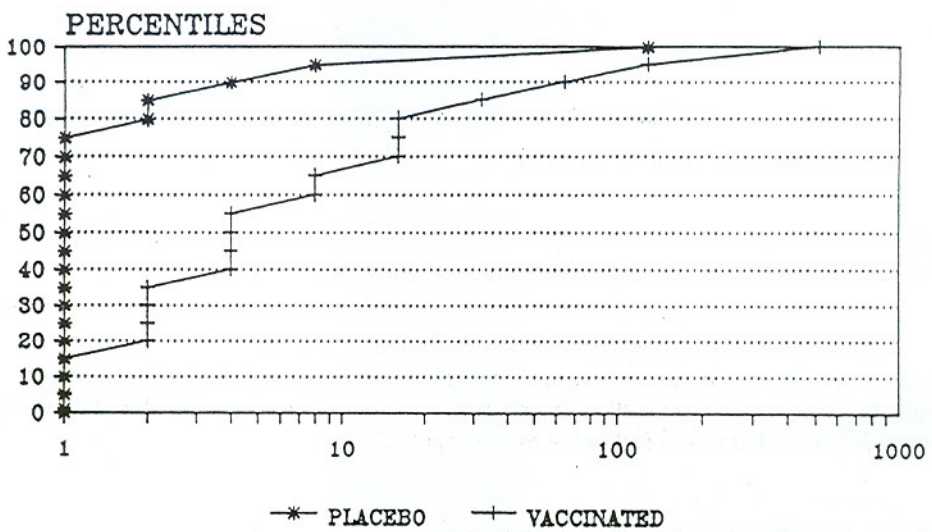

Figure 2
Cumulative distribution of the ratio BACTE2.Titer/BACTE1.Titer four weeks after vaccination

Seroconversion regarding bactericidal activity was detected in 85% of the vaccine group in which percentil 50 (median) shows a four-fold titer increase (Fig. 2). The difference with respect to the placebo group was highly significant, $p < 0.001$. Only percentil 85 shows two-fold increase in this group caused by normal contacts with meningococci in the population. The cumulative distribution of the ratio B2/B1 comparing placebo and vaccine group shows statistical significance.

This study lasted 16 months, during which 25 culture and latex positive confirmed cases occurred in the study population, four in the vaccine group and 21 in the placebo group. The timing of cumulative case occurrence among placebo and vaccine group, as seen further on, constitutes a good graphic demonstration of the vaccine effect (Fig.3). Case occurrence was distributed per school as indenpendent cases and fulfilling the criteria of «case definition» needed in order to be included in the efficacy calculation.

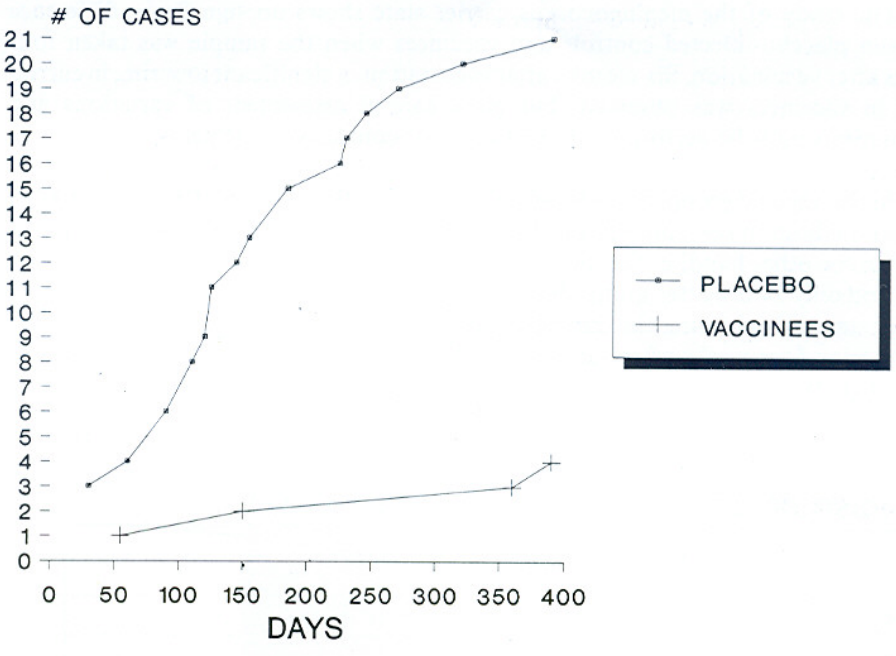

Figure 3
Timing of cumulative case occurrence

Further observation up to 24 months revealed the occurrence of five more bacteriologically confirmed cases, all in the placebo group and none among the vaccinees though not included in the following efficacy calculation:

