Am. J. Trop. Med. Hyg., 72(3), 2005, pp. 339–346

IMMUNOGENICITY AND SAFETY OF BERNA-YF COMPARED WITH TWO OTHER 17D YELLOW FEVER VACCINES IN A PHASE 3 CLINICAL TRIAL

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Abstract. BERNA-YF (Flavimun®) is a live, attenuated yellow fever (YF) vaccine of the 17D strain produced by Berna Biotech Ltd. following a transfer of technology from the Robert Koch Institute (RKI) in Berlin, Germany. In this phase 3 bridging study, the immunogenicity and safety of BERNA-YF were compared with the original RKI YF vaccine (RKI-YF) and to a current, commercially available YF vaccine, Stamaril® (AP-YF; Aventis Pasteur, Lyon, France), in 304 healthy, adult volunteers. All three vaccines elicited an effective immune response with seroprotection achieved in 100% of individuals in each vaccine group at a neutralizing antibody titer $\geq$ 1:10. BERNA-YF was shown to be comparable to the other two vaccine products, and subgroup analysis showed no differences in immune response between three consecutive production batches. The immune response to BERNA-YF and RKI-YF was very similar, with no significant difference in antibody titer between the two groups ($P = 0.4634$). However, AP-YF vaccination resulted in a significantly lower antibody titer ($P < 0.0001$ versus BERNA-YF). Males exhibited a higher antibody response than females to both BERNA-YF and RKI-YF, but not to AP-YF. All three vaccines were well tolerated and no serious adverse events were reported.

INTRODUCTION

Yellow fever (YF) is an acute hemorrhagic fever with symptoms that include hemorrhage, shock, hepatitis, and renal failure. In tropical areas of Africa and South America, where the disease is endemic with occasional epidemic outbreaks, there are up to 200,000 cases per year.[1,2] Infection rates in unvaccinated populations may be as high as 75% with a case-fatality rate of 20%, although estimates vary.[1,3,4] Prior to the development of a vaccine in 1936, YF was one of the world's most dreaded infectious diseases.[5] Unlike diseases such as smallpox, eradication of YF is not possible because it circulates independently of humans, in jungles or forests, with non-human primates serving as reservoir hosts.[2,4]

The YF infective agent is a single-stranded RNA virus belonging to the genus *Flavivirus*, family Flaviviridae, which contains approximately 70 viruses, including those responsible for West Nile fever and dengue fever.[2,3] Transmission of YF shows two epidemiologic patterns, the forest or jungle cycle and the urban cycle.[1,3] Endemic forest cycle YF accounts for up to 500 cases of infection in non-immunized forestry workers per year in South America.[3] In Africa, both forest and urban cycles are endemic, but there are occasional, dramatic major epidemics where large numbers of non-immunized individuals are infected.[3] Although YF does not occur in the Pacific region, Middle East, or Asia, the vector mosquito species are present and there are concerns that viremic human travelers could spread the disease to these regions as well as to Central and North America via air travel.[5] Historical examples in North America show that the impact of the spread of YF to susceptible populations should not be underestimated; for example, in 1793, 10% of the population of Philadelphia died as a result of an epidemic.[5]

The disease mechanisms of YF are not well understood and no specific treatment exists. Management of patients is notoriously difficult and even with intensive care, the course of the disease may not be positively influenced. As a result, prevention by vaccination is the most realistic option in terms of both effectiveness and cost.[2] Vaccination of travelers to certain YF-endemic or epidemic regions is mandatory by World Health Organization (WHO) conventions.[4,6] In 2002, the deaths were reported of two travelers who contracted YF while on holiday in endemic regions.[7,8] Neither of the individuals concerned had been vaccinated. Increasing travel to regions where there is a risk of contracting YF has resulted in greater demand for the vaccine, and there have been frequent supply problems.[4]

The only type of YF vaccine produced today is the live attenuated vaccine of virus strain 17D, which was originally produced in 1937 by Theiler and Smith.[9] This vaccine has had an excellent safety record since 1945, when the WHO introduced a mandatory primary and secondary seed lot system for manufacturing 17D-YF vaccine for immunization of international travelers.[3,5]

Berna Biotech Ltd. is taking over production of a YF vaccine previously manufactured by the Robert Koch Institute (RKI) in Berlin, Germany. This institute produced YF vaccine from 1960 until 2001, and marketed it in Germany from 1963 until 2003 (the RKI also developed the WHO YF primary seed lot 213-77[10]). As part of the transfer process, YF vaccine production from the RKI-17D seed virus has been scaled up to industrial levels. The derivation of the currently available 17D yellow vaccine strains is shown in Figure 1.

To satisfy regulatory requirements, Berna Biotech Ltd. also performed a limited clinical development program, which comprised the phase 3 bridging study that we describe in this report. The primary objective of this study was to demonstrate non-inferiority of Berna-YF (Flavimun®) to the original RKI YF vaccine (RKI-YF) and to a reference vaccine, Stamaril® (AP-YF), in terms of seroprotection rates (neutralizing antibodies) achieved one month after a single vaccination.

