The Phylogeny of Yellow Fever Virus 17D Vaccines

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Abstract

In recent years the safety of the yellow fever live vaccine 17D came under scrutiny. The focus was on serious adverse events after vaccinations that resemble a wild type infection with yellow fever and whose reasons are still not known. Also the exact mechanism of attenuation of the vaccine remains unknown to this day. In this context, the standards of safety and surveillance in vaccine production and administration have been discussed. Therein embodied was the demand for improved documentation of the derivation of the seed virus used for yellow fever vaccine production. So far, there was just a historical genealogy available that is based on source area and passage level. However, there is a need for a documentation based on molecular information to get better insights into the mechanisms of pathology. In this work we sequenced the whole genome of different passages of the YFV-17D strain used by Crucell Switzerland AG for vaccine production. Using all other publically available 17D full genome sequences we compared the sequence variance of all vaccine strains and oppose a phylogenetic tree based on full genome sequences to the historical genealogy.

1. Introduction

Yellow fever is still one of the major health problems in Sub-Saharan Africa and big parts of South America. It is caused by the yellow fever virus (YFV), a positive-sense, single-stranded RNA virus belonging to the family *Flaviviridae*. YFV is transmitted between humans and non-human primates through several species of the mosquito genus *Aedes* [1].

Despite the availability of the highly effective, live-attenuated vaccine 17D, there are still 200.000 new cases with 30.000 deaths each year worldwide as estimated by the WHO [2]. The lethality of YFV induced pansystemic disease with fever, jaundice, renal failure and haemorrhage is up to 50%. Especially an increase of cases in the last years and the lasting absence of a potent treatment make the illness an important reemerging disease [3].

The 17D vaccine was developed in 1937 and over 540 million doses have been administered since then with good results [4]. During this period there have only been little changes in the production process and the genetic stability of the 17D strains is contributing to the safety of the vaccine [5]. The vaccine is absolutely contraindicated for children under 6 months and people that are immunocompromised, which is a significant problem in Africa where over 20 million people are living with HIV (www.who.int). People over 60 years may be vaccinated in case of a medical need. Recently an increasing number of serious adverse events (SAE), occurring between two and 30 days after vaccination with YFV-17D, were reported [4, 5]. It can be distinguished between vaccine associated neurotropic disease (YEL-AND) and vaccine associated viscerotropic disease (YEL-AVD). YEL-AND causes encephalitis and occurs in 0.19 – 0.8 cases per 100.000 vaccinations with a case fatality rate (CFR) of less than 5%. YEL-AVD resembles an infection with wild type YFV, occurs with a chance of 0.004 – 11.7 per 100.000 and has a CFR of over 60% with a higher risk in women [4, 6]. The reasons for the occurrence of SAEs, as well as the mechanism of attenuation of the vaccine and its interaction with the immune system, are still poorly understood. There is a need for the

investigation of the possible influence of the YFV vaccine strain on the occurrence of the above mentioned serious adverse events. However, the benefit to risk ratio of YF vaccination is favorable in endemic areas.

To date, the genealogy of YFV-17D is well documented but is based on historical information of passaging. From today's perspective this kind of phylogeny is not up to date and should be revised with molecular information. Nonetheless, there is useful and important information such as passage level and worldwide distribution of the strains included in the historical flow sheet that need to be maintained [6].

In this work we sequenced the whole genome (10862 bp) of different passages of Crucell's YFV-17D strain, which was only sequenced partially until now [7]. This virus strain is used for vaccine production, but has not been licensed so far. As internal quality control we sequenced one vaccine lot of the vaccine Stamaril® by Sanofi Pasteur MSD that has already been published previously [8, 9]. Together with all other 17D full genome sequences available at the EMBL GenBank, we inferred a phylogenetic tree based on full genome sequences and compared it with the historical genealogy. Additionally we made a comparison of all sequence differences occurring between the vaccine strains for a better overview of mutations.

2. Materials and Methods

2.1 Sequenced virus strains

RKI YFV vaccine, Crucell Switzerland AG (strain 17D-204, substrain 112/95, passage 238) (Accession-no.: JN628279); Flavimun working seed lot (WSL), Crucell Switzerland AG (strain 17D-204, substrain 112/95, passage 237) (Accession-no.: JN628280); TVX Flavimun vaccine, Crucell Switzerland AG (origin 17D-204, substrain 112/95, passage 238)

(Accession-no.: JN628281); Stamaril® Yellow fever live vaccine, Sanofi Pasteur MSD (Ch.-Nr.: Z6329-2)

2.2 Sequences from EMBL GenBank

The following full genome sequences were obtained from EMBL GenBank (http://www.ncbi.nlm.nih.gov/genbank/):

YFV-17DD Brazil (DQ100292), YF-AVD2791-93F 04 from Spain (DQ118157), YFV 17D-Tiantan China (FJ654700.1), YFV-17DD case#1 YEL-AVD Peru (GQ379162.1), YFV-17DD case#2 YEL-AVD Peru (GQ379163.1), YFV-17DD (U17066), YFV-17D-213 (U17067), YFV French neurotropic strain (U21055), YFV-17D-204_1 (USA) (X03700), YFV-17D-204_2 (Pasteur) (X15062), YFV French viscerotropic strain (U21056), YFV Asibi (AY640589), Dengue 1 (NC_001477).

2.3 Sequencing

The cDNA synthesis from YFV RNA was performed using Superscript® III (Invitrogen™) according to the manufacturers' instructions. For the sequencing a minimum of 20 PCR products per virus strain were amplified with a set of YFV specific primers and Platinum®Taq polymerase (Invitrogen™) [10]. PCR products were analyzed by agarose gel electrophoresis and purified by gel extraction (Invisorb® Spin DNA Extraction Kit, Stratec Molecular; QIAquick Gel Extraction Kit, Qiagen) before sequencing. The sequence reaction was performed according to Sanger on a 3500 xL Dx Genetic Analyzer (Applied Biosystems™). Reagents were used from the Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™). Sequence analysis was done with the help of the Lasergene® SeqMan Pro Software Version 8.1.5 (DNASTAR Inc.).

2.4 Sequence alignments and phylogenetic analyses

We aligned the full genome nucleotide sequences of 16 YFV-strains using the muscle alignment tool from Geneious Pro™ Version 5.3.4, Biomatters LTD. The genome of the Dengue virus serotype 1 (Dengue 1) was used as outgroup.

The best fitting model of sequence evolution was determined using jModelTest 0.1 (http://darwin.uvigo.es/software/jmodeltest.html) with three substitution schemes. Model selection was computed using the Akaike information criterion (AIC).

