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RANDOMIZED, DOUBLE-BLIND, PHASE III, PIVOTAL FIELD TRIAL OF THE COMPARATIVE IMMUNOGENICITY, SAFETY, AND TOLERABILITY OF TWO YELLOW FEVER 17D VACCINES (ARILVAX™ AND YF-VAX®) IN HEALTHY INFANTS AND CHILDREN IN PERU

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Abstract. We conducted a randomized, double-blind, phase III yellow fever (YF) vaccine trial among 1,107 healthy children in Sullana in northern Peru. The safety and efficacy (by measurement of geometric mean neutralizing antibody titer responses) were determined for two YF vaccines, ARILVAX™ (n = 738) and YF-VAX® (n = 369). Seroconversion rates were higher (94.9%) in ARILVAX™ than in YF-VAX® (90.6%) recipients. The two-sided 95% confidence interval (YF-VAX®–ARILVAX™) was (−12.8% to −2.5%), indicating that the higher seroconversion rate for Arilvax™ was significant. Post-vaccination (30-day) mean $\log_{10}$ neutralization indices were found to be similar for both products: 1.32 for ARILVAX™ and 1.26 for YF-VAX® ($P = 0.1404$, by analysis of variance). A similar number of subjects in each group reported at least one adverse event (AE); 441 (59.8%) for ARILVAX™ versus 211 (59.9%) for YF-VAX®. Most (591; 96.7%) of these were of a mild nature and resolved without treatment. There were no treatment-related serious AEs. This is the first randomized, double-blind comparison of two YF vaccines in a pediatric population; both vaccines were shown to be highly immunogenic and well-tolerated.

INTRODUCTION

Yellow fever (YF) is a severe mosquito-borne hemorrhagic disease that is endemic and epidemic in tropical South America and Africa. It has the potential for introduction in other areas, including the United States, where the *Aedes aegypti* mosquito vector is present. The etiologic agent is the prototype of the family Flaviviridae of single-stranded RNA viruses that includes dengue and West Nile viruses.[1] The clinical spectrum of YF infections can vary from subclinical to severe systemic disease characterized by hepatitis, renal failure, bleeding diatheses, and cardiovascular collapse, and the case fatality rate can be as high as 20–50%.[1,2] The World Health Organization (WHO) estimates that 200,000 cases of YF occur in endemic areas each year.[3] In addition, there have been several reports in recent years of deaths among unvaccinated tourists traveling to endemic areas.[1,4]

An attenuated YF 17D vaccine strain was developed in 1936 by serial passage of the wild-type parental Asibi virus in mouse and chick embryo cells and is manufactured in a number of countries worldwide.[1] More than 400 million persons have been immunized with YF 17D vaccines since 1937. However, no controlled clinical studies of these vaccines have been conducted in infants and children, who represent the principal population in which vaccination is indicated in endemic areas. The origin, derivation, production, and genomic sequences of these vaccines have been previously described.[1,5] The vaccines, which are manufactured according to standards developed by WHO must be administered at approved vaccination centers. Despite increasing demands for the vaccine, the number of manufacturers has decreased from 13 in 1980 to 8 in 2000.[1] YF-VAX® (Aventis-Pasteur, Swiftwater, PA) is the only vaccine currently approved by the Food and Drug Administration (FDA) and is manufactured in the United States.

Adverse reactions to ARILVAX™ and YF-VAX® have generally been mild and of low frequency.[1] In published studies, self-limited mild and local reactions (erythema, swelling, and pain at the injection site) and systemic reactions (feeling ill, headache, myalgia, headache, and fever with or without symptoms) have occurred in a minority of adult subjects 5–7 days after immunization.[6–8]

In Peru, YF vaccination has been included in the Expanded Program of Immunization (EPI) of the Ministry of Health (MoH) since 2000 and is recommended for all children beginning at nine months of age.[9] Implementation of this vaccination policy has been highly variable, however, due in part to shortages of YF vaccine. Moreover, vaccine use varies across regions according to the risk of YF transmission. Continued occurrence of isolated and clusters of cases of YF among Peruvians has continued to occur during the past 25 years. Indeed, Peru has had the highest incidence of YF in South America during this interval.[1] The YF virus is maintained in enzootic/epizootic sylvatic cycles involving nonhuman primates and forest tree-top dwelling mosquitoes. Humans generally are infected when they intrude into forested areas (so-called jungle YF).[10]

Many cities in coastal areas of northern Peru are infested with *Ae. aegypti* and thus are receptive to virus introduction and resulting urban epidemics, in which humans rather than monkeys serve as the principal host. Cases of YF have also been increasing in Peru in the last few years in association with specific hydrographic river basins,[10,11] with some recent cases reported in regions of greater geographic proximity (within 100 miles) of the city of Sullana. Moreover, a pilot review of immunization records conducted in mid 2001 in five MoH immunization clinics in this *Ae. aegypti*-infested city indicated that less than 3% of age-eligible children had received YF vaccine (Sanchez JL and others, unpublished data). Since Sullana lies outside the YF enzootic area and YF vac-

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cine shortages have continued to exist, YF vaccination has not been a priority in coastal regions of Peru. Therefore, Sullana provided an ideal location for a clinical trial of YF vaccines because children who might travel to nearby enzootic regions of Peru or be exposed to urban outbreaks in the future would benefit from vaccination.