METHODS

Study design. The trial was an open-label, randomized, comparator-controlled, parallel group, single-center study, conducted in Switzerland in 304 healthy adult volunteers. This study was conducted in full compliance with the principles of the Declaration of Helsinki III and in accordance with the International Ethical Guidelines for Biomedical Re-

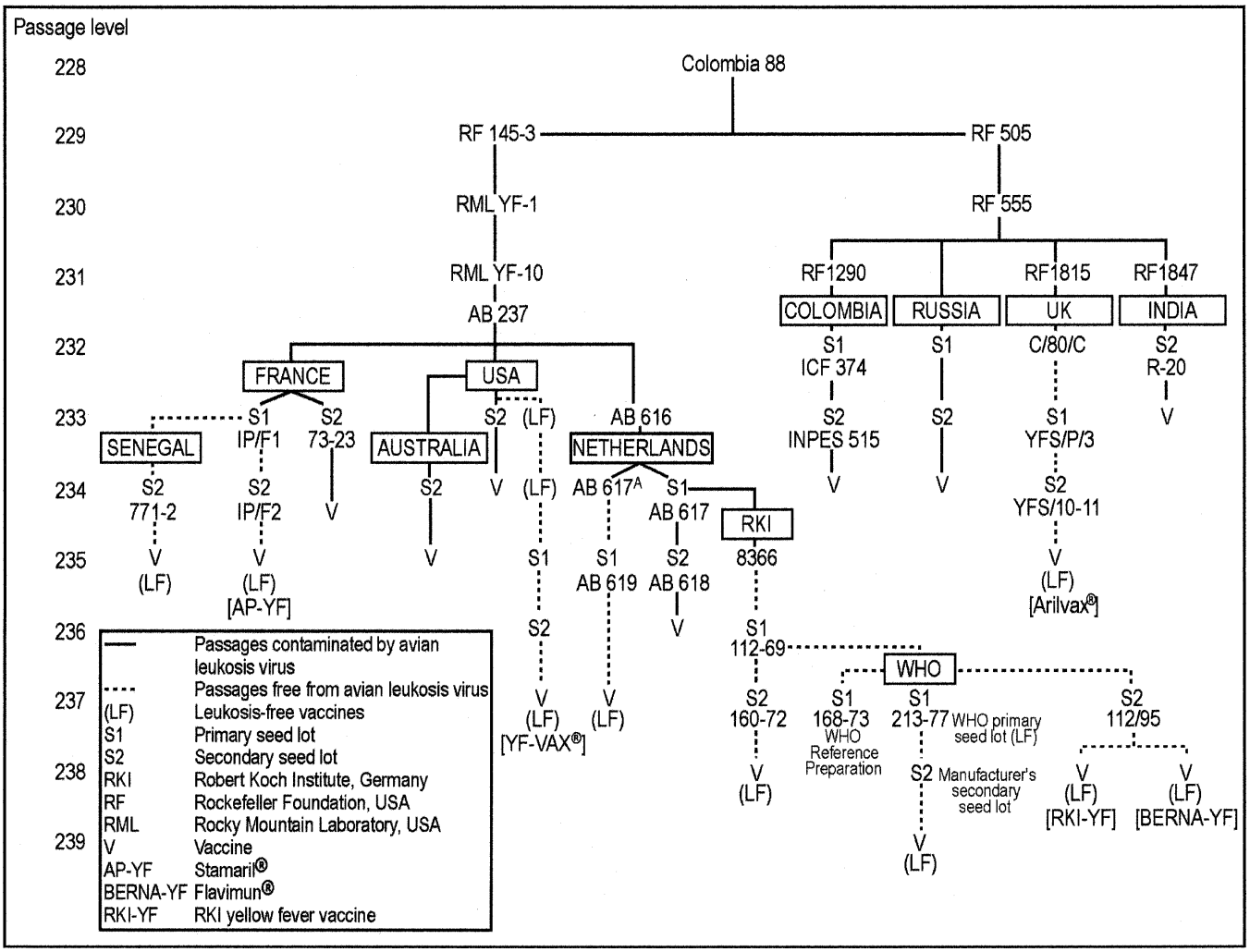

FIGURE 1. Derivation of the currently available 17D yellow fever vaccines (modified from Robertson[3]). WHO = World Health Organization.

search Involving Human Subjects as stated in the Good Clinical Practice guideline CPMP/ICH/135/95. The protocol was reviewed and approved by an independent ethics committee (Ethikkommission Beider Basel, Switzerland) prior to commencement of the study. After adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study, written informed consent was obtained from all subjects prior to participation.