Efficacy according to conglomerate approach

	Vaccine	Placebo	Total
Total of schools involved	99	98	197
Schools with cases	3	17	20
Total of cases	4	21	25

Analysis of Single Table
Relative risk-0.17 ($0.05 < RR < 0.58$)
Taylor series 95% confidence limits for RR

	Chi-squares	p-values
Uncorrected	11.07	0.0008791
Mantel-Haenszel	11.01	0.0009061
Yates corrected	9.55	0.0019968

Resultant efficacy: 83%
Confidence interval: 95-42%

Efficacy according to individual approach

Group	Cases	Not ill	Total
Vaccinee	4	52962	52966
Placebo	21	53264	53285
Total	25	106226	106251

Analysis of Single Table
Relative risk-0.19 (0.07< RR< 0.58)
Taylor series 95% confidence limits for RR

	Chi-squares	p-values
Uncorrected	11.46	0.0007107
Mantel-Haenszel	11.46	0.0007108
Yates corrected	10.15	0.0014457

Resultant efficacy: 81%
Confidence interval: 93-44%

Vaccine impact is also shown by demonstrating the significant reduction in case-occurrence probability in vaccine schools in comparison with placebo treated schools. Immediately before the starting point of the field trial the probability of one or more cases per school in the study was calculated (Po = 0,192). After 1.3 year of follow-up period 17 cases had occurred in 98 placebo schools. The probability of case occurrence was 0.173469 (Pp). The simultaneous case occurrence in vaccine schools was 3 in 99 schools.

Binomial distribution

	N = 99	P.1734693
X	P(X)	Cumulative probability
3	.00001	.00001
4	.00005	.00006
5	.00019	.00025
6	.00062	.00086
E(X) = 17.173346	std dev = 3.76754	variance = 14.19439

A way to evaluate the impact of the vaccine is by calculating under the hypothesis that Po = 0.192 (previous value) the probability of the happening in placebo and vaccine groups. The probability associated with the occurrence of three or less schools with at least one case in a set of 99 (vaccine groups) and 17 or less of a set of 98 (placebo groups) are 0.00001 and 0.38 respectively. These results suggest that p in the vaccine group is

significantly less than Po, but not so in the placebo groups. Another approach is using the placebo value of p in determining the probability of that happening in the vaccine group. Having p the value of 0.173 (17/98), then, P (X3)0.00001. This value of p is significantly less than 0.173.

The difference in case occurrence among placebo and vaccine groups was statistically significant, considering the cases individually or effecting a conglomerate (school distribution of cases). The vaccine proved to be effective in the study population in 81% (individual treatment of cases) or 83% (conglomerate treatment), and the confidence interval from 44 to 93% or 42 to 95% respectively.

Further follow-up studies

Furthermore, a good correlation between serological evolution and protection as well as the low reactogenicity and safety of the vaccine was confirmed.

The comparison of ELISA and bactericidal ratios E4/E1 and B4/B1 respectively constitutes an objective proof of duration of the vaccine protective capacity. E4 and B4 represent ELISA and bactericidal values one year after vaccination (Figs. 4 & 5).