We present the results of a randomized, double-blind, phase III vaccine trial conducted among 1,107 healthy children in Sullana in northern Peru. The objectives of the study were to determine the safety, tolerability, and efficacy (by measurement of neutralizing antibody responses) of two YF vaccines, ARILVAX™ and YF-VAX® and to assess the consistency in production of three different lots of ARILVAX™. This research was carried out under an Investigational New Drug Application approved by FDA in accordance with Good Clinical Practice regulations and clinical research guidelines established by the basic principles defined in the U.S. 21 Code of Federal Regulations Part 312 and the Declaration of Helsinki. Prior to study start, the protocol (and modifications) and the informed consent and assent forms were reviewed and approved by a properly constituted Institutional Review Board (IRB) as recognized by the FDA.

MATERIALS AND METHODS

Vaccines. The YF vaccines compared were ARILVAX™, produced by Evans Vaccines Limited (formerly Evans Medical Limited; now part of Chiron Vaccines, Speke, Liverpool, United Kingdom) and YF-VAX®, manufactured by Aventis-Pasteur Laboratories Inc., Swiftwater, PA). Both vaccines are manufactured in embryonated chicken eggs and both vaccines meet the international standards and requirements for YF vaccines specified by WHO.[12] There are minor differences in passage histories and final formulations of ARILVAX™ and YF-VAX®; however, the two vaccines are estimated to be greater than 99% homologous at the nucleotide sequence level.[13,14] The indication for use of these vaccines is the prevention of YF in persons traveling to, or living in endemic areas of, South America and Africa. Vaccine is also indicated for laboratory personnel who might be exposed to virulent YF virus. As for all live vaccines, neither ARILVAX™ nor YF-VAX® vaccine contains a bacteriostatic agent. ARILVAX™ is one of three YF 17D vaccines currently approved by WHO for supply to United Nations agencies.

Manufacture of YF 17D vaccine in the United Kingdom, performed according to WHO specifications, commenced in 1945 at the Wellcome Foundation Limited (Wellcome, London, United Kingdom). In 1964, a leukosis-free vaccine was introduced to the United Kingdom market and in 1976, the vaccine was improved with the addition of stabilizers. The vaccine was also approved for marketing in six other European Community Member States (Austria, Belgium, Finland, Ireland, The Netherlands, and Sweden) and in eight other countries (Hong Kong, Israel, Malaysia, Norway, Singapore, South Africa, Switzerland, and Thailand). In 1995–1996, manufacture of ARILVAX™ was initiated at Evans Medical Limited (Leatherhead, United Kingdom) by inoculation of chicken embryos with seed virus acquired from Wellcome. The only significant difference between the Wellcome and Evans Medical manufacturing processes, apart from the site of manufacture, is that the current vaccine does not contain antibiotics (polymyxin B sulfate and neomycin sulfate).

The three lots of ARILVAX™ used in this study were manufactured by Evans Vaccines Limited from seed virus acquired from Wellcome. ARILVAX™ is supplied as a freeze-dried powder in a single dose vial containing not less than 4.4 $\log_{10}$ plaque-forming units (PFU)/0.5-mL dose of YF 17D virus at release and which will contain not less than 3.7 PFU/0.5 mL at the end of shelf life. The virus content meets or exceeds the WHO specification for potency.[12] The vaccine also contains orthophosphate, potassium chloride, potassium dihydrogen orthophosphate, sorbitol, and porcine gelatin. The vaccine is reconstituted in 0.75 mL of diluent (water for injections) and 0.5 mL is injected by the subcutaneous route. After reconstitution, the vaccine appears opalescent and is pink-brown in color.

YF-VAX® is licensed by the FDA in the United States. It is also supplied as a freeze-dried powder in single dose or five-dose vials containing not less than 5.04 $\log_{10}$ PFU/0.5-mL dose of the attenuated 17D virus. The virus content meets or exceeds the WHO specification for potency.[12] The vaccine contains sorbitol and porcine gelatin without added salts. It is reconstituted in 0.6 mL of preservative-free sodium chloride United States Pharmacopeia, and 0.5 mL is injected by the subcutaneous route. After reconstitution, the vaccine appears slightly opalescent and is light orange in color.

Trial design and study population. This study was designed as a randomized, double-blind vaccine trial that was conducted in healthy children 9 months to 10 years of age (inclusive) in Sullana, a city in the northern coastal region of Peru. The study sponsor was Acambis Inc. The study was conducted by the U.S. Naval Medical Research Detachment (NMRCD) in Lima, Peru. The protocol was reviewed by the Ministry of Health of Peru and by the Scientific Review Committee of the Walter Reed Army Institute of Research. The protocol and supporting documents were reviewed and approved by three IRBs, including the Universidad Peruana Cayetano Heredia, the U.S. Naval Medical Research Center, and the Surgeon General's Human Subjects Research Review Board (HSRRB). Clinical monitoring of the study was conducted by staff of PRA International (Lenexa KS), a contract research organization. At monitoring visits, the study binder was reviewed and updated, verification of informed consent was performed, and a 100% source document verification of data in the case report form (CRF) was performed to ensure accuracy and reporting of adverse events (AEs). Drug accountability was also performed. A Peruvian physician served as Medical Monitor and was able to independently report AEs to the Peruvian IRB and the HSRRB. A Data and Safety Monitoring Board (DSMB) was established consisting of two physicians in Peru and one in the United States who reviewed all serious AEs. Data entry was the responsibility of Acambis and Syne qua non Limited (Norfolk, United Kingdom). Statistical analyses were provided by Syne qua non Limited and ViruStat Limited (North Wales, PA).

