Yellow fever, biomedical theories, and sanitary practices: an unfinished history
(seen through the Brazilian experience)
The indian (city of Rio de Janeiro) hands to the immigrant the prohibited fruit (miasmas) provide by the serpent (yellow fever). In the tree hangs the morbific agents: the city’s dirth, the medical school, the municipal chamber etc. Around the tree, the immigrants family drama, since departure from Europe until the orphanage of the children. *Revista Ilustrada*, 18.3.1876, n.12, p.4-5
Angelo Agostini, in *Revista Ilustrada* (1883), makes fun of the precaution taken by neighbour countries against yellow fever contagion from Brazil. Passengers are sprayed with phenic acid and hanged to dry over aromatic plants. Prisoners open letters sent from Brazil and exhibit them from a distance. Inhabitants jump over fires to purify themselves.
Manuel Carmona y Valle (1832-1902). President of the National Academy of Medicine, in México city. In the 1880s he developed a vaccine against yellow fever with a fungus found in the patients urine (http://institutodeoftalmologia.org/fap/wp-content/uploads/2010/08/drManuelCarmona.jpg)

Republican and abolitionist, Domingos Freire (1843-1899), professor of organic chemistry in Rio’s medical school. At least 12,329 people were vaccinated in Brazilian cities between 1883 and 1894 (Revista Ilustrada, 1889, ano 14, nº 533, p. 8)
A number of microbe hunters were converted to the hypothesis formulated by Robert Koch that yellow fever agent was similar to the cholera bacillus, discovered by him in 1883, since the prime symptom of first disease - black vomit - was also located in the intestine. Bacilli would compete in the 1890s as the yellow fever germ.
A new representation would replace the old image of death harvesting lives as in Rio’s Carnival, in 1876, when 3,476 of the city’s inhabitants (274,972) were killed by yellow fever.

(Images: Fièvre paludéene tueuse d’hommes. Desenho de A. Ehrmann. DAD – Casa de Oswaldo Cruz / Revista Ilustrada, 04.03.1876, ano 1, nº 10, p. 7)
TO THE MEMORY
OF
WALTER MYERS,
THE ONLY SON OF GEORGE AND FLORA MYERS,
BORN IN BIRMINGHAM, ENGLAND,
MARCH 28TH, 1872,
DIED AT PARÁ, JANUARY 20TH, 1901.
THIS STONE IS ERECTED BY THE
LIVERPOOL SCHOOL OF TROPICAL MEDICINE
ON A MISSION OF THE SCHOOL
TO INVESTIGATE YELLOW FEVER,
HE HIMSELF FELL A VICTIM TO THE DISEASE.
"THE REST IS SILENCE."
Oil Portrait by Dean Cornwell representing The Yellow Fever Commission sent to Cuba under the United States Government after the Hispanic-American War. On the left, Carlos Juan Finlay watches inoculation with the mosquito he had incriminated by the Reed Commission.

Below – *Stegomyia fasciata*, now known as *Aedes aegypti*.
Mosquito brigades at work. The buildings were covered and sealed before Clayton gas was injected or Pyrethrum (plant of the genus *Chrysanthemum*) burned to kill adult mosquitoes. Other teams took care of the larvae (Coc/Fiocruz.)

From his Institute, Oswaldo Cruz makes war against mosquitoes (yellow fever), rats & lice (bubonic pest) using syringes (smallpox vaccination) (Falcão, E. de C. Oswaldo Cruz. Monumenta História, 1971)
Howard Shearman Wolferstan Thomas (left) and Anton Brein (“founder” of the Australian tropical medicine). Both worked in the Runcorn Research Laboratory, where Thomas demonstrated that atoxyl was efficient in treating trypanosomiasis (LSTM Archives)
The Yellow Fever Research Laboratory in Manaus (LSTM Archives)

Malaria

1. Malaria can only be contracted by the bite of an infected mosquito.
2. The malaria mosquitoes are called Anopheles, and can be distinguished by their dappled wings, and by their attitude when resting on walls, etc. See small illustration.
3. Malaria can be cured by taking quinine, but it is necessary to take the drug for several months after the fever has disappeared. If treatment is discontinued because there is no more fever, a relapse will occur, and one attack of fever will follow another.
4. A patient who has not had sufficient quinine treatment, harbours the malarial parasite in his system, and is a source of danger to those around him. Mosquitoes can feed on this patient, and so become infected, and about 10 days later they will be capable of infesting other people.
5. A patient who suffers from relapses, loses his health very quickly, and is liable to contract other diseases.
6. Always sleep under a mosquito net and thus avoid contracting malaria.
In 1905, Fritz Richard Schaudinn announced the discovery of the agent of syphilis: *Spirochaeta pallida* (*Treponema pallidum*). In 1904, he wrote:

“My findings about the reduction in spirochetes during their reproduction led me to presume that they can also be the pathogenic agents in yellow fever. Since their intermediary host is known, it would be first necessary to carefully examine the Malpighi tubes (...). In any case, I would like to suggest starting research on yellow fever in the light of the viewpoints supplied by my results” (SCHAUDINN, F. Generations und Wirtswechsel bei Trypanosoma und Spirochaete. *Arbeiten aus dem Kaiserl. Gesundheitsamte*, v.20, n.3, 1904:52)
In 1915-1916, Ryukichi Inada and Yutaka Ido correlated Weil Disease (leptocariosis) to *S. icterohaemorrhagiae*. The disease that was epidemic in the European trenches, had an outstanding similarity to yellow fever: jaundice (Benchimol et al., Cherry trees and coffee farms, 2009)
In 1918, Hideyo Noguchi, from the Rockefeller Institute, was appointed member of a commission sent by Rockefeller Foundation to Equador, to investigate the etiology of yellow fever. Gorgas and other specialists believed it was caused by a Spirochaeta. Noguchi confirmed that and proposed a new genus (*Leptospira*) to accomodate the agents of yellow fever and infectious jaundice (*leptospirosis*).
“A curious phenomenon, the three things I most expected to find in Africa are conspicuous by their absence: neither yellow fever nor heat nor mosquitoes” — wrote Guiteras (1920-1921). The extinction of yellow fever in Havana, Rio de Janeiro and Yucatan and the scarcity of White people in Africa explained the limited extent of the disease there. His conclusion was based on two notions that would soon be revoked: yellow fever involved a cycle only in man and Stegomyia fasciata and had originally come from the American continent.

“If we add to this the (...) relative immunity of the negro (...) we should be prepared to admit the possibility and even the likelihood that yellow fever infection has been totally extinguished from there”
Emílio Goeldi, director of the Pará Museum, proposed in 1907 the African origin of *Stegomyia fasciata* (*Aedes aegypti*) and of yellow fever. On the left, first description of the disease in Brazil by João Ferreira da Rosa (1694). Above, geographical distribution of yellow fever and *Stegomyia fasciata* according to Kolle & Hetsche (1918).
At the beginning of the 1920s, Henry Rose Carter began researches that led him to endorse the theory that yellow fever was brought from Africa to the West Indies (photo: http://yellowfever.lib.virginia.edu/reed/images/02-panama.jpg)
In 1925, a second Commission was organized by the International Health Board, headed by Henry Beeuwkes.

“We did not manage to isolate *Leptospira icteroides* in West Africa,” — wrote Beeuwkes in June 1927 — “no clearly susceptible animal has yet been found”.

There was strong suspicion that the disease known as yellow fever in Africa was not the same as its American cousin.
In June 1927, while in Hamburg, Beeuwkes bought monkeys from India (*rhesus* e crown monkeys) and Brazil (*saguis*). On his way to Lagos with Adrian Stokes he also got chimpanzees. Stokes landed in Accra to help in the lab research. They had just received blood from patients with mild infections from Kpeve, in the Goald Coast. One of them was a 28-year-old man, Asibi. The virus strain extracted from him leads us to yellow fever vaccines used nowadays.
“I have been getting a definite organism (not a leptospira, but often forming spirals) which reproduces alterations very similar to experimental yellow fever (...) The organism is felterable” (Letter from Noguchi to Simon Flexner, 9.3.1928)

Adrian Stokes (left) death on 19th September 1927 speeded up the pace of the work. Noguchi was already preparing for his return to New York when he died on 21st May 1928. William Alexander Young (right), director of the British hospital in Accra, followed him eight days later.
Scenes of the campaign against yellow fever in Rio de Janeiro in 1928-1929

Images: Academia Nacional de Medicina / COC-Fiocruz / Film Yellow Fever - Cinemateca Brasileira / O Cruzeiro, 23.03.1929
The viscerotome should be introduced in the abdominal wall for puncture of the liver. Samples from Brazil and other countries were sent to Bahia’s and Rio’s laboratories. The Viscerotomy Service made possible the mapping of other diseases, such as visceral leishmaniasis (calazar) identified in Brazil by Henrique Penna (1934).
In 1949, there were 1,349 viscerotomy stations throughout Brazil. The viscerotomist could be any one in town with minimum manual ability. A liver sample had to be taken whenever happened a death by a suspect febrile disease. There were violent reactions to the profanation of corpses, especially after a government decree prohibited funerals without Viscerotomy Service permission.