Phylogenetic analyses were performed with Bayesian and Maximum likelihood (ML) methods to compare the support values of both mathematical methods.

The Bayesian analyses were performed using MrBayes 3.1.2 (http://mrbayes.csit.fsu.edu/index.php) [11] and consisted of two runs of four chains each. The GTR+G model of sequence evolution (General Time Reversible-model + Gamma-distribution) was applied according to the results from jModelTest. The two runs were monitored for 10 million generations, sampled every 100th generation and the temperature coefficient of the chain-heating scheme was set to 0.1 to ensure sufficient chain-swapping. All runs reached stationarity within the calculation (average standard deviation of split frequencies <0.01). The program Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer) was used to check for convergence of the model likelihood and parameters between the two runs and 10% of all trees were discarded as burn-in. A 50% majority-rule consensus tree was calculated in MrBayes 3.1.2.

The ML analyses were carried out using the program Seaview 4.2.12 (http://pbil.univ-lyon1.fr/software/seaview.html). Again, a GTR model was selected and further settings were left at the default. Two separate analyses were performed: one with 500 bootstraps and one with 1000 bootstraps.

The resulting Bayesian and ML consensus trees were visualised with the software Figtree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree) and rooted to midpoint. For visual clarity, the branches were transformed to a cladogram style.

3. Results and Discussion

For all newly sequenced yellow fever strains full genome sequences could be obtained. The sequences of the vaccine strains RKI YFV Vaccine, Flavimun® WSL and TVX Flavimun®-product (provided by Crucell Switzerland AG) were identical.

The sequencing of the Stamaril® vaccine yielded the same result as the internal sequencing by Sanofi Pasteur for the investigation of the stability of the vaccine [8]. Compared to the sequence of strain 17D-204 from Pasteur, we found four silent point mutations including one heterogeneity at nucleotide position 4054.

The comparison of the nucleotide sequences of all 17D vaccines showed an overall homology of 99.2 % (Table 1). A total of 84 sequence differences are existent throughout the whole genome, whereof 29 have an effect on the protein level and are accumulating within the range of the E-protein. This comparison essentially supports the genetic stability of YF vaccine strains [5].

The phylogenetic trees inferred from the different analyses (Bayesian, ML) show a high overall similarity concerning topology and statistical support. Figure 1 shows the consensus tree of the Bayesian analysis. Node labels describe the appropriate posterior probability values (pp). Additionally, the support values for the ML analyses with 500 and 1000 bootstraps (bs) are indicated in parentheses.

In all analyses, the YF-17DD group administered in South America is clearly separated with high support (0.96pp, 97bs, 98bs) from the YF-17D group used in all other parts of the world [6, 12, 13]. There are 56 nucleotide differences between 17DD- and 17D-vaccines that appear

only in the 17DD-group and of which 18 have an effect on the protein level. Only 12 of these nucleotide differences are common in all four 17DD-strains, leading to six amino acid changes (Table 1).

Within the YF-17D group, the strains YFV-17D-204 deriving from USA and Pasteur form one well-supported clade (1pp, 89bs, 88bs). The strain 17D-204-Pasteur is the vaccine strain from the Institute Pasteur [9] whereas the strain 17D-204-USA derived from the American Type Culture Collection in passage number 234 and was not used for vaccine production [14]. The sequences of these strains have already been compared [9].

Further on, the vaccine strains produced by Crucell Switzerland AG [7] together with the former vaccine strain from the Robert Koch-Institute (RKI, Germany) and the strain YFV-17D-213 which is provided by the World Health Organization (WHO, Switzerland) [12, 15] form a clade which is especially strongly supported in the Bayesian analysis (0.99pp, 69bs, 70bs). These two 17D subgroups are nested in a polytomy with the vaccine strain Stamaril® (France), an isolate from a YEL-AVD from Spain after vaccination with Stamaril® [16] and the Chinese vaccine strain YFV 17D-Tiantan. Regarding the historical genealogy of 17D-passaging (Fig.2), YFV 17D-Tiantan was separated from the other strains at passage 229 and has with 13 nucleotide differences, leading to six amino acid changes, a lot of differences in its sequence compared to the other strains (Table 1) belonging to this polytomy. These differences do not have an influence on the placement of the strain in the phylogenies, because all sequence deviations occurring in YFV 17D-Tiantan are phylogenetically uninformative autapomorphies, meaning that they are unique characteristics in this strain.

The sister group relationship of the wildtype strain Asibi towards the 17D and 17DD vaccine strains corresponds with the fact that 17D and 17DD arose from the Asibi strain through serial passaging [4, 17]. The YFV French viscerotropic strain is also a wild isolate and led to the vaccine YFV French neurotropic strain through 237 intracerebral passages in mice, which

was taken off the market due to severe side effects [17, 18]. These two strains form one clade that appears as sistergroup to Asibi in our analysis. However, this relationship is not statistically supported by any of the phylogenetic methods.

Compared to the historical genealogy, the phylogenetic tree based on full genome nucleotide sequences shows the same clustering of subgroups, with exception of the Chinese vaccine YFV 17D-Tiantan, which clusters in one polytomy with all other 17D vaccines in our analyses. Generally, the phylogenetic analysis reflects the historical way of 17D-distribution (Fig.1 and 2).

During the last meeting of the Working Group on Technical Specifications for Manufacturing and Evaluating Yellow Fever Vaccines, several topics regarding vaccine safety, surveillance and production have been discussed [6]. One issue was the historical record of derivation of seed virus for the production of YF-vaccines. In Fig.2, the source and passage levels of the vaccine strains of all manufacturers are documented which is important for the surveillance of vaccine production. However, for vaccine safety as well as for the investigation of SAEs and vaccine attenuation, all master and working seeds should be sequenced and made publicly accessible. Up to now no link could be established between the occurrence of SAEs and YF-vaccine genotypes. Indication exists that certain genetic predispositions of the vaccinees trigger the symptoms [19, 20]. Age is considered as risk factor.

Previous studies that dealt with full genome analyses of yellow fever vaccine strains focused either on the comparison between wildtype and vaccine strains and by this on possible mutations leading to attenuation [12, 21, 22], or discussed the comparison of only few vaccine strains [9, 22, 23]. In this work we sequenced three more vaccine strains whose sequence has not been published until now and together with all other known full genomes of YFV-17D viruses we compared the sequences of overall 13 17D vaccine strains. The focus was on

differences among the vaccines themselves and not in relation to the wildtype strains. Furthermore we open a new perspective of vaccine surveillance with a phylogenetic tree based on the full genome of all available YFV-vaccine strains, opposing the historical genealogy. More sequences are needed to assure the exact evolutionary history of YFV strains which is still unresolved, especially in the clade including the two 17D subgroups, the Stamaril® strain, the YEL-AVD isolate and the Chinese vaccine YFV 17D-Tiantan. Furthermore it would be useful for everyone who is working in this field to have access to more sequences with exact information on sequence differences as shown in Table 1.