Subjects. All of the subjects who were invited to participate in the study were healthy adults 18–60 years old who had not been previously vaccinated against YF. Women who were pregnant or breastfeeding were not included. Subjects were also excluded if they were participating in another clinical trial, had acute febrile illness (body temperature $\geq 38.0°C$), a known sensitivity to any vaccine component, a history of egg protein allergy, severe atopy, blood clotting abnormalities, or in the three months prior to the study, had received a blood transfusion or immunoglobulin treatment. Furthermore, subjects were excluded if they had a known immunodeficiency (including leukemia, cancer, or human immunodeficiency virus), or were receiving treatment with immunosuppressive drugs, such as systemic corticosteroids (equivalent of > 20 mg of prednisolone per day).

Vaccines. Three vaccines were used in this study: BERNA-YF (Berna Biotech Ltd., Berne, Switzerland), RKI-YF (Robert Koch Institute, Berlin, Germany), and AP-YF (Aventis Pasteur, Lyon, France). All of the vaccines were produced by propagation of live-attenuated YF virus (17D strain) in chick embryos free from avian leukosis virus in accordance with WHO criteria.[11]

The trial vaccine (BERNA-YF) was prepared from a reference stock (RKI-17D 112/95 passage 237 of strain 17D, substrain 204) produced by the RKI in 1995 and subsequently transferred to Berna Biotech (Figure 1). The batches of vaccine used in this study were produced to commercial standards according to Good Manufacturing Practice. Three consecutive production batches (Lot nos. 3000129, 3000130, and 3000131) were supplied freeze-dried in single-dose vials, each provided with a ready to use syringe containing diluent. Each 0.5 mL reconstituted dose contained at least 1.5×10^4 plaque-forming units (PFU) of 17D virus (strain 112/95), as well as chick protein and excipients (sodium chloride, sodium dihydrogen phosphate, sorbitol, and inositol).

The comparator vaccines (RKI-YF and AP-YF) were both commercial batches. The RKI-YF (Lot no. 189/00/1) was supplied freeze-dried in single-dose ampules, each provided with

TABLE 1
Primers used for RT-PCR and/or sequencing of the protein gene*

Primer	Purpose	Sequence
YF up	RT-PCR/sequencing	5′-GAG TCG TGA TTG CCC TAC TGG TC-3′
YF up 2	Sequencing	5′-CTA CAC TGG AAT GCC AGG TG C-3′
YF up 3	Sequencing	5′-CAA CCA ATG ATG ATG AAG TG-3′
YF down	RT-PCR/sequencing	5′-AGT CTC TAA ATA TGA AGA TAC CAT CTC-3′
YF down 2	Sequencing	5′-TCA CCT GCA TCA CAA CAG TG-3′
YF down 3	Sequencing	5′-CAA TGA ACT CGA CTT CCT G-3′

* RT-PCR = reverse transcriptase–polymerase chain reaction; E = envelope; YF = yellow fever.

a ready to use syringe containing diluent. Each 0.5 mL reconstituted dose contained at least 1.5×10^4 PFU of 17D virus (strain 112/95), as well as chick protein and excipients (sodium chloride, sodium dihydrogen phosphate, sorbitol, and inositol). The AP-YF (Lot no. U6218-3) was supplied freeze-dried in single-dose ampules, each provided with a ready to use syringe containing diluent. Each 0.5 mL reconstituted dose contained at least 6.3×10^3 PFU of 17D virus, as well as chick protein, amino acids and excipients (sodium chloride, lactose and sorbitol).

The subjects were screened according to the inclusion/exclusion criteria and assigned on a 2:1:1 basis to receive BERNA-YF, RKI-YF, or AP-YF using a computer-generated randomization number. The study was double-blinded with respect to the three production batches of BERNA-YF, but was open-label with respect to the different brands of vaccine. On day 1, the investigator or designated person administered reconstituted vaccine (0.5 mL) to each subject subcutaneously over the deltoid muscle in either the right or left upper arm. The first five subjects allocated to each of the three BERNA-YF dose groups were also included in a subgroup, from which blood was collected five and seven days after administration of vaccine to allow sequencing of the gene encoding the viral coat envelope (E) protein from isolated virus.

Immunogenicity. Serum samples for assessment of immunogenicity were obtained from all subjects at baseline (day 1, pre-vaccination) and on day 29 ± 3 (one month post-vaccination). Neutralizing antibody titers were quantified with a constant virus serum-varying plaque-neutralization test (PNT) procedure that was developed and validated using in-house positive and negative sera. The in-house sera were standardized against the first international reference preparation of monkey YF serum (National Institute for Biologic Standards and Control, South Mimms, Potters Bar, Hertfordshire, United Kingdom). Validation was performed according to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines Q2A and Q2B. The test was performed in Vero cell 24-well microplate cultures of 15–100 PFU of BERNA-YF 17D virus with various serum dilutions. Neutralizing antibody titer was defined as the reciprocal of the highest dilution of serum that neutralized at least 50% of the viral plaques. The criterion for seroprotection was defined as a neutralizing antibody titer $\geq$ 1:10.[12] We have also expressed our results in international units per milliliter (IU/mL).