(Images: Smith in Strode, 1951, p. 594 / Collection Family Penna)
In the building in which the yellow fever inspector entered had to be hanged a flag. Its colour and drawing varied according to his hierarchical position and duty. The map illustrates area to be inspected after identification of a *Aedes* focus

(Images: Coc-Fiocruz / Manual de instruções técnicas e administrativas do Serviço Nacional de Febre Amarela - Bio-Manguinhos-Fiocruz)
Santa Teresa, in Espírito Santo, Brazil, is characterized by steep valleys formed by rivers that run down the forested mountains with few human habitations.

In 1932, Fred Soper, Henrique Penna and other workers collected more than 600 blood samples in the region. Not being able to find *Aedes aegypti* in the houses they began to investigate triatomes, bedbugs, fleas, ticks and forest mosquitoes…

http://www.treknature.com/gallery/South_America/Brazil/photo221753.htm
Blood extraction in Minas Gerais, 1937. The blood was sent to the lab for protection test. A technician fills a syringe with a mixture of virus and serum. If the serum contained antibodies for yellow fever, it would neutralize the virus action after intracerebral inoculation in mice.
House were a man died of yellow fever in a rural zone, recurrent image after the discovery of the selvatic form of the disease (1933). Above, typical environment until then associated with yellow fever (Rio de Janeiro in the 1890s)

Family of victim of selvatic yellow fever. Brejão farm, Anápolis, Goiás, 31.8.1935
In 1938, the virus was isolated in *Haemagogus capricorni* and *Aedes leucocelaenus*, and also in *Sabethines*. In the crowns of trees were found other species associated with the transmission of yellow fever and other arboviruses. Protection tests were important in the search for vertebrate hosts to the yellow fever virus, like howler monkeys (genus *Alouata*)
Truck with boys used to capture mosquitoes in Minas Gerais, 1948. On the right: *Cebus*, a species used in protection tests as an alternative to *rhesus*. The diagram shows experiences of virus transmission from infected mosquitoes to marmosets (1945).
Cycles and bridges of sylvan yellow fever in South American and Africa

WHO’s panel on yellow fever, in 1949, proposed four types of yellow fever areas in the African and American continents, approved by the III World Health Assembly, in May 1950. Endemic and epidemic, with transmission man to man through *Aedes aegypti*; enzootic and epizootic areas without the classic vector. Such distinctions had practical relevance, since countries with endemic and epidemic areas were subject to quarantine restrictions.
Asibi’s blood was inoculated into a rhesus monkey on June 30, 1927, and then virus made numerous passages through mosquitoes and other monkeys.

One route yielded 17D, after *in vitro* passages through embryonic chick tissue without the central nervous system. Using subculture 214 – counting from the original Asibi – many parallel series were begun, some in embryonated eggs. The “friendly” virus (1937) protected rhesus monkeys and no longer occasioned encephalitis when injected into their brains (although it still did so in mice).
Extremely sensitive to direct light and to heat, the vaccine was rehydrated and diluted just before inoculation. To make sure the virus had survived the trip, when a session began, the first dose from each vial was used for intracerebral inoculation in mice, while the last dose was used on a second group, both of which were observed for 21 days. Ending up with an inactive virus wasn’t the only risk: the wild virus of yellow fever would kill mice as of the third day. If a mouse died then, the problem was far more serious.
At Instituto Oswaldo Cruz, Rockefeller’s Yellow Fever Laboratory, opened in March 1, 1937
Photos: Coc - Fiocruz
During 8 to 9 days before inoculation, eggs are analysed by transillumination to see condition of embryo. Eggs are then marked so as to indicate the position of air chamber and embryo. The blowlamp prepares the egg shell for perforation (1941-1943)

Photos: Coc - Fiocruz
Virus is injected near the embryo and hole is sealed. After incubation, egg is ready to be opened with the flame of blowtorch (1941-1943). Embryos are removed to flask and triturated in the coloidal mill.