Five recruitment centers (health posts and clinics) within the city were selected based on staff availability and logistical considerations. The initial objective was to be able to vaccinate and complete follow-up of 1,050 subjects assuming a loss to follow-up of no greater than 5–10%. Planned recruitment was for 750 children in the nine month to five years of age group (cut-off at the fifth birthday) and for an additional 300 children in the 5–10-year-old age group (cut-off at the 10th birthday). Within each of the two groups, a 2:1 ratio of

ARILVAX™ to YF-VAX® was followed. Vaccination was conducted between the months of May and November 2002, inclusive.

Randomization. A stratified randomization procedure was followed to ensure equal distribution of vaccination by age group and sex. Additionally, for the ARILVAX™ vaccine product only, randomization was also stratified according to production lot (that is, to achieve enrollment and follow-up of at least 167 subjects for each of the three conformance lots). All study staff who dealt with subjects in the study, as well as co-investigators and laboratory personnel who performed laboratory testing at NMRCD-Lima and Acambis Inc., remained blinded to the vaccine type and lot assignments.

Study enrollment criteria and follow-up procedures. Participation in the study was limited to infants and children meeting the inclusion and exclusion criteria. Exclusion criteria included children falling outside of the age parameters, those who were unwell or demonstrated poor growth, those who had received another vaccine in the previous 30 days or who had documented YF vaccination in their personal or clinic immunization records, those who had immunosuppressive illness or were taking immunosuppressive medications, and those who gave reliable histories of egg allergies. Children who were due for routine childhood vaccinations would receive them according to Peruvian MoH guidelines and were re-scheduled for participation in the study once the 30-day period elapsed and if the parents still wished to have their child participate.

The study procedures are outlined in Table 1. The vaccination center was equipped with a full-time study pediatrician and nurse as well as with the necessary emergency facilities for maintenance care in response to a serious allergic reaction. Children were vaccinated with one of the two vaccines administered subcutaneouly into the deltoid region of the upper arm. They were kept at the center for a minimum of 30 minutes to monitor for any systemic allergic reactions. Children failing to present for scheduled clinic appointments were visited in their homes.

Safety was assessed by recording all AEs that occurred after vaccination. On all clinic visits (Table 1), a structured interview was conducted for AEs and the diary cards were reviewed. All AES were recorded in the case report forms, including severity, the investigator's assessment of causality (relationship to study vaccine), start date and end date, and whether treatment was required. Children failing to present for scheduled appointments were visited in their homes. Parents/guardians were instructed to return to the clinic if the child developed a fever (oral temperature $\geq 38°C/100.4°F$) or if they were in any way concerned with the health of their child during the follow-up period (days 1–31). Subjects who developed a generalized febrile illness within the first 10 days post-vaccination were carefully evaluated. The study's on-site investigator (VEB-W) determined if there was a plausible explanation for the illness, such as a respiratory infection. If there was no plausible explanation, a blood sample was taken to help determine the nature of illness (by liver function tests [LFTs] and viremia).

Demographic and clinical information was recorded on standardized data forms (source documents) and transcribed into CRFs, which were then sent in hard copy for data entry and statistical analysis with copies retained at the Sullana study site under lock and key until study termination.

All subjects completing the 31-day follow-up received confirmation of immunization against YF, which was deemed valid by Peruvian MoH authorities.

Antibody tests. Neutralizing antibody is the principal mediator of immunity elicited by YF 17D vaccines and has been proven to be correlated with protection from disease in nonhuman primates.[6,15] Sera collected on days 1 and 31 were tested for neutralizing antibodies to YF and serum collected on day 1 was tested for evidence of pre-existing immunity to dengue virus serotypes 1, 2, 3, and 4 by an enzyme-linked immunosorbent assay and a plaque-reduction neutralization test (PRNT) at NMRCD-Lima. Neutralizing antibody titers to YF were measured at Acambis Inc. using a constant serum varying virus assay performed in cell culture previously standardized by Bureau of Biologics (FDA) (Rockville, MD).[15,16] Acambis Inc. has used this method to establish a validated

TABLE 1
Study procedures and schedule

		On study period day (range)		
Study day→	1	4	11 (11–14)	31 (24–38)
Clinic visit	X	X	X	X
Informed consent	X			
Inclusion/exclusion criteria	X			
Medical history	X			
Physical examination	X	X*	X*	X*
Vital signs	X			
Blood collections				
Serum for yellow fever neutralizing antibody	X			X
Serum for dengue neutralizing antibody	X			
Baseline AST, ALT (retention sample)	X†			
AST, ALT, quantitative viremia		At any time days 2–10‡		X§
Randomization	X			
Vaccination	X			
Diary card	Days 1–10			
Subject interviews for adverse events	X	X	X	X

* Interim physical examinations are performed at other visits at the discretion of the investigator, if necessary, to investigate adverse events or clinical laboratory abnormalities.
† Serum for liver function (aspartate aminotransferase [AST], alanine aminotransferase [ALT]) frozen for subsequent analysis as required to assist in the interpretation of adverse events during days 2–10.
‡ Blood tests (AST, ALT, and viremia) to investigate unexplained febrile illness occurring between days 2 and 11.
§ AST and ALT repeated on day 31 if abnormal results were obtained on samples tested between days 2 and 11 and repeated at day 31 to determine resolution.

neutralization test in Vero cells. This test measures the neutralizing capacity of serum.