Safety. All subjects were given a diary card and instructed to record daily (according to solicited questions) any local or systemic adverse events which occurred within 14 days of vaccination. The intensity of each adverse event was assessed using a four-point rating scale ranging from 0 = none to 1 = mild (not interfering with daily activities), 2 = moderate (interfering with daily activities), and 3 = severe (prohibiting normal daily activities). For erythema and induration/swelling, the diameter in millimeters of the affected area was measured. The health status of the volunteers and assessment of adverse events was conducted at the follow-up visit on day 29.

Serious adverse events were defined as any event, irrespective of treatment relationship, that required unplanned hospitalization (or prolonged existing hospitalization), resulted in persistent/significant disability/incapacity, and was life-threatening or resulted in death. Furthermore, an event that although not life-threatening and did not require hospitalization, was defined as serious if it jeopardized the patient, or required intervention to prevent hospitalization or a life-threatening condition. All serious adverse events were required to be reported immediately to the investigator.

Body temperature (tympanal) was recorded prior to vaccination, and oral or axillary body temperature was recorded daily up to day 14 after vaccination.

Genetic stability. Blood samples for virus isolation were collected from the 15 volunteers in the BERNA-YF genetic stability subgroup on days 6 and 8. The E (envelope) protein gene from the isolated viral particles was sequenced as follows. The 17D virus in the samples was amplified for eight days in Vero cells. Supernatants were collected, and RNA was extracted from the supernatant using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA). The RNA was stored at –20°C prior to reverse transcription using ImProm-II™ (Promega, Madison, WI). Amplification by reverse transcriptase–polymerase chain reaction (RT-PCR) was carried out using the Expand High Fidelity^{PLUS} PCR system (Roche, Basel, Switzerland). The reaction products were subsequently purified using the QIAquick PCR purification kit (Qiagen). Sequencing was performed by primer walking at Microsynth GmbH (Balgach,

TABLE 2
Baseline characteristics of the study population*

Vaccine	No. ITT	Sex (%)		Age (years)		Ethnic origin
		Male	Female	Mean	Range	
BERNA-YF	151	50.3	49.7	35.3	18–60	98.7% Caucasian 1.3% Afro-Caribbean
RKI-YF	77	51.9	48.1	35.1	18–58	98.7% Caucasian 1.3% Afro-Caribbean
AP-YF	76	47.2	52.8	35.1	18–60	98.7% Caucasian 1.3% Afro-Caribbean

* ITT = intent-to-treat; YF = yellow fever; RKI = Robert Koch Institute; AP = Aventis Pasteur.

TABLE 3
Neutralizing antibody titers at day 29 (one month post-vaccination) by treatment group*

Vaccine group	Day	No. PP	Geometric mean titer	Lower 95% CI	Upper 95% CI	P†
BERNA-YF						
1:dilution	29	143	1,184	982	1,426	
IU/mL	29	143	102	85	122	
RKI						
1:dilution	29	73	1,050	795	1,386	0.4634
IU/mL	29	73	90	68	118	0.4303
AP-YF						
1:dilution	29	72	612	474	791	< 0.0001
IU/mL	29	72	54	42	68	< 0.0001

* PP = per protocol; CI = confidence interval; YF = yellow fever; RKI = Robert Koch Institute; AP = Aventis Pasteur.
† T-test for pairwise comparisons between the BERNA-YF group and the control groups based on analysis of variance.

Switzerland). The primers used for the RT-PCR and/or sequencing of he E protein gene are shown in Table 1.

Statistical methods. Based on the assumption of a seroprotection rate of 95% for the original RKI-YF vaccine and a clinically non-significant non-inferiority limit of 10%, 300 subjects would provide 90% power to show the non-inferiority of BERNA-YF compared with the original RKI-YF vaccine (the primary efficacy variable) using the chi-square test.

The non-inferiority criterion for BERNA-YF compared with RKI-YF vaccine was that the lower one-sided 95% confidence limit for the difference in seroprotection rates between the two groups exceeded the non-inferiority limit $-\delta = -10\%$ using the chi-square test (normal approximation with the Hauck-Anderson correction). This analysis was also conducted to test the secondary hypothesis of non-inferiority of BERNA-YF compared with AP-YF and to compare the three different production batches of BERNA-YF (using three pairwise comparisons). Geometric mean titers (GMTs) were analyzed using analysis of variance for $\log_{10}$-transformed titers.

No formal statistical testing was done for non-inferiority of BERNA-YF compared with RKI-YF vaccine and AP-YF in terms of tolerability. Descriptive statistics were used (95% confidence interval [CI]) for incidence rates.