The sequence data of all YF 17D vaccine strains should also be used to confirm the identity and consistency of the vaccine production. Confirmed sequence identity might then also be used to replace the very laborious and difficult safety tests for the preparation of the 17D working seed, i.e. intracerebral injection of working seed lots into monkeys followed by an extensive histological examination which is still mandatory for vaccine production according to WHO recommendations [16]. A substitution with the new and easy sequencing techniques as reliable tool for vaccine characterisation should be considered and should also lead to corresponding adaptations of the safety regulations as part of the vaccine production in the near future.

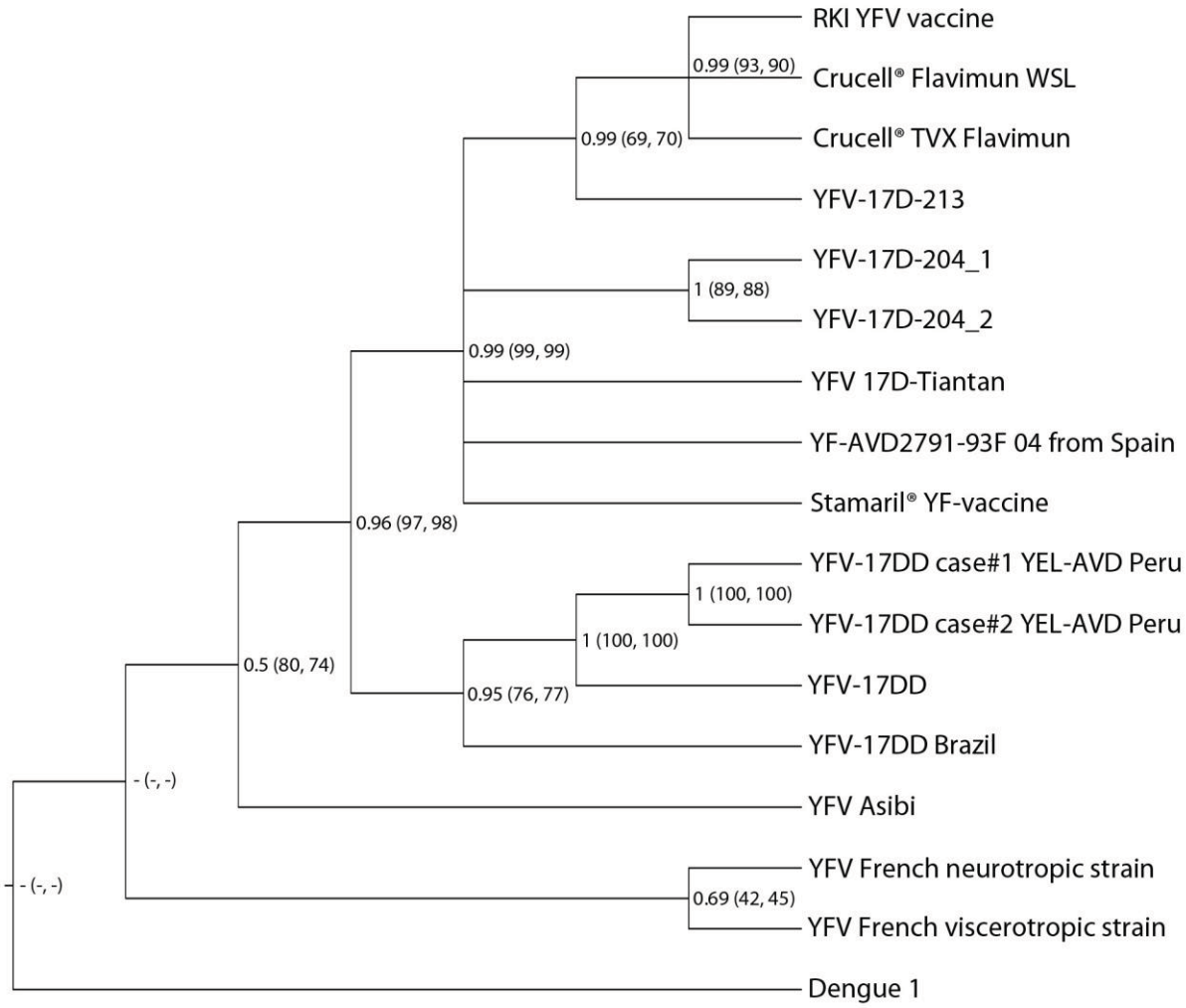

Fig. 1: Majority-rule consensus tree of the Bayesian analysis with 10 million generations based on full genome nucleotide sequences of the Yellow fever virus 17D vaccine strains. Posterior probability values are given on the right side of the related node. Bootstrap values of the ML analysis with 500 and 1000 bootstraps are displayed in parentheses. A '-' indicates that a certain branch was not supported by bootstrap or posterior probability values.