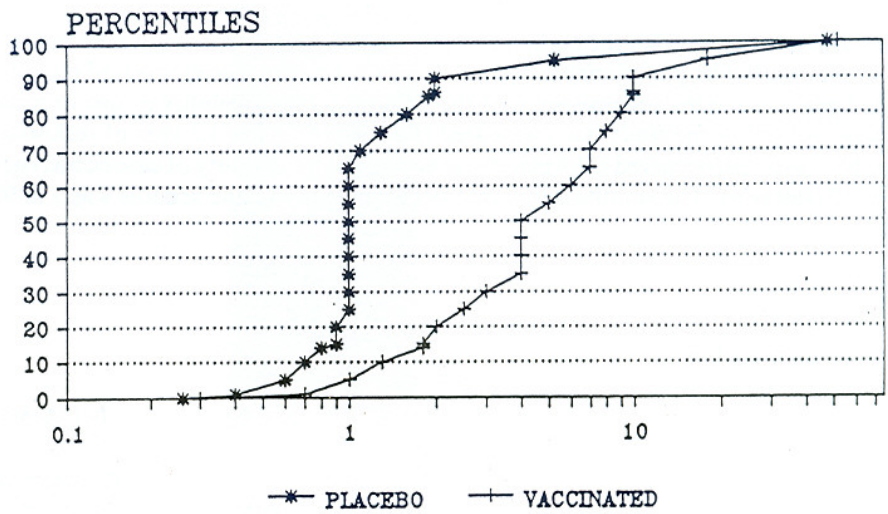

Figure 4
Cumulative distribution of the ratio ELISA4/ELISA1 one year after vaccination

The anamnestic antibody response was studied in army recruits, in parallel. A booster dose was tested in one group after one year and another group after 18 months. Before boostering (12 to 18 months) blood plasma from the vaccine group of the parallel study was collected, and the IgG fraction was purified and tested by ELISA and bactericidal activity (Figs. 6 and 7).

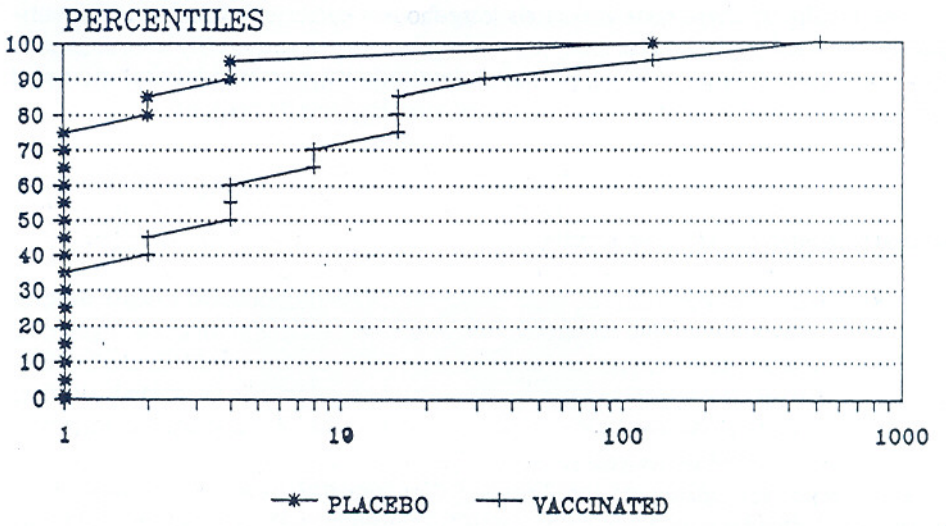

Figure 5
Cumulative distribution of the ratio BACTE4.Titer/BACTE1.Titer one year after vaccination