RESULTS

Subjects. A total of 304 subjects were recruited into this study, 151 in the BERNA-YF group, 77 in the RKI-YF group, and 76 in the AP-YF group (Table 2). The groups were well-matched in terms of baseline characteristics. In each group, approximately half of the subjects were female, the majority (98.7%) were Caucasian, and 1.3% were Afro-Caribbean. The mean age was 35 years, with a range of 18–60 years in all groups other than the RKI-YF group (18–58 years). Two subjects did not complete the study, one in the BERNA-YF group because he failed to provide safety data (did not return his diary card nor attend the last follow-up visit) and one in the RKI-YF group who was lost to follow-up. The safety population included all randomized subjects, with the exception of the individual described above who failed to provide safety data. Both individuals who failed to complete the study were excluded from the immunogenicity analyses. A total of 14 subjects who were seropositive at baseline (seven subjects in the BERNA-YF group, three in the RKI group, and four in the AP-YF group) were excluded from the per protocol (PP) for immunogenicity population, but were included in the intent-to-treat (ITT) for immunogenicity population. Subsequent inquiries showed that of the baseline seropositive individuals, one had received a YF vaccination in 1992 (but had not admitted this during enrollment), and six had visited potentially YF-endemic regions. Four of the subjects had never visited a potentially YF-endemic area, and the remaining three did not provide a response.

Immunogenicity. The PP population comprised 288 seronegative (antibody titer < 1:10) subjects. At day 29, the GMTs of the neutralizing antibodies were 1,184 and 1,050 in the BERNA-YF and RKI-YF groups, respectively, and the difference was not significant ($P = 0.4634$) (Table 3). For AP-YF, at day 29 the GMT was 612, which was significantly lower than for BERNA-YF ($P < 0.0001$). It should be noted that when the AP-YF 17D virus was used as the challenge strain in

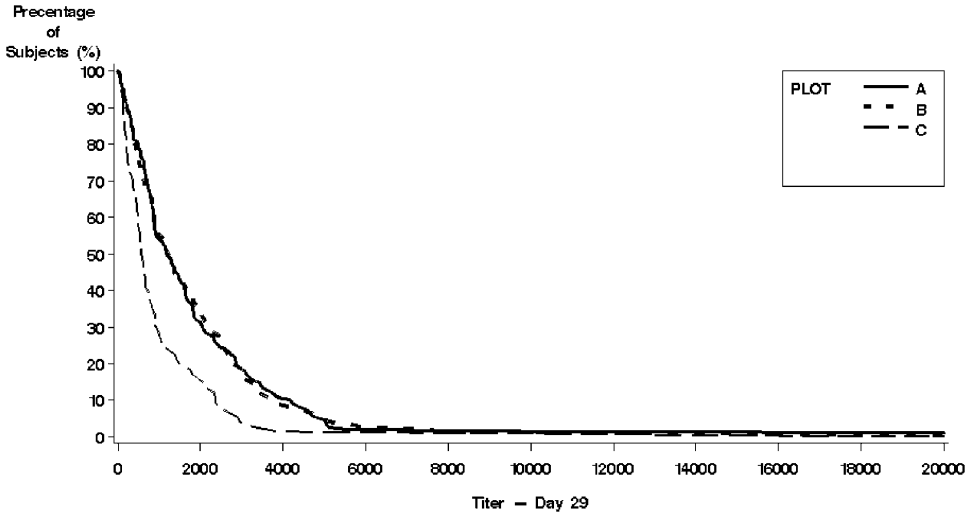

FIGURE 2. Reverse cumulative distribution curve of day 29 yellow fever (YF) neutralizing antibody titers for **A**, BERNA-YF; **B**, RKI-YF; and **C**, AP-YF. RKI = Robert Koch Institute; AP = Aventis Pasteur.

TABLE 4
Neutralizing antibody titers at day 29 (one month post-vaccination) by BERNA-YF subgroup*

BERNA-YF subgroup batch no.	Day	No. pp	Geometric mean titer	Lower 95% CI	Upper 95% CI
3000129					
1:dilution	29	48	1,195	867	1,646
IU/mL	29	48	102	73	142
3000130					
1:dilution	29	48	1,187	818	1,724
IU/mL	29	48	102	72	144
3000131					
1:dilution	29	47	1,168	872	1,566
IU/mL	29	47	102	77	134

* YF = yellow fever; PP = per protocol; CI = confidence interval.

the PNT assay, almost identical titer differences were found in a subgroup of vaccines (data not shown). The reverse cumulative distribution of the antibody titers measured for the three study groups is presented in Figure 2.

The GMT values for the three BERNA-YF subgroups on day 29 were 1,194, 1,187, and 1,168, with no significant difference between batches (Table 4).

Seroprotection rates in the PP immunogenicity population for the BERNA-YF, RKI-YF, and AP-YF groups were 100% (Table 5). The between-group difference for both BERNA-YF versus RKI-YF and BERNA-YF versus AP-YF was 0.0% (90% CIs = –0.69, 0.69). As a result, BERNA-YF was concluded to be non-inferior to the other two vaccine products. Comparable results were obtained using the ITT for immunogenicity population, which included YF antibody-seropositive subjects. Again, seroprotection rates were 100% in each of the three groups. The between-group difference for both BERNA-YF versus RKI-YF and BERNA-YF versus AP-YF was 0.0% (90% CIs = –0.66, 0.66).