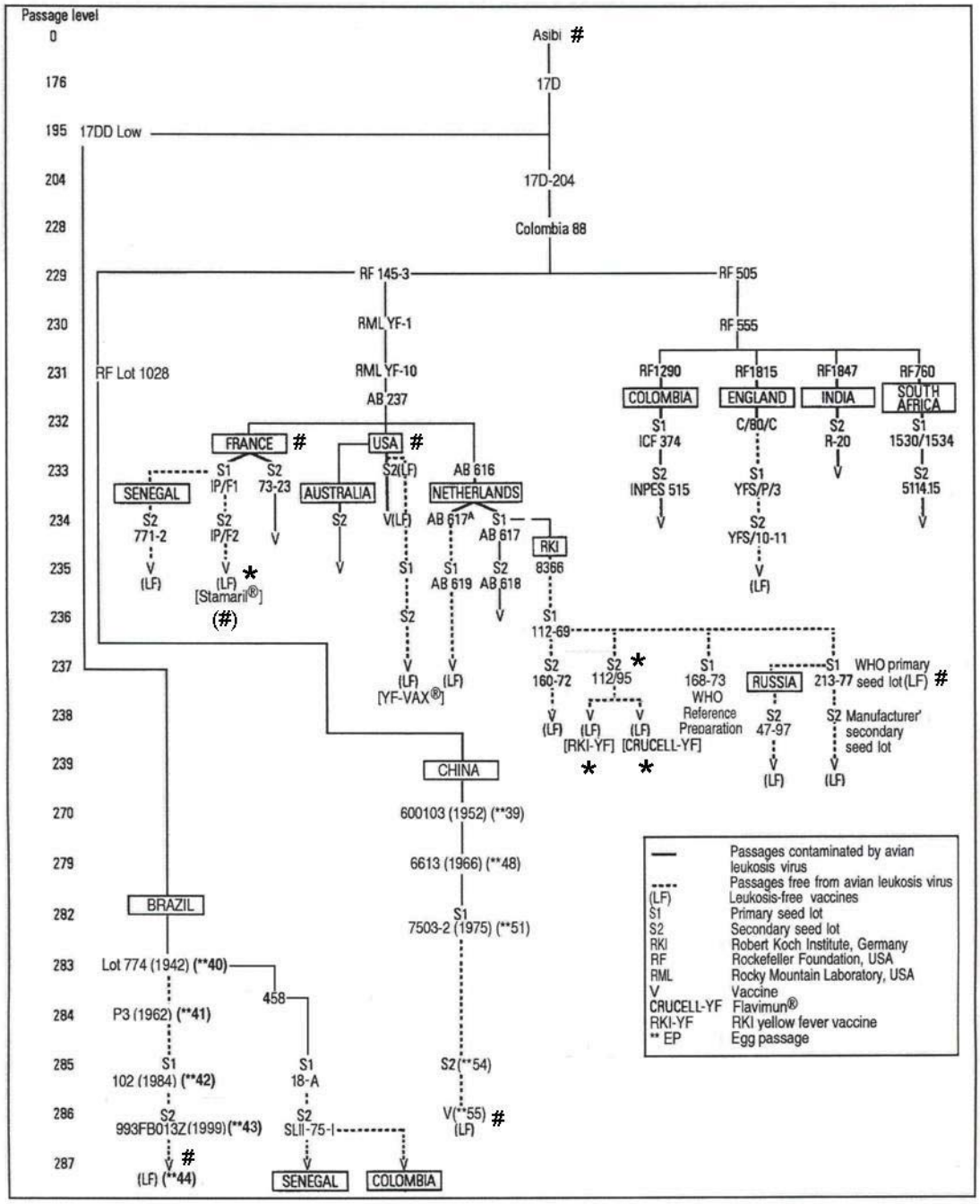

Fig. 2: YFV-17D genealogy based on historical data of passaging and transfer (modified from WHO [15]). Newly sequenced vaccine strains are indicated with an asterisk *; sequences derived from Genbank are highlighted with a rhombus #.

Table 1: Sequence differences between all available YFV vaccine strains. Divergent nucleotides are highlighted in bold. Positions that have an effect on the protein level are shaded in grey. (Y = C or T)

		C			PrM			E															NS1	
	Nucleotideposition	142	237	370	490	643	883	1003	1140	1150	1431	1436	1437	1491	1558	1692	1946	2003	2110	2219	2220	2356	2677	3470
17D	Stamaril® YF-vaccine	A	C	C	G	A	G	T	T	G	A	G	A	T	C	C	T	A	G	A	C	T	C	A
17D	YF-AVD2791-93F 04 from spain	A	C	C	G	A	G	T	T	G	A	G	A	T	C	C	T	A	G	A	C	T	C	A
17D	Crucell® TVX Flavimun	A	C	C	G	A	G	T	T	G	**C**	G	A	T	C	C	T	A	G	A	C	T	C	A
17D	Crucell® Flavimun WSL	A	C	C	G	A	G	T	T	G	**C**	G	A	T	C	C	T	A	G	A	C	T	C	A
17D	RKI YFV vaccine	A	C	C	G	A	G	T	T	G	**C**	G	A	T	C	C	T	A	G	A	C	T	C	A
17D	YFV-17D-213	A	C	C	G	A	G	T	T	G	**C**	G	A	T	C	C	T	A	G	A	C	T	C	A
17D	YFV-17D-204_2	A	C	C	G	A	G	T	T	G	A	G	A	T	C	C	T	A	G	A	C	T	C	A
17D	YFV-17D-204_1	A	C	C	G	A	G	T	T	G	A	G	A	T	C	C	T	A	G	A	C	T	C	A
17D	YFV 17D-Tiantan China	**G**	**T**	C	**A**	A	G	T	T	**A**	A	G	A	**C**	C	**T**	T	A	G	A	C	T	C	**G**
17DD	YFV-17DD Brazil	A	C	C	G	A	**A**	T	**C**	G	A	G	A	T	C	C	**C**	**G**	G	A	C	**C**	C	A
17DD	YFV-17DD	A	C	**T**	G	**G**	**A**	T	**C**	G	A	**A**	**G**	T	**A**	C	**C**	A	**A**	**G**	**T**	T	C	A
17DD	YFV-17DD case#1 YEL-AVD Peru	A	C	**T**	G	**G**	**A**	T	**C**	G	A	**A**	**G**	T	**A**	C	**C**	A	G	**G**	**T**	**C**	**T**	A
17DD	YFV-17DD case#2 YEL-AVD Peru	A	C	**T**	G	**G**	**A**	**Y**	**C**	G	A	**A**	**G**	T	**A**	C	**C**	A	G	**G**	**T**	**C**	**T**	A

		NS2a					NS2b				NS3													
	Nucleotideposition	3599	3637	3668	4013	4054	4204	4222	4523	4559	4612	4804	4873	4921	4942	4948	4957	4972	5115	5123	5153	5161	5225	5362
17D	Stamaril® YF-vaccine	T	C	C	T	**Y**	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	C
17D	YF-AVD2791-93F 04 from spain	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	C
17D	Crucell® TVX Flavimun	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	**T**
17D	Crucell® Flavimun WSL	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	**T**
17D	RKI YFV vaccine	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	**T**
17D	YFV-17D-213	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	**T**
17D	YFV-17D-204_2	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	C
17D	YFV-17D-204_1	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	C
17D	YFV 17D-Tiantan China	T	C	**T**	T	T	C	G	C	**A**	C	T	G	G	A	C	C	G	A	C	G	T	A	C
17DD	YFV-17DD Brazil	T	C	C	**C**	**C**	C	**A**	C	G	**T**	**C**	**T**	G	A	C	C	G	A	**T**	**A**	**C**	A	C
17DD	YFV-17DD	**C**	**T**	C	**C**	**C**	**T**	G	C	G	**T**	T	**T**	G	**G**	C	**T**	**A**	**G**	C	**A**	T	**C**	**A**
17DD	YFV-17DD case#1 YEL-AVD Peru	**C**	**T**	C	**C**	**C**	**T**	G	**Y**	G	**T**	T	**T**	G	**G**	C	**T**	**A**	**G**	C	**A**	T	**C**	C
17DD	YFV-17DD case#2 YEL-AVD Peru	**C**	**T**	C	**C**	**C**	**T**	G	**Y**	G	**T**	T	**T**	**A**	**G**	**Y**	**T**	**A**	**G**	C	**A**	T	**C**	C