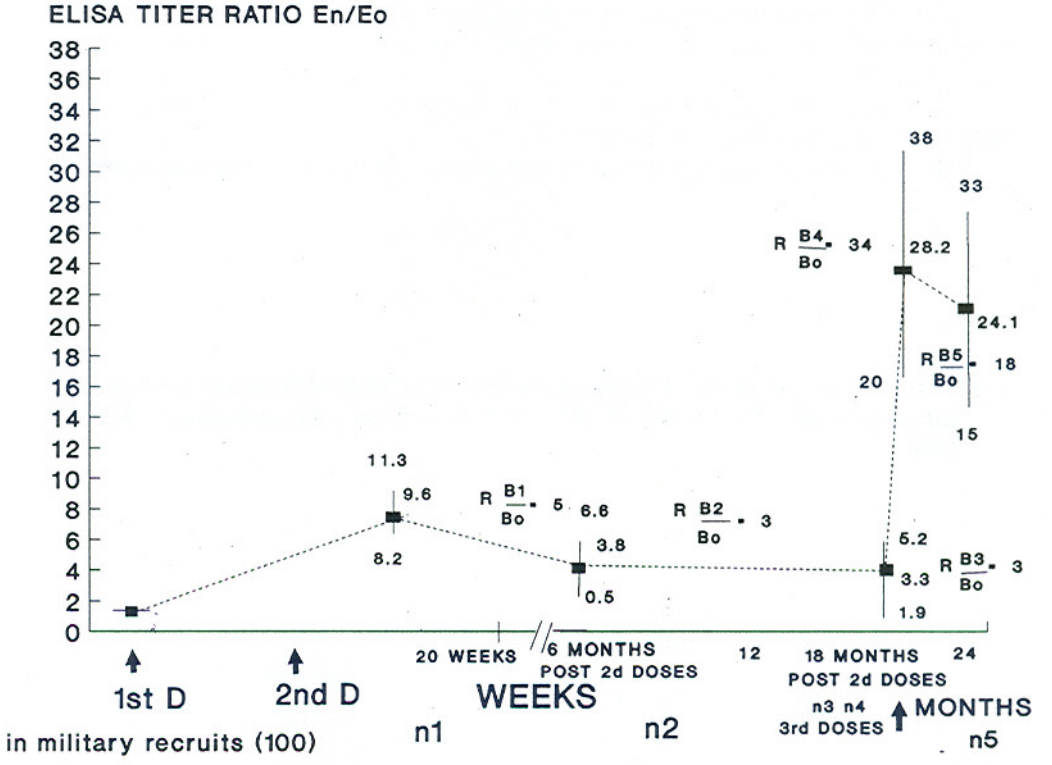

Figure 6
Increasing bactericidal and specific antibodies with a booster

The results of these tests indicated long-lasting bactericidal and specific antibodies against some of the most frequent pathogenic B serotypes. After boostering the specific antibody increased by more than 5 times and the bactericidal titer increased more than 10 times, showing an excellent anamnestic specific response.

Summary of mass vaccination results

Taking into consideration the high incidence of meningococcal disease among children up to six years of age occurring in Cuba and the satisfactory reactogenicity and efficacy studies with the Antimeningococcal BC Vaccine «VA-MENGOC-BC^{R}» evaluated during the last years, a mass vaccination campaign was organized, starting with this age-group (1989-90). Table 1 shows the percentage of vaccination per province in the age-group below six years.

An independent group of the Tropical Medicine Institute «Pedro Kouri» of Ministry of Public Health evaluated in a carefully conducted study, the effectivity of the mass vaccination campaign. Table 2 shows the summary of efficacy, in general reaching 92.5%.

One very clear example of the vaccine impact was observed in the province Havana City, Fig. 8, where the incidence rate among children below six years rose constantly (1986 - 27.5/10^5, 1987 - 30/10^5, 1988 - 42.5/10^5) and after the vaccination the strong epidemic wave was stopped (1989 - 15/10^5, 1990 - 3.8/10^5, 1991 - 1/10^5) and among vaccinees less than 0.08/10^5).

Actually in Cuba the population between 3 months and 20 years is vaccinated; the VA-MENGOC-BC^{R} vaccine is incorporated to the infancy vaccine scheme (for 3 months children).

Also the vaccination of other risk-groups is practically concluded; elderly homes, military camps, big schools, industries, *etc.*

In a very short time the meningococcal disease will be completely controlled.

Reference

(1) Sierra GVG, Campa HC. Preclinical and clinical studies with the antimeningococcal vaccine BC: VA-MENGOC-BC^{R}. Rev Interferon y Bio-tecnologia, Special. 1990.