Seroconversion to YF virus was defined as a $\log_{10}$ neutralization index (LNI) ≥ 0.7. The LNI is calculated as the $\log_{10}$ difference in virus titer of a mixture of serum and virus between baseline (pre-immunization) and post-immunization samples. The post-vaccination serum from a subject who did not seroconvert had to exhibit an LNI ≤ 0.7 compared with the pre-vaccination serum. The cut-off value for a positive LNI ≥ 0.7 was established by protection studies in non-human primates and represents the antibody titer required to protect against lethal challenge.[15] In addition, an LNI cut-off ≥ 0.7 was used as the endpoint for seroconversion in a previous clinical trial comparing ARILVAX™ with YF-VAX® conducted in the United States by Acambis Inc.[6]

Statistical methods. *Sample size.* We estimated that a sample size of 144 subjects per treatment group (all age ranges) would be required to show a study power of 0.80 in a one-sided test for non-inferiority at a significance level (alpha error) of 0.05 and with a 5% exclusionary percentage when the underlying seroconversion rates are equal and 97% in each group. All 700 subjects in the ARILVAX™ treatment group and 350 in the YF-VAX® treatment group (total = 1,050) were to be tested for a YF serologic response. The sample size for efficacy exceeded that required based on statistical assumptions because it could not be predicted in advance what proportion of subjects would not be evaluable for efficacy (seronegative to YF at baseline). Moreover, prior dengue immunity may interfere with the immune response to YF vaccine, reducing the seroconversion rate and increasing the sample size for demonstrating non-inferiority.

The sample size estimations also established an upper bound of 0.004 for the 95% confidence interval (CI) for the incidence of a severe AE (SAE) in the case that such an event was not observed among the 700 subjects in the ARILVAX™ treatment group and an upper bound of 0.009 for the 95% CI for the 350 subjects in the YF-VAX® treatment group.

For the objective of demonstrating clinical consistency of three ARILVAX™ conformance lots considered in a pairwise analysis, a sample size for conformance of 62 subjects per group was estimated to provide a study power of 0.90 in a one-sided test of non-inferiority at an alpha significance level of 0.025 (or an overall 0.025 level alpha test of equivalence) with a 10% exclusionary percentage when the underlying seroconversion rates are equal and 97% in each group.

Primary analysis. Since the primary goal of this study was to demonstrate non-inferiority in immunogenicity of ARILVAX™ compared with YF-VAX®, the primary efficacy analysis consisted in comparing the proportion of subjects who seroconverted to YF virus 30 days post-vaccination in the two vaccine groups. Initially, non-inferiority was defined as the seroconversion rate following ARILVAX™ not being lower than the seroconversion rate following YF-VAX® by more than a clinically acceptable difference of 5%. Following subsequent discussions with the FDA, a narrower limit of confidence for non-inferiority was applied. The non-inferiority test was repeated using a one-sided test at a significance level of 0.025. Moreover, the upper limit for inferiority was calculated as the one-sided 97.5% confidence limit. Finally, the two-sided 95% CI for the difference of proportions between treatment groups was also calculated. These tests were performed to evaluate the proportion of subjects seroconverting to YF following vaccination with either ARILVAX™ or YF-VAX® up to the day 31 visit from the combined age groups, 9 months to 10 years inclusive. These analyses were repeated for the following age groups: 9–18 months, > 18–36 months, > 36–60 months, and 60 months plus one day to 10 years. Only study subjects without prior YF immunity at baseline, and who actually received one of the two study vaccines and were tested for YF neutralizing antibody on day 31, were included in the per-protocol population.

Secondary analysis. The secondary goal of this study was the comparison of geometric mean titers (GMTs) between the two vaccine groups. Analyses were performed to show that the 95% CI on the ratio of the two treatment group geometric mean YF neutralizing antibody titers (expressed as mean LNI), rules out a half-fold decrease and a two-fold increase (ARILVAX™ to YF-VAX®). Analyses were repeated for the following age groups: ≥ 9–18 months, > 18–36 months, > 36–60 months, and > 60 months to 10 years.

Clinical consistency. A pairwise comparison of the geometric mean YF neutralization titer (expressed as mean LNI) was used to test the three ARILVAX™ clinical consistency lots for homogeneity. Use of this analysis shows that the 95% CI on the ratio of means rules out both a half-fold decrease and a two-fold increase in geometric mean YF neutralizing antibody titers from the combined age groups (9 months to 10 years inclusive) between each ARILVAX™ lot on day 31. A secondary analysis of ARILVAX™ lot homogeneity was carried out using equivalence tests using the 95% CI for the difference of proportions of subjects who seroconverted. Where pairwise CIs all fall between −10% and +10%, the limits identified in the study protocol, the three lots were to be considered equivalent.

Supplemental analyses. Supplemental analyses were performed to address the role of host factors (sex, age, and sex by age) and consistency of YF antibody response between the three ARILVAX™ lots. Other analyses included a comparison of the mean LNI on day 31 between age groups with and without adjustment for pre-existing dengue immunity (present or absent), and ARILVAX™ conformance lot effects.