		NS3					NS4a				NS4b					NS5							
	Nucleotideposition	5393	5641	6070	6280	6418	6514	6529	6625	6673	6758	6947	7319	7496	7497	7571	7701	7975	8029	8099	8808	9397	9522	9523
17D	Stamaril® YF-vaccine	T	G	C	C	T	T	T	A	T	A	C	A	T	T	A	G	C	T	G	A	A	T	G
17D	YF-AVD2791-93F 04 from spain	T	G	C	C	**C**	T	T	A	T	A	C	A	T	T	A	G	C	T	G	A	A	T	G
17D	Crucell® TVX Flavimun	T	G	C	C	T	T	T	A	T	A	C	A	**C**	T	A	G	C	T	G	A	A	T	G
17D	Crucell® Flavimun WSL	T	G	C	C	T	T	T	A	T	A	C	A	**C**	T	A	G	C	T	G	A	A	T	G
17D	RKI YFV vaccine	T	G	C	C	T	T	T	A	T	A	C	A	**C**	T	A	G	C	T	G	A	A	T	G
17D	YFV-17D-213	T	G	C	C	T	T	T	**C**	T	A	C	A	T	**C**	A	G	C	T	G	A	A	T	G
17D	YFV-17D-204_2	T	**A**	C	C	T	T	T	A	T	A	C	A	T	T	A	G	C	T	G	A	A	T	G
17D	YFV-17D-204_1	T	**A**	C	C	T	T	**C**	A	T	**G**	C	**G**	T	T	A	G	C	T	G	A	A	T	G
17D	YFV 17D-Tiantan China	T	G	C	**G**	T	T	T	A	T	A	C	A	T	T	A	G	C	T	G	A	A	T	G
17DD	YFV-17DD Brazil	**C**	G	**T**	C	T	T	T	A	T	A	**T**	A	T	T	**C**	**A**	C	T	**A**	**G**	A	**A**	**T**
17DD	YFV-17DD	T	G	**T**	C	T	**C**	T	**C**	T	A	C	A	T	T	**C**	**A**	**T**	**C**	G	**G**	**G**	T	G
17DD	YFV-17DD case#1 YEL-AVD Peru	T	G	**T**	C	T	**C**	T	**C**	**Y**	A	C	A	T	T	**C**	**A**	**T**	**C**	G	**G**	**G**	T	G
17DD	YFV-17DD case#2 YEL-AVD Peru	T	G	**T**	C	T	**C**	T	**C**	**Y**	A	C	A	T	T	**C**	**A**	**T**	**C**	G	**G**	**G**	T	G

		NS5							3'-NTR							
	Nucleotideposition	9605	9783	9988	10144	10174	10243	10291	10367	10454	10550	10675	10722	10815	10847	10860
17D	Stamaril® YF-vaccine	A	A	C	A	A	G	C	C	A	C	A	G	G	C	A
17D	YF-AVD2791-93F 04 from spain	A	A	C	A	A	G	C	C	A	C	A	G	G	C	A
17D	Crucell® TVX Flavimun	A	A	C	A	A	G	C	C	A	C	A	G	G	C	A
17D	Crucell® Flavimun WSL	A	A	C	A	A	G	C	C	A	C	A	G	G	C	A
17D	RKI YFV vaccine	A	A	C	A	A	G	C	C	A	C	A	G	G	C	A
17D	YFV-17D-213	A	A	C	A	A	**A**	C	C	A	C	A	G	G	C	A
17D	YFV-17D-204_2	A	A	C	A	A	**A**	C	C	A	C	A	**A**	G	C	A
17D	YFV-17D-204_1	**G**	A	C	A	A	**A**	C	C	**G**	C	A	G	G	C	A
17D	YFV 17D-Tiantan China	**A**	A	C	**G**	A	G	C	C	A	C	A	G	**A**	C	**T**
17DD	YFV-17DD Brazil	A	**G**	C	A	A	G	C	**T**	A	**T**	A	G	G	C	A
17DD	YFV-17DD	A	A	C	A	A	**A**	C	C	A	**T**	A	G	G	C	A
17DD	YFV-17DD case#1 YEL-AVD Peru	A	A	C	A	A	G	C	**T**	A	**T**	A	G	G	**A**	A
17DD	YFV-17DD case#2 YEL-AVD Peru	**A**	A	**Y**	A	**R**	G	**Y**	**T**	A	**T**	**R**	G	G	**A**	A

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Table(s)

		C			PrM			E															NS1	
	Nucleotideposition	142	237	370	490	643	883	1003	1140	1150	1431	1436	1437	1491	1558	1692	1946	2003	2110	2219	2220	2356	2677	3470
17D	Stamaril® YF-vaccine	A	C	C	G	A	G	T	T	G	A	G	A	T	C	C	T	A	G	A	C	T	C	A
	YF-AVD2791-93F 04 from spain	A	C	C	G	A	G	T	T	G	A	G	A	T	C	C	T	A	G	A	C	T	C	A
	Crucell® TVX Flavimun	A	C	C	G	A	G	T	T	G	**C**	G	A	T	C	C	T	A	G	A	C	T	C	A
	Crucell® Flavimun WSL	A	C	C	G	A	G	T	T	G	**C**	G	A	T	C	C	T	A	G	A	C	T	C	A
	RKI YFV vaccine	A	C	C	G	A	G	T	T	G	**C**	G	A	T	C	C	T	A	G	A	C	T	C	A
	YFV-17D-213	A	C	C	G	A	G	T	T	G	**C**	G	A	T	C	C	T	A	G	A	C	T	C	A
	YFV-17D-204_2	A	C	C	G	A	G	T	T	G	A	G	A	T	C	C	T	A	G	A	C	T	C	A
	YFV-17D-204_1	A	C	C	G	A	G	T	T	G	A	G	A	T	C	C	T	A	G	A	C	T	C	A
	YFV 17D-Tiantan China	**G**	**T**	C	**A**	A	G	T	T	**A**	A	G	A	**C**	C	**T**	T	A	G	A	C	T	C	**G**
17DD	YFV-17DD Brazil	A	C	C	G	A	**A**	T	**C**	G	A	G	A	T	C	C	**C**	**G**	G	A	C	**C**	C	A
	YFV-17DD	A	C	**T**	G	**G**	**A**	T	**C**	G	A	**A**	**G**	T	**A**	C	**C**	A	**A**	**G**	**T**	T	C	A
	YFV-17DD case#1 YEL-AVD Peru	A	C	**T**	G	**G**	**A**	T	**C**	G	A	**A**	**G**	T	**A**	C	**C**	A	G	**G**	**T**	**C**	**T**	A
	YFV-17DD case#2 YEL-AVD Peru	A	C	**T**	G	**G**	**A**	**Y**	**C**	G	A	**A**	**G**	T	**A**	C	**C**	A	G	**G**	**T**	**C**	**T**	A