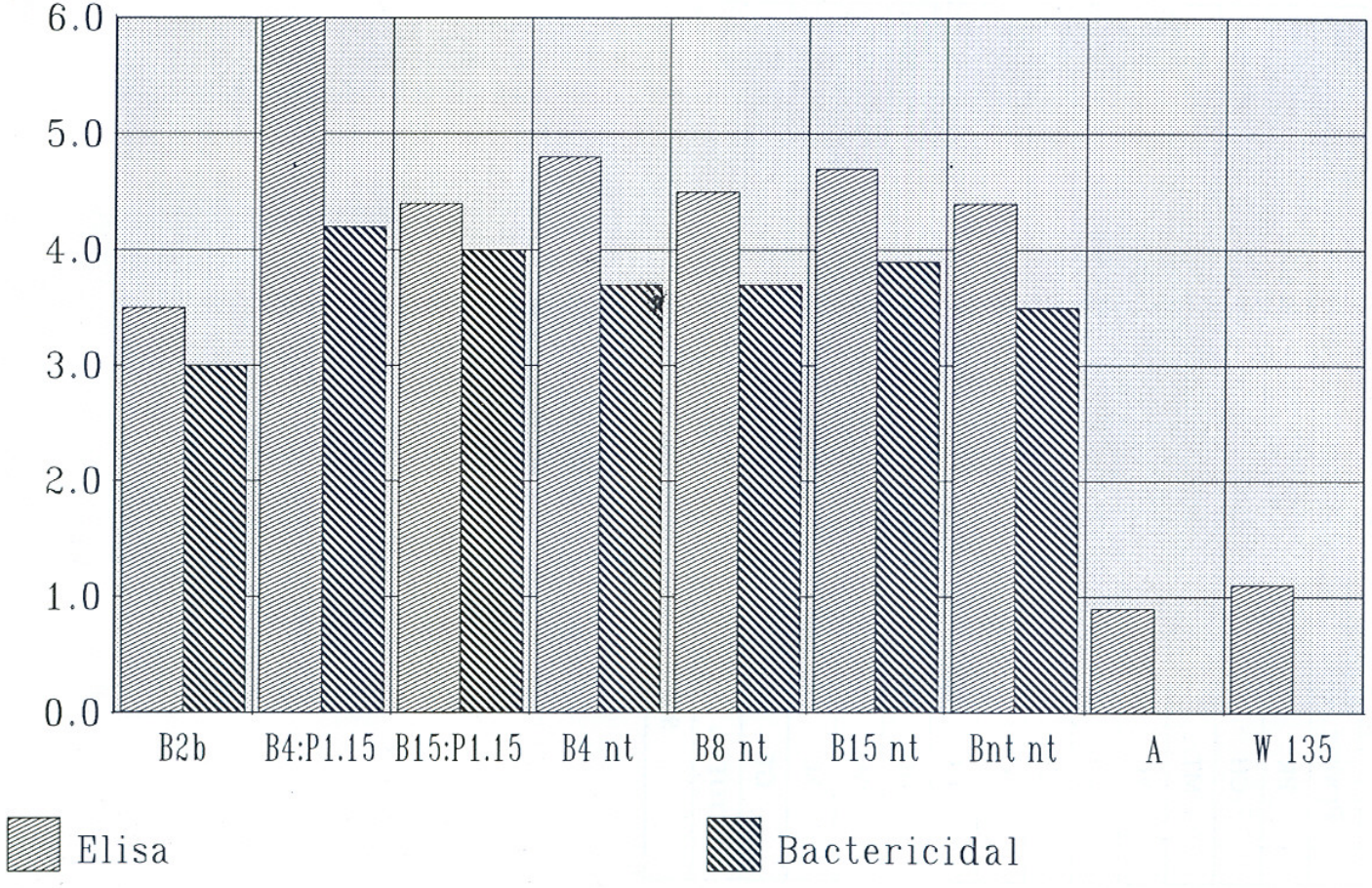

Figure 7
Specific and bactericidal antibody titer increases maintained up to 18 months after vaccination in military recruits against different pathogenic serotypes

Table 1
Percentage of vaccination per province in age group < 6 years

Province	Population	Vaccinees	%
PR	67 500	56 623	83.8
CH	182 400	161 670	88.6
MT	53 000	48 815	92.1
VC	70 000	61 047	87.2
CF	33 800	32 094	95.0
SS	37 700	31 935	84.7
CM	73 500	61 917	84.2
LT	53 100	43 808	82.5
HO	102 900	91 191	88.6
GM	94 800	72 898	76.9
SC	116 600	100 820	86.5
GT	61 100	53 679	87.9
TOTAL	1 013 900	866 148	85.4
Source: Division of Epidemiology, Ministry of Public Health			