Photos: Coc - Fiocruz
a) Extraction of embryonic juice from triturator flask. After addition of human serum to the embryonic mass, the suspension is distributed to tubes for centrifugation. B) Over the table, equipment for vaccine dessication, with vacuum and sulphuric acid (1941-1943). c) Dessicator that replaced the earlier one: mounted with Megavac pump and thermal bottle. d) Distribution of vaccines in ampoules.

Photos: Coc - Fiocruz
In 1941, cases of encephalitis were detected among the immunized. The cause was a mutation in the virus itself and so the seed lot system was conceived: successive batches of a product are derived from the same master seed lot at a given passage level. The final product is derived from the working seed lot and has not undergone more passages from the master seed lot than the vaccine shown to be satisfactory with respect to safety and efficacy.
Rio de Janeiro / Laboratory of Yellow Fever: Production of yellow fever vaccine in doses 1944-2000

Stock, production and delivery of doses 1944-2000

![Graph showing destination of vaccines from Rio de Janeiro](image)


### Estimated stocks of yellow fever vaccine (17D) in July 1978 (in doses)

<table>
<thead>
<tr>
<th>Institution</th>
<th>Stock (in doses)</th>
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<tbody>
<tr>
<td>Instituto Pasteur de Dacar — Senegal</td>
<td>2,500,000</td>
</tr>
<tr>
<td>Laboratório de Vacina Contra a Febre Amarela da Fiocruz — Rio de Janeiro, Brasil</td>
<td>2,000,000</td>
</tr>
<tr>
<td>Unité d’Ecologie Virale, Institut Pasteur — Paris, França</td>
<td>1,400,000</td>
</tr>
<tr>
<td>Laboratorio de Producción de Vacuna de Fiebre Amarilla do Instituto Nacional de Salud — Bogotá, Colômbia</td>
<td>500,000</td>
</tr>
<tr>
<td>Connaught Laboratories Inc. — Swiftwater, Pennsylvania, Estados Unidos</td>
<td>224,000</td>
</tr>
<tr>
<td>Departamento de Saúde do National Institute for Virology — Transvaal, África do Sul</td>
<td>220,000</td>
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<tr>
<td>Commonwealth Serum Laboratories — Victoria, Austrália</td>
<td>80,000</td>
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<tr>
<td>Koninklijk Instituut voor de Tropen — Amsterdã, Holanda</td>
<td>70,000</td>
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<tr>
<td>Instituto Central de Pesquisas — Kasauni, India</td>
<td>60,000</td>
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<tr>
<td>Laboratório de Febre Amarela do Instituto Robert Koch — Berlim, República Federal da Alemanha</td>
<td>20,000</td>
</tr>
<tr>
<td>Biological Products, Wellcome Research Laboratories — Beckenham, Londres</td>
<td>-</td>
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<tr>
<td>Federal Vaccine Production Laboratory — Aba, Nigéria</td>
<td>-</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td><strong>7,074,000</strong></td>
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*Fonte: OPAS/OMS, abril de 1980, p. 34.*
Guard spraying tires (1982), one of the main villains of Aedes aegypti campaigns since the 1980s
Photo: Coc-Fiocruz
The crash of sylvatic yellow fever in the interior of Brazil and foci of *Aedes aegypti* along the coast produced did not the feared urban yellow fever but Brazil’s first dengue outbreaks. In Rio de Janeiro (1986), *Aedes albopictus* was detected for the first time. Originating in Asia, where it transmits dengue fever and Japanese encephalitis, this mosquito entered Brazil via ports that were exporting iron to Japan.

Maps: http://ars.els-cdn.com/content/image/1-s2.0-S0025712508000989-gr1.jpg / http://ars.els-cdn.com/content/image/1-s2.0-S0025712508000989-gr1.jpg
Confirmed cases of yellow fever and vaccine doses used
Phenotypic and Molecular Analyses of Yellow Fever 17DD Vaccine Viruses Associated with Serious Adverse Events in Brazil

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Clinical and Immunological Insights on Severe, Adverse Neurotropic and Viscerotrophic Disease following 17D Yellow Fever Vaccination

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Above: Yellow fever risk areas (endemic, transition, potential risk and undamaged), 1997 to 2008
On the left: Cases and letality. Brazil, 1982 to 2010 (Source: Sinan/SVS/MS / slide made by Reinaldo de Menezes Martins)