Analyses of seroprotection rates at higher neutralizing antibody titers ≥ 1:50, ≥ 1:150, and ≥ 1:450 for both the PP and ITT for immunogenicity populations also demonstrated that BERNA-YF was non-inferior to the other two vaccines (Table 5). Furthermore, at the ≥ 1:450 level, BERNA-YF was associated with a significantly higher seroprotection rate than AP-YF ($P = 0.0025$).

Seroprotection rates in the three BERNA-YF subgroups, based on a neutralizing antibody titer ≥ 1:10, were all 100%, with no difference between groups.

Effect of sex on neutralizing antibody titer. The GMTs were higher in males than in females in both the BERNA-YF and RKI-YF groups (Table 6). In the BERNA-YF group, the day 29 GMT was 1,465 in males and 953 in females ($P = 0.022$). Similarly, in the RKI-YF group, the day 29 GMT was 1,364 in males and 790 in females ($P = 0.049$). However, for AP-YF the day 29 GMT was 520 in males, which was slightly lower than for females (709) ($P = 0.2293$).

Level of neutralizing antibodies measured in IU/mL. The cut-off antibody titer of 1:10 corresponds to 0.7 IU/mL. When all titers were expressed as IU/ml, equivalent results were obtained, i.e., there was basically no difference between BERNA-YF and RKI-YF vaccine, antibody levels were higher compared with AP-YF, and there was an effect of sex on the antibody levels (Tables 3, 4, and 6).

Safety. None of the subjects in any of the treatment groups experienced a serious adverse event. The incidence of solicited local reactions in the AP-YF group (36.8%) was comparable with that in the BERNA-YF group (36.7%; $P = 0.9794$), but was significantly higher in the RKI-YF group (57.1%, $P = 0.0032$) (Table 7). This higher incidence in the RKI-YF group resulted from a significantly greater rate of both injection site erythema and pain. At least one unsolicited local reaction was reported by 6.0% of the BERNA-YF group, 7.8% of the RKI-YF group, and 5.3% of the AP-YF group. The most common unsolicited local reaction in all three groups was injection site pruritus.

The most commonly reported solicited systemic adverse events (AEs), regardless of relationship to treatment, were headache, asthenia and myalgia (Table 8). Headache was reported by 30.7% of subjects in the BERNA-YF group, 26.0% in the RKI-YF group, and 39.5% in the AP-YF group. The incidence of asthenia was 27.3%, 20.8%, and 19.7%, respectively. Myalgia, arthralgia, and gastrointestinal disorders were also relatively common. A pairwise comparison between the BERNA-YF and each of the two comparator groups showed no statistically significant differences in the incidence of any of the solicited systemic AEs.

Unsolicited systemic AEs were reported by 20.7% of subjects in the BERNA-YF group, 16.9% of subjects in the RKI-YF group, and 14.5% of subjects in the AP-YF group. The most common unsolicited systemic AE was fatigue, which was reported by 6.7% of subjects in the BERNA-YF group and 5.2% of subjects in the RKI-YF group, but was not reported by any subjects in the AP-YF group.

Age correlated negatively with the incidence of any systemic adverse events (logistical regression analysis, categorical < and ≥ 45 years or with age as continuous variable) with an odds ratio of 2.21 ($P = 0.0027$). This difference was also statistically significant for headache.

Genetic stability. Following cultivation in Vero cells and subsequent amplification of the YF virus E protein locus us-

TABLE 5
Seroprotection at day 29 (one month post-vaccination) by treatment group*

Neutralizing titer	BERNA-YF no. PP = 143	RKI-YF no. PP = 73	AP-YF no. PP = 72	Difference BERNA-YF %	Difference RKI-YF 90% CI	Difference† BERNA-YF %	Difference† AP-YF 90% CI
≥ 1:10	100.0	100.0	100.0	0.00	–0.69, 0.69	0.00	–0.69, 0.69
≥ 1:50	99.30	98.63	100.0	0.67	–2.54, 3.89	–0.70	–2.54, 1.15
≥ 1:150	95.10	90.41	87.50	4.69	–2.43, 11.82	7.60	–0.20, 15.41
≥ 1:450	84.62	79.45	66.67	5.16	–4.80, 15.13	17.95	6.79, 29.11‡

* YF = yellow fever; RKI = Robert Koch Institute; AP = Aventis Pasteur; PP = per protocol; CI = confidence interval.
† If the lower one-sided CI for the difference is greater than –10%, then non-inferiority is shown.
‡ $P = 0.0025$ for superiority test.