Table 2
Relative risk and efficacy per province in age group < 6 years

Province	Relative Risk	Efficacy %
PR	9.37	89.3
CH	39.06	97.44
MT	23.33	88.57
VC	24.55	95.90
CF	23.00	95.68
SS	11.08	90.97
CM	7.48	86.60
LT	23.60	89.28
HO	97.49	98.97
GM	29.99	96.66
SC	6.84	85.47
GT	18.11	94.47
TOTAL	13.44	92.56
Source: Tropical Medicine Institute «PEDRO KOURI», Ministry of Public Health		

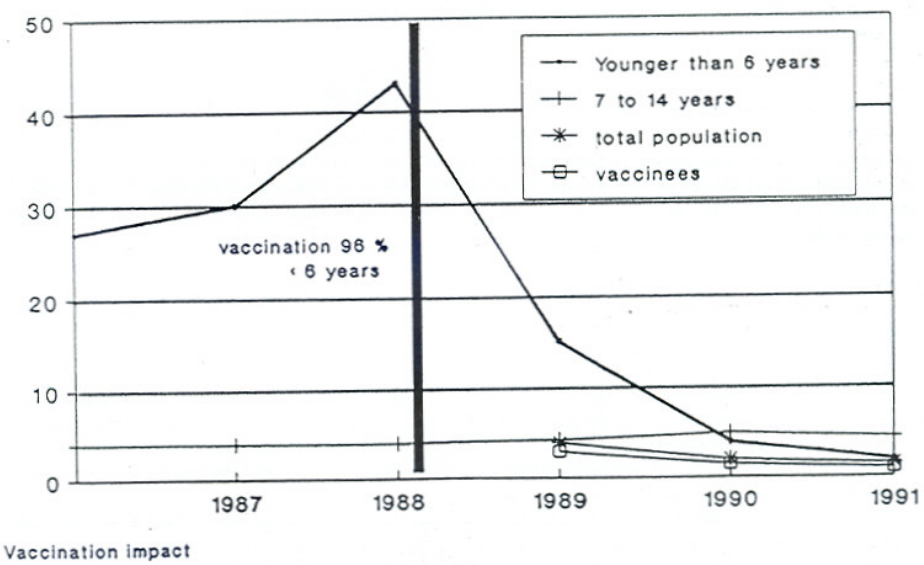

Figure 8
Meningococcal Disease in Havana city: indicence rate per 100 000

DISCUSSION

Gotschlich:
To what do you attribute the broad specificity of the vaccine? What strains were tested for bactericidal effect, and what is the relative bactericidal effect against other strains?

Sierra:
All the results are related to the vaccine strain B:4:P1.15. We have also evaluated the spectrum of specific antibodies in ELISA and in bactericidal assay using different strains.

Gotschlich:
Could you just give us a list of the strains against which the bactericidal test was performed?

Sierra:
We have used B:4:P1.15, B:15:P1.15, B:4:NT, B:2a, B:2b, B:2c, B:8.

Gotschlich:
Could you try to speculate on the reason for the wide cross reactivity; which antigens do you think this is due to?

Sierra:
We think that this long lasting cross reactivity is due to the presence of high molecular weight proteins. There are six important proteins in this rank between 65 to 95 000 daltons. Two or three of these are iron-regulated proteins.

Gotschlich:
Which ones are iron-regulated?

Sierra:
One is a protein of 80 000 daltons, the other is very near to 90 000 daltons. We have now cloned, expressed, and purified some of these high molecular weight proteins, and we have characterized them. It is very difficult to obtain these in sufficient amount for characterization. We have demonstrated the importance of these proteins, especially for the immunological memory and for the cross reactivity.

Gotschlich:
There was yesterday a remarkably careful follow-up of patients that had been vaccinated, in terms of long term sequelae. Have you had the opportunity to investigate this in the same details as what was presented yesterday?

Sierra:
Yes, we studied it in Cuba very carefully. The first group of people was vaccinated in Cuba in 1985, and we have until now followed all the groups of the phase 1, phase 2, and phase 3 trials. We have no long-lasting sequelae at all. We have no disease caused and established through the vaccine. The most important reactions we have observed are the purpuric reactions. Very few cases have occurred, all over the country, especially in very small children. They were very short-lasting reactions. There were no sequelae and no neurologic diseases at all.