Safety analysis. Safety and tolerability were assessed by comparison of the incidence (expressed in percent) of AEs across the two treatment groups by a chi-square test (Fisher's exact test as required). Separate analyses were performed to determine whether subjects with and without antibodies to YF or dengue at baseline differed with respect to the incidence of AEs in the two vaccine groups.

RESULTS

Demographic and baseline characteristics of participants. A total of 1,107 children were eligible for participation and were randomly assigned to a study group at the time of vaccination. Of these, 738 received ARILVAX™ and 369 received YF-VAX® (an exact 2:1 ratio of vaccine group randomization was achieved). There were no age and sex differences in terms of the population receiving vaccination at baseline (Table 2). At baseline, 36 (26 receiving ARILVAX™ and 10 receiving YF-VAX®) children were found to be YF immune; 82 (54 and 28 in the two treatment groups, respectively) did not have two samples taken for serologic testing,

TABLE 2
Description of the study population (for all vaccinees)

Demographic feature	Statistic	ARILVAX™ (n = 738)	YF-VAX® (n = 369)	P*
Ethnicity	Caucasian	8 (1.1)	4 (1.1)	1.0000
no. (%)	Black	1 (0.1)	0 (0)	
	Mixed	729 (98.8)	365 (98.9)	
Age (months)	Mean	48.6	50.1	0.4421
	Standard deviation	30.44	30.67	
Age group†	9 months to 18 months	144 (19.5)	59 (16.0)	0.3999
no. (%)	18 months + 1 day to 36 months	131 (17.8)	77 (20.9)	
	36 months + 1 day to 60 months	243 (32.9)	123 (33.3)	
	60 months + 1 day to 10 years	220 (29.8)	110 (29.8)	
Sex	Male	357 (48.4)	178 (48.2)	1.0000
no. (%)	Female	381 (51.6)	191 (51.8)	

* *T*-tests used for continuous variables; chi-square tests for categorical variables. YF = yellow fever.
† Age derived from date of day 1 visit and date of birth. Years/months have been rounded down to the lowest whole number, where applicable.

one child had a baseline titer that could not be determined, and seven children otherwise did not complete the protocol. This left a final (per protocol population) sample of 981 ($n_{ARILVAX} = 652$; $n_{YF\text{-}VAX} = 329$) children for endpoint analyses.

There were also no statistically significant differences between treatment (vaccine) groups in terms of the proportion of children randomized to each vaccine by study site, mean age, sex, weight (in kg), height (in cm), body mass index (kg/m^2), pulse (beats per minute), allergy history, anaphylactic reaction history, or significant pre-existing medical conditions. No differences were found for the pre-existing (baseline) prevalences of antibody to YF (4.1% versus 3.0%) or antibody to dengue by $PRNT_{50}$ (14.3% versus 15.0%) between the ARILVAX™ and YF-VAX® treatment groups, respectively.

Efficacy. Overall, 619 (94.9%) of the 652 ARILVAX™ and 298 (90.6%) of the 329 YF-VAX® recipients in the per protocol final sample seroconverted to YF virus. The seroconversion rate for ARILVAX™ was statistically non-inferior to that for YF-VAX® ($P < 0.0001$, by one-sided test of non-inferiority). Moreover, the upper limit of the 97.5% CI for YF-VAX® minus ARILVAX™ was –0.025%, indicating that ARILVAX™ actually produced a significantly higher response rate than YF-VAX®. The two-sided 95% CI for YF-VAX® minus ARILVAX™ ranged from –12.8% to –2.5%, which also indicates that ARILVAX™ produced a significantly higher seroconversion rate than YF-VAX® ($P < 0.05$). Analysis for each of the four age groups indicated in all cases a significantly higher seroconversion rate, as well as non-inferiority between treatment groups (Table 3). The difference between seroconversion rates for the two vaccines was most striking in the lowest age groups. In addition, no differences in seroconversion rates were detected between the male and female participants ($P = 0.82$).

Notably, the seroconversion rates did not vary according to baseline level of dengue immunity or vaccine administered (Table 4). Among dengue immune and non-immune participants, 93.6% and 94.4% in the ARILVAX™ group versus 82.7% and 92.0% in the YF-VAX® group, respectively, seroconverted. Baseline immunity to specific dengue serotypes was not found to affect the YF seroconversion rates following vaccination.

Yellow fever antibody titer response. The absolute level (GMT) of the YF vaccine-induced antibody response (expressed as the mean YF LNI) was not found to differ in dengue immune versus non-immune individuals (Table 4). Overall, the mean (± SD) LNI was 1.32 (± 0.56) for ARILVAX™ compared with 1.26 (± 0.65) for YF-VAX® (Table 5). Using analysis of variance to calculate the 95% CI for the difference in mean LNI, we found that there was no difference between the ARILVAX™ and YF-VAX® treatment groups at day 31 ($P = 0.1404$). More importantly, the 95% CI on the ratio of geometric mean YF neutralizing antibody titers was 0.9563–1.3706, which falls within the interval 0.5–2.0, thus establishing equivalence of ARILVAX™ and YF-VAX® in terms of neutralizing antibody titer levels. Additional sub-analysis for each of the four age groups showed no differences between the two vaccine groups (Table 5).