		NS2a					NS2b				NS3													
	Nucleotideposition	3599	3637	3668	4013	4054	4204	4222	4523	4559	4612	4804	4873	4921	4942	4948	4957	4972	5115	5123	5153	5161	5225	5362
17D	Stamaril® YF-vaccine	T	C	C	T	**Y**	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	C
	YF-AVD2791-93F 04 from spain	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	C
	Crucell® TVX Flavimun	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	**T**
	Crucell® Flavimun WSL	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	**T**
	RKI YFV vaccine	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	**T**
	YFV-17D-213	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	**T**
	YFV-17D-204_2	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	C
	YFV-17D-204_1	T	C	C	T	T	C	G	C	G	C	T	G	G	A	C	C	G	A	C	G	T	A	C
	YFV 17D-Tiantan China	T	C	**T**	T	T	C	G	C	**A**	C	T	G	G	A	C	C	G	A	C	G	T	A	C
17DD	YFV-17DD Brazil	T	C	C	**C**	**C**	C	**A**	C	G	**T**	**C**	**T**	G	A	C	C	G	A	**T**	**A**	**C**	A	C
	YFV-17DD	**C**	**T**	C	**C**	**C**	**T**	G	C	G	**T**	T	**T**	G	**G**	C	**T**	**A**	**G**	C	**A**	T	**C**	**A**
	YFV-17DD case#1 YEL-AVD Peru	**C**	**T**	C	**C**	**C**	**T**	G	**Y**	G	**T**	T	**T**	G	**G**	C	**T**	**A**	**G**	C	**A**	T	**C**	C
	YFV-17DD case#2 YEL-AVD Peru	**C**	**T**	C	**C**	**C**	**T**	G	**Y**	G	**T**	T	**T**	**A**	**G**	**Y**	**T**	**A**	**G**	C	**A**	T	**C**	C

		NS3					NS4a					NS4b					NS5							
	Nucleotideposition	5393	5641	6070	6280	6418	6514	6529	6625	6673	6758	6947	7319	7496	7497	7571	7701	7975	8029	8099	8808	9397	9522	9523
17D	Stamaril® YF-vaccine	T	G	C	C	T	T	T	A	T	A	C	A	T	T	A	G	C	T	G	A	A	T	G
	YF-AVD2791-93F 04 from spain	T	G	C	C	**C**	T	T	A	T	A	C	A	T	T	A	G	C	T	G	A	A	T	G
	Crucell® TVX Flavimun	T	G	C	C	T	T	T	A	T	A	C	A	**C**	T	A	G	C	T	G	A	A	T	G
	Crucell® Flavimun WSL	T	G	C	C	T	T	T	A	T	A	C	A	**C**	T	A	G	C	T	G	A	A	T	G
	RKI YFV vaccine	T	G	C	C	T	T	T	A	T	A	C	A	**C**	T	A	G	C	T	G	A	A	T	G
	YFV-17D-213	T	G	C	C	T	T	T	**C**	T	A	C	A	T	**C**	A	G	C	T	G	A	A	T	G
	YFV-17D-204_2	T	**A**	C	C	T	T	T	A	T	A	C	A	T	T	A	G	C	T	G	A	A	T	G
	YFV-17D-204_1	T	**A**	C	C	T	T	**C**	A	T	**G**	C	**G**	T	T	A	G	C	T	G	A	A	T	G
	YFV 17D-Tiantan China	T	G	C	**G**	T	T	T	A	T	A	C	A	T	T	A	G	C	T	G	A	A	T	G
17DD	YFV-17DD Brazil	**C**	G	**T**	C	T	T	T	A	T	A	**T**	A	T	T	**C**	**A**	C	T	**A**	**G**	A	**A**	**T**
	YFV-17DD	T	G	**T**	C	T	**C**	T	**C**	T	A	C	A	T	T	**C**	**A**	**T**	**C**	G	**G**	**G**	T	G
	YFV-17DD case#1 YEL-AVD Peru	T	G	**T**	C	T	**C**	T	**C**	**Y**	A	C	A	T	T	**C**	**A**	**T**	**C**	G	**G**	**G**	T	G
	YFV-17DD case#2 YEL-AVD Peru	T	G	**T**	C	T	**C**	T	**C**	**Y**	A	C	A	T	T	**C**	**A**	**T**	**C**	G	**G**	**G**	T	G

		NS5							3'-NTR							
	Nucleotideposition	9605	9783	9988	10144	10174	10243	10291	10367	10454	10550	10675	10722	10815	10847	10860
17D	Stamaril® YF-vaccine	A	A	C	A	A	G	C	C	A	C	A	G	G	C	A
	YF-AVD2791-93F 04 from spain	A	A	C	A	A	G	C	C	A	C	A	G	G	C	A
	Crucell® TVX Flavimun	A	A	C	A	A	G	C	C	A	C	A	G	G	C	A
	Crucell® Flavimun WSL	A	A	C	A	A	G	C	C	A	C	A	G	G	C	A
	RKI YFV vaccine	A	A	C	A	A	G	C	C	A	C	A	G	G	C	A
	YFV-17D-213	A	A	C	A	A	**A**	C	C	A	C	A	G	G	C	A
	YFV-17D-204_2	A	A	C	A	A	**A**	C	C	A	C	A	**A**	G	C	A
	YFV-17D-204_1	**G**	A	C	A	A	**A**	C	C	**G**	C	A	G	G	C	A
	YFV 17D-Tiantan China	A	A	C	**G**	A	G	C	C	A	C	A	G	**A**	C	**T**
17DD	YFV-17DD Brazil	A	**G**	C	A	A	G	C	**T**	A	**T**	A	G	G	C	A
	YFV-17DD	A	A	C	A	A	**A**	C	C	A	**T**	A	G	G	C	A
	YFV-17DD case#1 YEL-AVD Peru	A	A	C	A	A	G	C	**T**	A	**T**	A	G	G	**A**	A
	YFV-17DD case#2 YEL-AVD Peru	A	A	**Y**	A	**R**	G	**Y**	**T**	A	**T**	**R**	G	G	**A**	A