TABLE 6
Neutralizing antibody titers at day 29 (one month post-vaccination) by sex and treatment group*

Sex	Vaccine group	Day	No. PP	Geometric mean titer	Lower 95% CI	Upper 95% CI
Male	BERNA-YF					
	1:dilution	29	72	1,465†	1,154	1,861
	IU/mL			126	100	159
	RKI-YF					
	1:dilution	29	38	1,364‡	890	2091
	IU/mL			117	77	176
	AP-YF					
	1:dilution	29	34	520§	356	758
	IU/mL			46	33	64
Female	BERNA-YF					
	1:dilution	29	71	953†	718	1,265
	IU/mL			82	62	108
	RKI-YF					
	1:dilution	29	35	790‡	560	1,115
	IU/mL			67	47	96
	AP-YF					
	1:dilution	29	38	709§	49	1,013
	IU/mL			62	43	88

* PP = per protocol; CI = confidence interval; YF = yellow fever; RKI = Robert Koch Institute; P = Aventis Pasteur.
† $P = 0.022$ by t-test for pairwise comparisons between males and females derived from an analysis of variance model.
‡ $P = 0.049$ by t-test for pairwise comparisons between males and females derived from an analysis of variance model.
§ $P = 0.2293$ by t-test for pairwise comparisons between males and females derived from an analysis of variance model.

ing RT-PCR, amplification of the corresponding approximately 1,600-basepair PCR fragment was possible in 6 of the 15 volunteers evaluated. The six volunteers had been vaccinated with BERNA-YF batch 3000129 in one case, batch 3000130 in two cases, and batch 3000131 in three cases. The consensus sequence of each of the six PCR fragments was determined without any prior cDNA cloning. The cDNA sequences of the six PCR fragments were compared with the cDNA sequences of the RKI-17D 112/95 YF seed virus and the three clinical batches of BERNA-YF. The comparison showed that the consensus sequences of the PCR fragments obtained from the sera of the vaccine recipients were identical to the consensus sequences of both the seed and vaccine virus, as well as to the published 17D-213 sequence (GenBank Accession no. U17067). As mentioned earlier, heterogeneity of the vaccine virus population has not been assessed by sequencing of cDNA clones. However, chromatograms of the sequences did not show any evidence of heterogeneity. It cannot be excluded that variations in the sequences of small sub-populations of vaccine viruses might have been masked by the consensus sequencing approach. Nevertheless, the sequencing data reported herein support the genetic stability of the YF fever seed virus RKI-17D 112/95 when used as a live vaccine.

DISCUSSION

The results of this controlled trial showed that BERNA-YF and the two comparator vaccines elicited an effective immune response and were well tolerated. Seroprotective antibody titers were achieved in 100% of individuals in each vaccine group, and BERNA-YF was shown to be non-inferior (comparable) to both its predecessor (RKI-YF vaccine) and a currently marketed product (AP-YF) at a neutralizing antibody titer $\geq$ 1:10. This was confirmed at neutralizing antibody titers of $\geq$ 1:50, $\geq$ 1:150 and $\geq$ 1:450. Indeed, at an antibody titer of 1:450, BERNA-YF was shown to be superior to AP-YF ($P = 0.0025$). Subgroup analysis of different production batches of BERNA-YF showed no inter-batch variability in neutralizing antibody response or seroprotection.

The immune response to BERNA-YF and RKI-YF was very similar, with no significant difference in antibody titer between the two dose groups ($P = 0.4634$), whereas the antibody titer following AP-YF vaccination was significantly lower ($P < 0.0001$). Further analysis of the sera from a subgroup of vaccinees showed that the titer differences were not a consequence of the challenge virus used in the PNT assay.

In the present study, the neutralizing antibody response in males was higher than in females for both BERNA-YF ($P = 0.022$) and RKI-YF ($P = 0.049$). A similar finding was observed in an earlier study which compared YF-VAX® and Arilvax®.[13] The authors of the earlier study cited historical data that described a higher incidence of postvaccinal encephalitis in males than in females following inoculation with an early YF vaccine (French neurotropic),[5] and concluded that infection may elicit a stronger immune response in males. However, in our study, given the lower neutralizing antibody response seen in males than in females for AP-YF, it appears probable that other factors are also involved.[14]

In our study, BERNA-YF was as well tolerated in terms of safety as RKI-YF and AP-YF. The incidence of adverse events that we observed was similar to the results of the largest YF virus clinical trial conducted to-date.[13] Higher age correlated with less systemic adverse events, as has been reported for Arilvax® and YF-Vax.[13]

There is only one known 17D-YF virus-associated fatality that resulted from a mutation of the 17D virus. This was the case of a three-year-old child who died of encephalitis.[14] The reversion to virulence was caused by substitution of two

TABLE 7
Solicited local reactions by treatment group*

Adverse event	BERNA-YF (n = 150) Subjects No.	BERNA-YF (n = 150) Subjects %	BERNA-YF (n = 150) Events No.	RKI 17D (n = 77) Subjects No.	RKI 17D (n = 77) Subjects %	RKI 17D (n = 77) Events No.	AP-YF (n = 76) Subjects No.	AP-YF (n = 76) Subjects %	AP-YF (n = 76) Events No.	P† BERNA-YF versus RKI-YF	P† BERNA-YF versus AP-YF
Subjects with at least one reaction	55	36.7	91	44	57.1	87	28	36.8	48	0.0032	0.9794
Erythema	37	24.7	39	33	42.9	38	14	18.4	14	0.0050	0.2886
Induration	23	15.3	23	18	23.4	20	12	15.8	12	0.1359	0.9286
Pain	27	18.0	29	24	31.2	29	18	23.7	22	0.0244	0.3120

* YF = yellow fever; RKI = Robert Koch Institute; AP = Aventis Pasteur.
† Fisher's exact test for incidence rate (percentage of subjects).