Tests for ARIVAX™ lot consistency. Six hundred fifty-two doses of ARILVAX™ were administered from three conformance lots as follows: lot 760467, n = 216; lot 760468, n = 217; and, lot 761294, n = 219. The 95% CI on the ratio of means ruled out both a half-fold decrease and a two-fold

TABLE 3
Yellow fever (YF) seroconversion rates at day 31 (per protocol population) by age groups

Feature	ARILVAX™ % (no./No.)	YF-VAX® % (no./No.)	Non-inferiority P*	One-sided 97.5% for inferiority*	Two-sided 95% confidence interval
Total evaluated	94.9 (619/652)	90.6 (298/329)	< 0.0001	–0.025	(–0.128, –0.025)
Age group					
9–18 months	95.8 (115/120)	88.5 (46/52)	0.0016	0.010	(–0.191, 0.010)
> 18–36 months	94.6 (106/112)	86.2 (50/58)	0.0018	0.006	(–0.201, –0.006)
> 3–5 years	93.1 (202/217)	92.3 (108/117)	0.0231	0.049	(–0.076, –0.049)
> 5–10 years	96.6 (196/203)	92.2 (94/102)	0.0003	0.009	(–0.114, –0.009)

* One-sided test of non-inferiority at the 2.5% level of significance with 5% non-inferiority bound based on Chan's test.

TABLE 4
Comparison of yellow fever (YF) immune response 30 days after vaccination for subjects with and without baseline dengue (any serotype) immunity with paired samples (by vaccine study group)*

Baseline dengue immunity	ARILVAX™ group		YF-VAX® group	
	Seroconversion rate	Mean YF LNI	Seroconversion rate	Mean YF LNI
Present	88/94 (93.6%)	1.449	43/52 (82.7%)	1.332
Absent	32/573 (94.4%)	1.336	23/288 (92.0%)	1.254

* No statistically significant difference for comparison of seroconversion rates (P = 0.0980) or mean YF $\log_{10}$ neutralization index (LNI) (P = 0.1659) between vaccine study groups within a linear regression model accounting for dengue immunity as a host factor.

increase in geometric mean YF neutralizing antibody titers from the combined age groups (9 months to 10 years inclusive) between each ARILVAX™ lot on day 31, establishing equivalence of the three lots. Moreover, the proportion of children who seroconverted to each lot was very similar ranging from 94.0% to 96.3%.

Effect of host (sex and age) factors. In a model of host factors including age and sex analyzed using unconditional logistic regression, the overall YF seroconversion rate for subjects vaccinated with ARILVAX™ continued to be significantly higher compared with that after vaccination with YF-VAX® (P = 0.01). Moreover, when stratifying for dengue immunity using the model applied to the entire per protocol population (n = 981), we found that the difference in seroconversion rates between ARILVAX™ and YF-VAX® was retained (P = 0.03). Within the model, none of the host factors had a statistically significant impact on the YF antibody seroconversion rate. The significance (P) values for the host factors that included sex, age, and sex by age were 0.82, 0.49, and 0.46, respectively. Similar regression analysis based on log mean YF neutralizing index (LNI) antibody titers at day 31 also indicated that the role of host factors did not influence final YF antibody response (P = 0.50).

Safety assessments. No SAEs were experienced by children receiving ARILVAX™; however, two children had three unrelated, though serious, AEs after YF-VAX®. One child had an episode of bronchial pneumonia and another a urinary tract infection as well as a documented diarrheal episode attributed to an enteropathogenic *Escherichia coli* infection.

The first subject was a six-year-old girl who developed fever 13 days after vaccine administration and was subsequently hospitalized. The workup showed a urine culture positive for *Klebsiella*, as well as a stool culture positive for enteropathogenic *E. coli*. Results of liver function studies were normal. She was treated with appropriate antibiotics and recovered uneventfully.

The second subject was a 10-month-old boy who was hospitalized the day after vaccination with a bronchial obstruction, which was diagnosed as focal pneumonia by chest radiograph. Further inquiry showed that the subject had developed a cough two days before vaccination, suggesting that the process was occurring prior to vaccine administration. The subject was treated with antibiotics and also recovered uneventfully. No subjects had a febrile syndrome clinically suspicious of YF vaccine–associated viscerotropic disease (YEL-AVD) requiring liver function or viremia investigations.

A third subject who did not meet the criteria for an SAE presented on day 31 follow-up with scleral icterus. Preliminary LFTs showed mildly elevated levels of liver enzymes and subsequent blood work showed IgM for hepatitis A. Hepatitis A is endemic throughout this population and given the time frame of the event (approximately 29 days after vaccine administration), it is highly unlikely that the jaundice was vaccine related. This child was followed until resolution of symptoms was documented.

The incidences of reporting one or more AEs were almost identical (Table 6) between the two vaccine study groups. Of these, investigators blinded to the vaccine identity determined approximately half the subjects were reporting AEs related to vaccination. The majority of related AEs were mild in nature and resolved within 24–48 hours post-vaccination.

The profile of commonly reported (> 5% of subjects) AEs following each vaccine was similar (Table 7). Given the fact that these reports include events that may or may not be related to vaccination, the profile includes common pediatric conditions. In addition to these common AEs, it should be noted that only 28 (3.8%) reports of injection site pain were received following ARILVAX™ vaccination and 5 (1.4%) following YF-VAX® administration, although it is accepted that because of the age of the subjects in this study, underreporting of such symptoms most probably occurred.