Figure(s)

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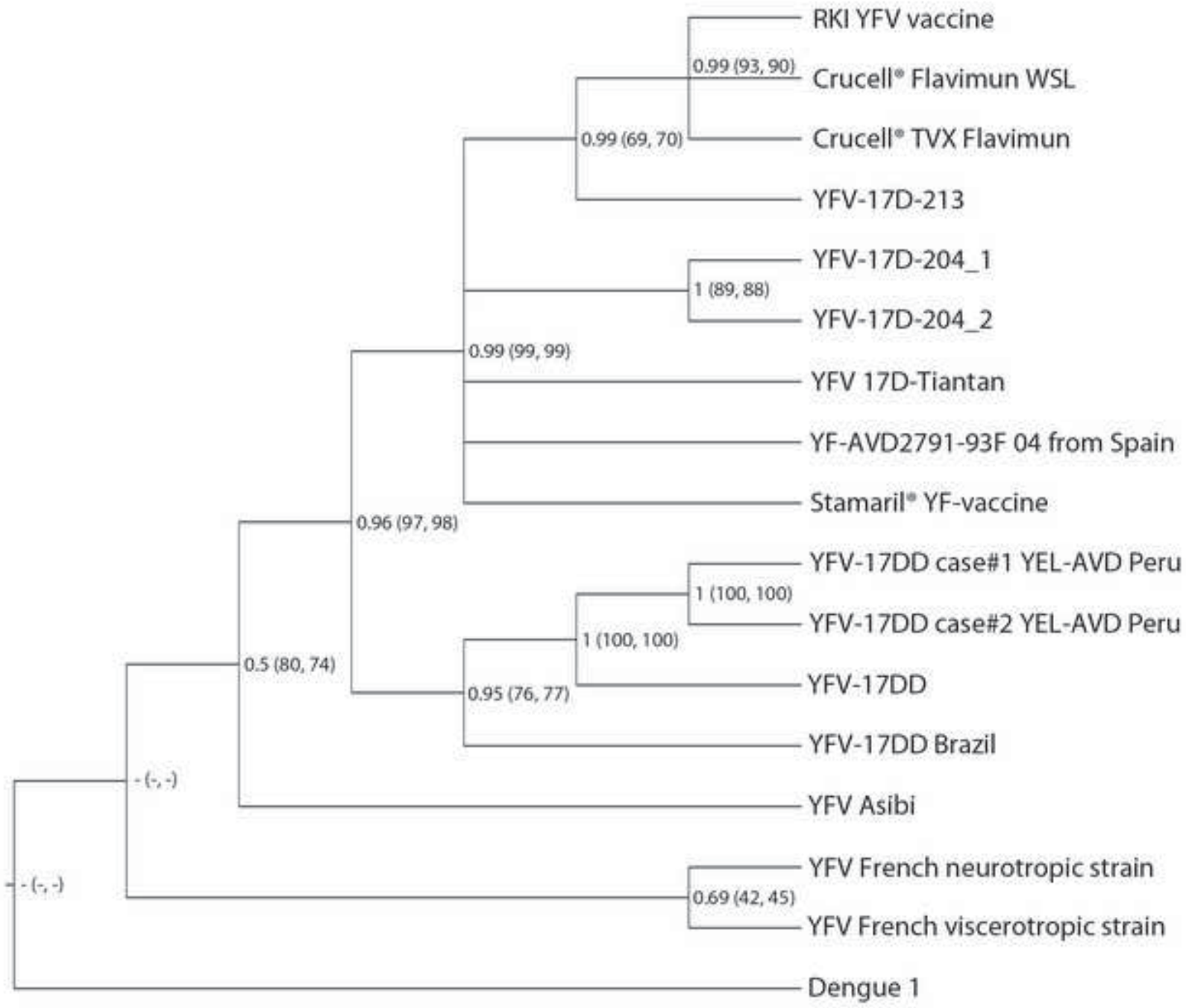

Figure(s)

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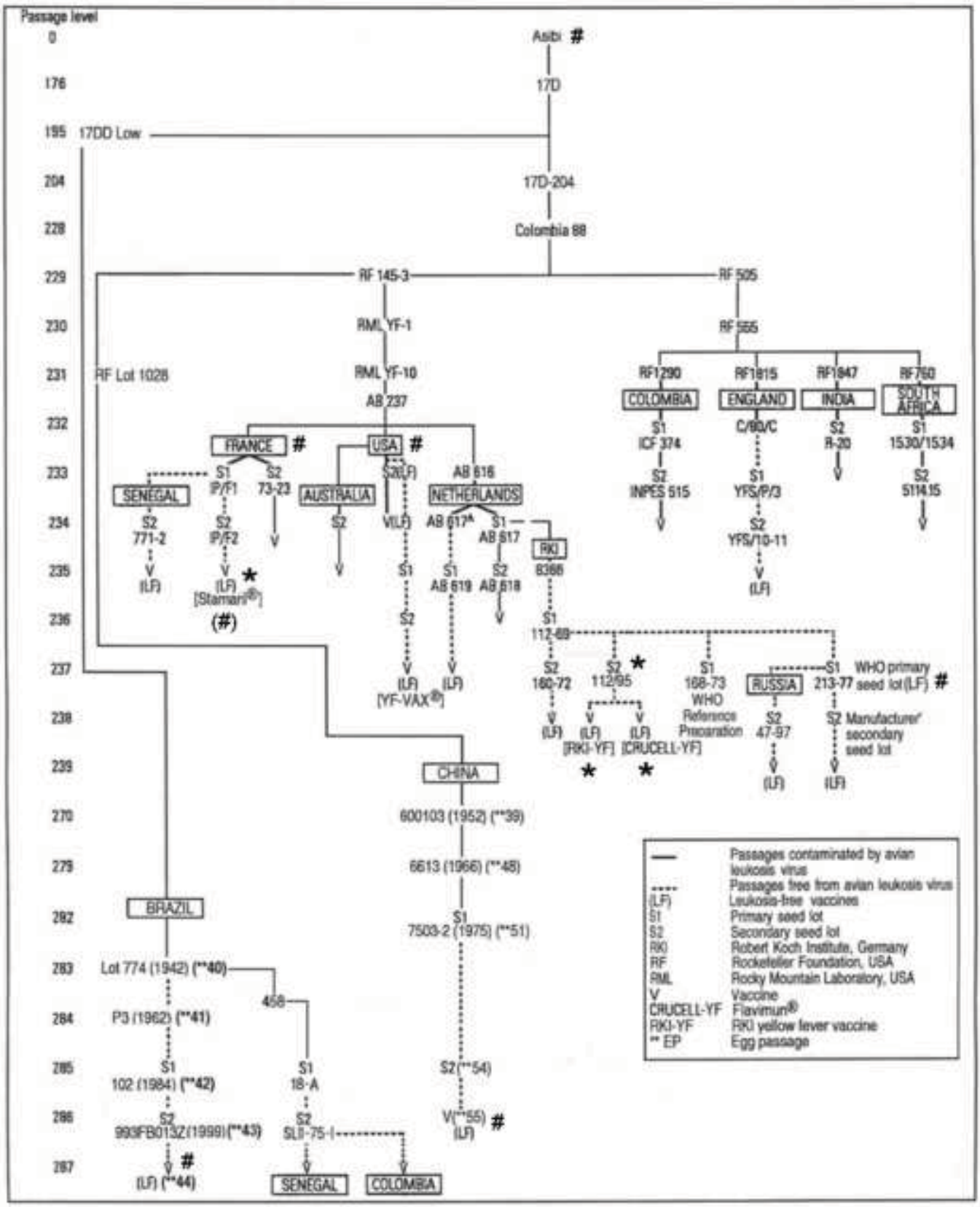