TABLE 8
Solicited systemic reactions by treatment group*

Adverse event	BERNA-YF (n = 150) Subjects No.	BERNA-YF (n = 150) Subjects %	BERNA-YF (n = 150) Events No.	RKI-YF (n = 77) Subjects No.	RKI-YF (n = 77) Subjects %	RKI-YF (n = 77) Events No.	AP-YF (n = 76) Subjects No.	AP-YF (n = 76) Subjects %	AP-YF (n = 76) Events No.	P† BERNA-YF versus RKI-YF	P† BERNA-YF versus AP-YF
Subjects with at least one reaction	82	54.7	237	36	46.8	112	37	48.7	100	0.2658	0.4019
Arthralgia	17	11.3	20	14	18.2	16	7	9.2	8	0.1595	0.8197
Asthenia	41	27.3	50	16	20.8	22	15	19.7	19	0.3333	0.2546
Fever‡	14	9.3	19	6	7.8	8	6	7.9	6	0.8079	0.8085
GI disorders	21	14.0	24	9	11.7	10	10	13.2	11	0.6841	1.0000
Headache	46	30.7	69	20	26.0	28	30	39.5	40	0.5377	0.2331
Lymphadenopathy	12	8.0	14	4	5.2	4	4	5.3	4	0.5868	0.5872
Myalgia	29	19.3	33	18	23.4	22	8	10.5	9	0.4925	0.1270
Skin rash	8	5.3	8	2	2.6	2	3	3.9	3	0.5010	0.7545

* GI = gastrointestinal. For definitions of other abbreviations, see Table 7.
† Fisher exact test for incidence rate (percentage of subjects).
‡ Body temperature > 37.5°C.

amino acids in the E protein. A subsequent study that investigated mutations in virus collected from the sera of six healthy volunteers five days after YF vaccination concluded that 17D virus accumulated few mutations, and those which did occur tended to be in genes which code for non-structural proteins.[15] It was suggested that this may in part explain the excellent safety record of 17D vaccines. In our study, analysis of the E protein gene sequence in the 17D virus isolated from the volunteers showed a perfect match with clinical batches of BERNA-YF, clearly demonstrating its genetic stability.

Literature data show that 17D vaccines in general have an excellent safety and efficacy record. The six recent deaths from viscerotropic disease[16–18] have tarnished the reputation of the vaccine, but nonetheless, no new fatal cases have been reported since 2001, which given increased vigilance, suggests that these deaths were not simply "the tip of the iceberg."[19] In the context of the estimated 400 million YF vaccinations administered since the introduction of the 17D vaccine, the risk of viscerotropic disease appears to be very low,[20] approximately 1.3 cases per 1 million doses according to a recent retrospective analysis of post-marketing safety surveillance data for Arilvax®.[21] Half of the deaths, a non-fatal case in a United States citizen and one suspected non-fatal case in Germany (which was not associated with the RKI-YF vaccine) occurred in individuals of advanced age, which has been identified as a potential risk factor for adverse events following 17D vaccination.[16–18,22,23] For this reason, we chose an upper age limit of 60 years in our study. While not a contraindication, the judicious use of vaccine in this age group should help minimize the risk. Of the remaining three reported deaths, two were in healthy adults with no obvious risk factors, but the third case was in a five-year-old girl, who although apparently healthy at the time of vaccination, had a history of poor health from birth.[16] None of the deaths occurred in individuals who were vaccinated with RKI-YF. It has also been proposed that despite the cases of viscerotropic disease, there should be no change in vaccination policy for travelers to YF-endemic areas because the risk of illness resulting from wild-type YF is greater than the risk from vaccination.[4,20,24]

In this study, BERNA-YF was shown to be as safe and effective as both its predecessor (RKI-YF) and AP-YF. With the increased incidence of YF and the known international shortage of vaccine, an additional source of 17D vaccine to respond to epidemics and to ensure a reliable supply for travelers is timely.

Received April 15, 2004. Accepted for publication October 20, 2004.

Acknowledgments: We thank J. Signer, D. Buchs, and E. Kehrli-Gygax for their excellent technical assistance and R. Ibanéz for his valuable help in conducting this study.

Disclosure: The authors wish to disclose that Markus Pfister, Oliver Kursteiner, Helene Hilfiker, Didier Favre, Peter Durrer, Achim Kaufhold, and Christian Herzog are employees of Berna Biotech Ltd., the company that proposes to market Flavimun®. This statement is made in the interest of full disclosure and not because the authors consider this to be a conflict of interest.

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