DISCUSSION

For the purposes of international travel certification, YF revaccination is required every 10 years.[1,17] However, immunity has been documented to last more than 35 years[18] and is probably life long. Thus, durability of immunity could not be tested in a clinical trial setting. In our study, we demonstrated that children receiving ARILVAX™ met the primary endpoint showing statistical non-inferiority in seroconversion rates compared with the active control, YF-VAX®. Indeed,

TABLE 5
Geometric mean yellow fever (YF) neutralization antibody titer on day 31 (per protocol population) for age groups*

Feature	ARILVAX™ Mean LNI (SD)	YF-VAX® Mean LNI (SD)	P†	Mean difference	95% CI
Total evaluated	1.32 (0.56)	1.26 (0.65)	0.1404	0.059	–0.0194, 0.1369
Age group					
9–18 months	1.31 (0.51)	1.16 (0.61)	0.0850	0.157	–0.022, 0.337
> 18–36 months	1.36 (0.58)	1.24 (0.62)	0.2086	0.122	–0.069, 0.313
> 3–5 years	1.27 (0.56)	1.23 (0.51)	0.4549	0.047	–0.076, 0.169
> 5–10 years	1.33 (0.56)	1.35 (0.80)	0.8398	–0.016	–0.171, 0.139

* LNI = $\log_{10}$ neutralization index; CI = confidence interval.
† By analysis of variance (analysis one-way for difference between treatments).

TABLE 6
Summary of adverse events (AEs) by age group and severity

Parameter	ARILVAX™ n = 738 (%)	YF-VAX® n = 369 (%)
Number of reported AEs	1,365	612
Subjects with ≥ 1 AE	441 (59.8)	221 (59.9)
9–18 months old reporting AE	98 (68.1)	49 (83.1)
18–36 months old reporting AE	90 (68.7)	54 (70.1)
36–60 months old reporting AE	156 (64.2)	62 (50.4)
60–120 months old reporting AE	97 (44.1)	56 (50.9)
Subjects reporting related AE	216 (29.3)	111 (30.1)
Mild related AE	396 (53.7)	195 (52.8)
Moderate related AE	12 (1.6)	8 (2.2)
Severe related AE	0	0

the upper limit of the 97.5% CI for YF-VAX® minus ARILVAX™ was –2.5%, indicating that ARILVAX™ actually produced a significantly higher response rate than YF-VAX®. The two vaccines elicited equivalent neutralizing antibody titers.

Interestingly, for both ARILVAX™ and YF-VAX® vaccines, seroconversion rates in Peruvian children were lower than those recently reported in adults in the United States (99%).[6] The overall seroconversion rate in children who received ARILVAX™ (94.9%) was higher than that in children who received YF-VAX® (90.6%), and the difference in seroconversion rate was most pronounced in the two youngest age groups (95.8% versus 88.5% in children 9–18 months old and 94.6% versus 86.2% in children 18–36 months old). In Africa, young children represent the age group at highest risk, and this would also be true in South America if YF virus were introduced into coastal regions and transmitted by the urban vector *Ae. aegypti*. The reason for the higher seroconversion rates in adults[6] than in children is unclear. It is possible that both the ethnic background of the population and age may have played a role. In the adult study reported by Monath and others,[6] Hispanics had slightly lower antibody titers than Caucasians. One previously published report from Latin America (Brazil) noted that young children had lower immune responses than older persons to YF vaccine.[19]

Factors that can potentially affect the immune response to YF 17D vaccine include 1) pre-existing immunity to antigenically related, cross-protective flaviviruses, such as dengue viruses; 2) immunosuppression due to underlying diseases or drug treatment; 3) severe malnutrition; and 4) pregnancy. The incidence of illness following infection with wild-type YF virus is higher in males, but it is uncertain whether this is due to epidemiologic or host susceptibility factors.[1] In the phase III trial conducted among adults in the United States, males had higher mean neutralizing antibody titers than females.[6] However, in this study among young children in Peru, no such effect was observed.

Many areas of YF endemicity overlap with those of dengue transmission, particularly in South America. Therefore, dengue immunity was investigated to determine whether this modulates the immune response to YF vaccine. Previous wild dengue infection with residual immunity has been shown to reduce the immune response to YF vaccine,[20] and dengue immunity was shown to partially cross-protect against YF in a monkey model.[21] There is no evidence of this effect in the data collected here, and quite the contrary, it would appear that the mean YF antibody (LNI) response for dengue-immune children after vaccination is higher than that among children which are not dengue-immune, suggesting flavivirus antigenic cross-reactivity. Dengue infection, particularly with serotypes 1 and 2, is evidently common among young children of the Sullana city area. While the response to ARILVAX™ is comparable to that observed among children not immune to dengue, the proportions of children responding to YF-VAX® is less than 90%. The trend to lesser seroconversion rates for YF-VAX® in dengue-immune individuals compared with ARILVAX™ is not statistically significant. This may be due to the small sample sizes or reflects the overall lesser response to YF-VAX® regardless of dengue immunity.

The underlying reasons for dengue modulation of YF infection or vaccination deserves further study, and is particularly relevant in Peru and other South American countries where since the early 1990s, dengue has invaded the YF-endemic region. As early as 1923, dengue immunity was suggested as the basis for resistance to YF in long-term residents of endemic areas, and was later proposed as a barrier to introduction of YF into Asia.[22] However, the evidence for interference with 17D vaccine in humans is conflicting and discrepancies across studies may be due to the number of prior Flavivirus infections, the breadth of heterotypic response, or the identity of the viruses responsible for prior infection.