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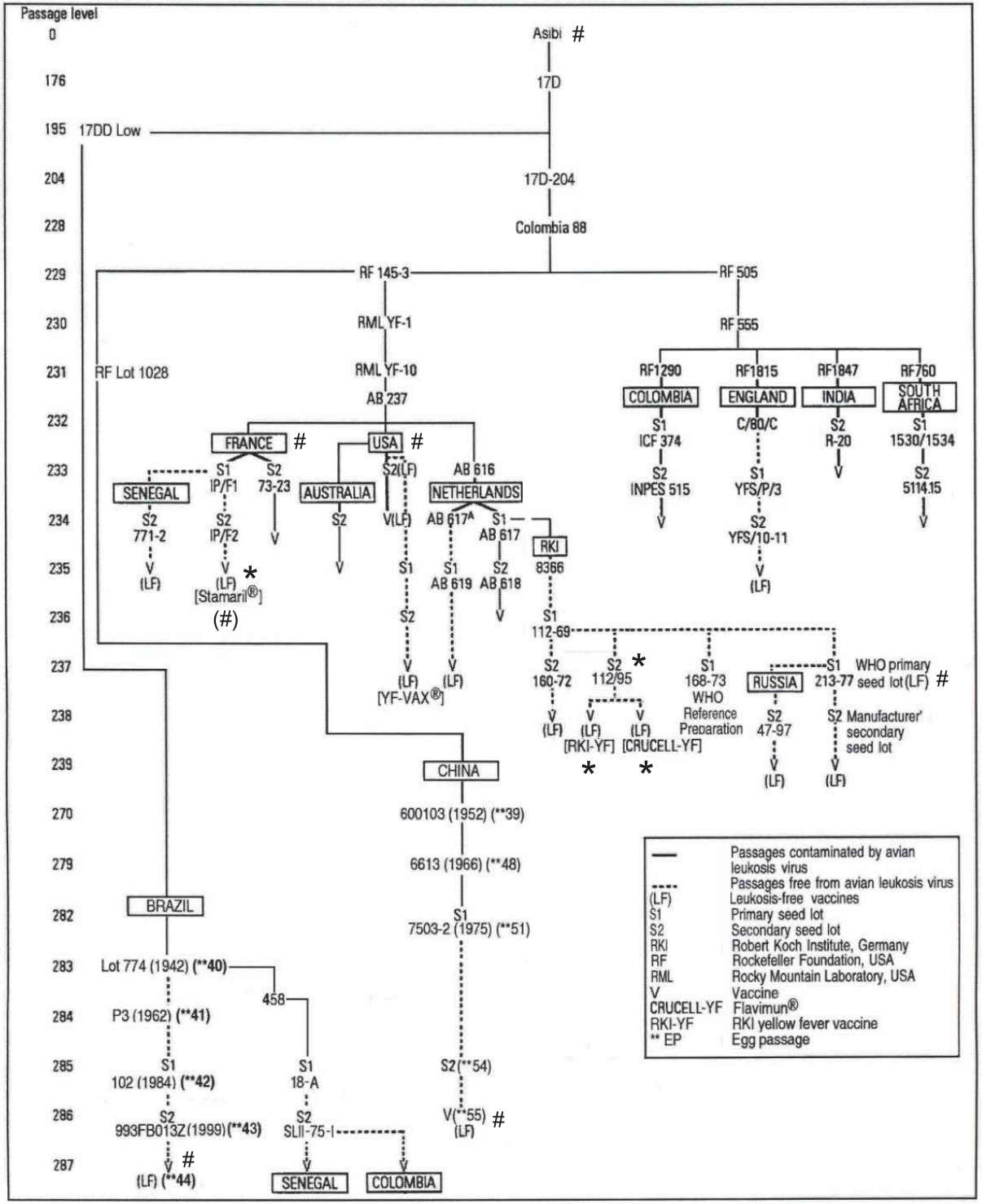
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RF1815
RF1847
RF760
COLOMBIA
ENGLAND
INDIA
SOUTH AFRICA
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C/80/C
S2 R-20
S1 1530/1534
S2 INPES 515
S1 YFS/P/3
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S2 AB 618
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6613 (1966) (**48)
S1 7503-2 (1975) (**51)
BRAZIL
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P3 (1962) (**41)
S1 102 (1984) (**42)
S1 18-A
S2 993FB013Z (1999) (**43)
S2 SLII-75-I
S2 (**54)
V(**55) #
(LF) (**44)
SENEGAL
COLOMBIA
Passages contaminated by avian leukosis virus
Passages free from avian leukosis virus
(LF) Leukosis-free vaccines
S1 Primary seed lot
S2 Secondary seed lot
RKI Robert Koch Institute, Germany
RF Rockefeller Foundation, USA
RML Rocky Mountain Laboratory, USA
V Vaccine
CRUCELL-YF Flavimun®
RKI-YF RKI yellow fever vaccine
** EP Egg passage