Peltola:
What do you mean by purpuric reactions? Do you mean petecchiae? If so, they usually are not very short-lived.

Sierra:
There are two types of reactions. One type of rash seems to be some type of allergy to some of the components and the other type of reaction consists of very small petecchiae. Both are short lasting. I think it is very important also to take into consideration the experience that we have in collaboration with Brazil in São Paulo. They have vaccinated nearly two and a half million children below six-seven years of age within in one week. They have experience in evaluating these side reactions.
They also observed a small number of these purpuric reactions or petecchiae. As far as I know, in Cuba or in Brazil we don't have long-lasting sequelae disease or severe reactions caused through the vaccine.

Griffiths:
You think that these iron-regulated proteins are important in your vaccine. Do you take any special measures to insure that they are expressed?

Sierra:
I have not said that the iron regulated proteins are the only ones responsible for the efficacy. I have said that among the four most important high molecular weight proteins responsible for the long lasting bactericidal response, at least two proteins were related to iron metabolism.

Gotschlich:
The question was: Do you purposely limit iron for growth?

Sierra:
We have performed some changes in media regarding the concentration of some nutrients, but not especially iron limitation.

Wetzler:
Were there any non-volunteers in your study, and if there were, what was the incidence of disease in that group?

Sierra:
The incidence among people not volunteering in the study, in the same region or in the neighbourhood of these schools, has remained on the same level. In that age group we have studied this question intensively. We are going to publish all these results very soon.

Robbins:
I have two questions. The dose of the vaccine you mentioned was 50 micrograms. Was that based upon protein? And if it was based upon the protein, what was the total weight of all the material in the vaccine per dose? And the second question: what percentage of cases of bacterial meningitis were you unable to diagnose either by culture or by serological methods?

Sierra:
The vaccine composition is 50 micrograms of proteins per dose. Between 12 and 18% of these are proteins of the high molecular weight complex. We purify the outer membrane proteins, purify the proteoliposomes and we enrich these preparations with high molecular weight proteins. This is not simply a purification route. The composition is approx. 85% of the normal serotype proteins and approx. 15% of high molecular weight proteins. And we try to avoid the presence of class 4 and class 5 proteins in the vaccine because we have found a great variability in the presence and expression of these proteins in the strains circulating in our country, even before the P1.15 epidemic. We have a limit for tolerance of these proteins in the vaccine. We also avoid the B polysaccharide in the vaccine. We test the preparation chemically and also for the capacity to induce antibodies. We have found that the preparation is not able to induce significant anti-B antibodies.
To the second question, related to the incidence: we were not able to find the causal agent in three cases among vaccinees and in two cases of placebo. But after these we have applied different serological analytical methods. We have tested very carefully for the presence of antigens.
A case of meningococcemia in a vaccinee in the Olgin province was excluded because through culture, latex and ELISA methods we were not able to find B antigen.

Broome:
I would like to look a little further into these excluded cases. There were clinically diagnosed two cases of meningococcemia, one fatal in vaccinees and one also fatal in the placebo recipients. They certainly did not have culture confimation, so they did not meet your primary case definition. But in adolescents vaccinated against group C experiencing a group B epidemic, I think those cases are of some consideration as potential group B cases. My expericence with group B antigen testing is that is is not particularly sensitive, certainly not as sensitive as group A or group B antigen detection. I wonder if you could tell us what is the LPS content of the vaccine.

Sierra:
The LPS content of the vaccine is 1% of the total protein amount. If we include the cases without culture the efficacy is around 75%. In one of the three cases mentioned we had not demonstrated B antigens but we have demonstrated other bacterial antigens.

Gotschlich:
You attribute a high importance to the high molecular weight proteins that you add to you vaccine. Could you outline for us the principles of how you obtain these?

Sierra:
We have found one strain with very important growth characteristics in our culture media. We limit in our culture media some of the nutrients, but not iron especially. We are thinking also of doing that in the future. We found that this strain is able to produce higher concentrations of high molecular weight proteins than the other strains. We perform very difficult steps in purification. We purify these high molecular weight proteins, with successive chromatographic steps, and precipitation, and concentration steps. Then, we use them to enrich the preparation of vesicles.