In a phase III clinical trial of ARILVAX™ and YF-VAX® conducted in the United States in 1999,[6] significantly more subjects in the YF-VAX® group (71.9%) experienced one or more drug-related AEs when compared with subjects in the ARILVAX™ group (65.3%; $P = 0.008$). The difference between subjects receiving these treatments was due to a higher rate of local reactions in the YF-VAX® group. Interestingly, we did not find such AE rates to be any different among young Peruvian children.

In the United Kingdom and the United States, approximately 6,000–9,000 infants and 3,000–4,000 children between 9 months and 10 years of age annually receive ARILVAX™ or YF-VAX®, respectively. There is no evidence that the safety profile of these vaccines differs in children who are ≥ 9 months old from that of adult subjects. Yellow fever vaccine–associated neurotropic adverse events (YEL-AND, formerly called post-vaccinal encephalitis) are a rare, age-related complications of YF vaccination. Encephalitis has been reported in the published literature in 25 cases since standardization of YF vaccine manufacture in 1945, of which 16 cases were in infants ≤ 7 months old, prior to establishment of a

TABLE 7
Common adverse events reported (incidence ≥ 5%)*

MedDRA (diagnostic) terminology	ARILVAX™ n = 738 (%)	YF-VAX® n = 369 (%)
Pyrexia	197 (26.7)	98 (26.6)
Pharyngitis	129 (17.5)	54 (14.6)
Diarrhea NOS	91 (12.3)	40 (10.8)
Nasopharyngitis	64 (8.7)	32 (8.7)
Appetite decreased NOS	62 (8.4)	34 (9.2)
Malaise	57 (7.7)	26 (7.0)
Cough	55 (7.5)	20 (5.4)
Pharyngotonsillitis	45 (6.1)	21 (5.7)
Headache	44 (6.0)	26 (7.0)
Vomiting NOS	43 (5.8)	11 (3.0)

* MedDRA = Medical Dictionary for Regulatory Activities; NOS = not otherwise specified.

minimum age for vaccination.[1] The incidence of encephalitis following YF vaccination in children ≥ 9 months of age is not known with precision, but is believed to be very low. Full recovery from encephalitis is the rule, but one fatal case occurred in the United States in a three-year-old child.[23]

Hypersensitivity reactions to egg proteins, gelatin, or other allergens contained in YF 17D vaccine are rare. The incidence of such reactions has historically been estimated to be approximately 1 per million,[17] but recent data suggest that this may be an underestimation. Post-marketing reports of generalized allergic reactions to YF-VAX® between 1991 and 1997 indicate an incidence of 1 in 131,000.[1,24] An analysis of Vaccine Adverse Event Reporting System data for YF-VAX® was conducted by the Centers for Disease Control and Prevention. During the interval 1990–1997, there were an estimated 1.3 million doses administered to civilians and 31 SAEs defined as "neurologic or systemic reactions persisting greater than 48 hours", giving a rate of 2.3 per 100,000 vaccinees. The incidence of SAEs was significantly higher in elderly subjects.[25]

During a mass immunization campaign in 1999–2000 in Brazil, deaths associated with YF 17D vaccination occurred in a five-year-old child and a 22-year-old adult.[22] The clinical presentation and pathologic evidence suggested that the vaccine caused hepatitis and a syndrome similar to wild-type YF. These reactions appeared to be host-related rather than due to mutation(s) in the vaccine virus. Serious AEs of this kind appear to be extremely rare.[27,28] Unfortunately, the sample size in this study was too small to identify rare serious AEs such as systemic allergic reactions, YEL-AND, or YEL-AVD.

Finally, the lot-to-lot consistency of production of ARILVAX™ was demonstrated in this study by comparing geometric mean (LNI) antibody titers and seroconversion rates to the three lots. Each lot of ARILVAX™ produced seroconversion rates in excess of 94%, all greater than levels achieved by YF-VAX®. Therefore, no lot of ARILVAX™ was considered inferior to YF-VAX® and the ARILVAX™ lots were assessed to be equivalent.

No controlled clinical trials of ARILVAX™ and YF-VAX® (or any of the other YF 17D vaccines) had been previously conducted among infants and children. This is the largest such study ever conducted and as recently shown in adults,[6] confirms that these two vaccines are well tolerated and highly immunogenic. It is noteworthy that YF 17D vaccines, including ARILVAX™ and YF-VAX®, have been used for decades in travelers ≥ 9 months of age without recognized safety problems, and YF 17D vaccines produced by major manufacturers (Biomanguinhos, Rio de Janeiro, Brazil; Institute Pasteur, Dakar, Senegal; and Aventis-Pasteur, Lyon, France) are used in infants starting at nine months of age undergoing routine immunization in the EPI in South America and Africa. There have been recent increases in demand for YF vaccines associated with a concomitant expansion of YF virus circulation in endemic countries, as well as an increase in travel by non-immune persons from developed countries. ARILVAX™ represents an additional, well-tolerated and immunogenic vaccine, which can now be added to the armamentarium of YF vaccines available to travelers and other populations at risk of exposure. This study was one of several trials that were conducted for the purposes of obtaining approval from the U.S. FDA for use in the United States. The clinical data on safety and immunogenicity in children contained in this report will be summarized on the product label. If approval is granted, the vaccine will be sold and distributed in the United States for protection of travelers, military, and laboratory personnel.

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