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A CONTINUING FOCUS OF YELLOW FEVER IN THE APURIMAC RIVER VALLEY, AYACUCHO, PERU, AND THE FIRST ISOLATION OF YELLOW FEVER VIRUS IN THAT COUNTRY[1]

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Extensive outbreaks of hemorrhagic disease compatible with yellow fever (YF) occurred in southern Peru in 1977, 1978, and 1981. This article describes the epidemiologic circumstances involved and the procedures applied to isolate YF virus from six patients—the first time this virus has been isolated in Peru.

Introduction

Clinical sylvatic yellow fever (YF) in Peru may have been reported as early as 1913 by Rojas *(1)*; however, the earliest known outbreak that has been reported was that cited by Giles and Pinto *(2)* as occurring in Chanchamayo (Junín Department) in 1925. It has also been shown that children living along the upper Amazon River in Brazil and Peru during 1931 had antibody to YF virus *(3)*; and serologic surveys for antibody to YF virus in 1933–1934 in the same general area indicated that the antibody prevalence increased with age—10.6% of the study subjects under 15 years old being found with antibody to YF virus, as compared to 50.2% of those 15 years and older *(4)*. That this immunity was not due to infection with attenuated strains of YF virus was demonstrated by the fact that fatal cases, confirmed by liver examination, were reported from Peru and elsewhere in the Amazon region. More recently, Morales-Ayala found neutralizing antibody to YF virus in 64% of 208 individuals living in San Martín Department, two years before a 1969–1970 outbreak of jungle yellow fever that produced 86 reported cases and 61 deaths *(5)*. In 1975, one case of yellow fever was reported in Peru *(6)*.

In 1977, an extensive outbreak of a hemorrhagic disease compatible with YF occurred in the Apurímac River Valley of south-central Peru's Ayacucho Department. A massive vaccination program was initiated, but the disease reappeared in Ayacucho Department and the adjacent department of Cuzco in 1978 (see Figure 1). This situation afforded an opportunity to study the outbreak and the attendant epidemiologic circumstances. That study, the results of which are reported here, indicated a situation involving the introduction of susceptible migrant workers into an enzootic focus and provided specimens which, for the first time, yielded YF virus isolates from Peru.

The Study Area

The regions involved in the outbreaks were Huanta and La Mar provinces in Ayacucho Department and La Convención Province in Cuzco Department, all of which lie at approxi-

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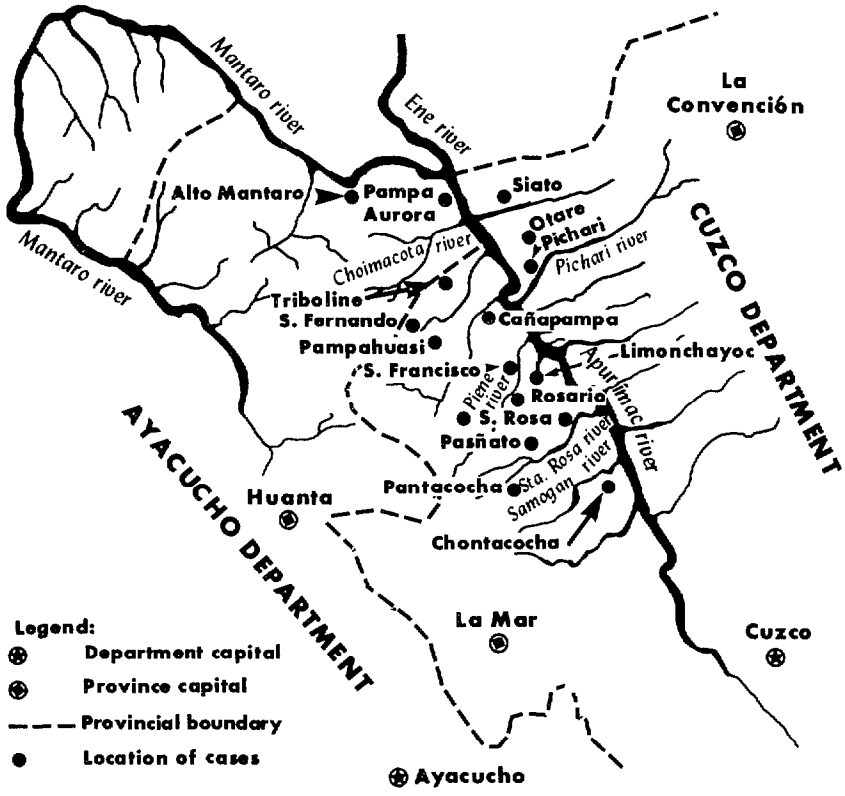
Figure 1. A map of Peru showing departmental boundaries and the cities of Ayacucho, Cuzco, and Lima.

mately 13° S, 73° W in an area near the confluence of the Apurímac and Mantaro rivers (Figure 2). The Apurímac River Valley in this region extends over approximately 500 km^2, ranges in altitudes from 650 to 1,000 m above sea level, and has ecologic characteristics of both high rain forest *(7)* and dry tropical forest *(8)* (see photo). High temperatures in the valley tend to fluctuate around 32°C in June and July, and precipitation is between 1,000 and 2,000 mm from December to March, with an attendant mean relative humidity of 80%.

Within this region are 420 towns with an approximate permanent population of 40,000 people living in about 18,000 residences. However, from January to March a massive immigration occurs. The migrants—contracted temporarily to harvest coffee, cocoa, and other crops—are principally from the departments of Junín, Huancavelica, and Ayacucho. Languages spoken in the study region are Spanish, Quechua, and local dialects. Common illnesses of the region include intestinal parasitism, gastroenteritis, malaria, leishmaniasis, and respiratory infections.

It should also be noted that this is a very isolated region by reason of its mountainous terrain. The straight-line distance between the city of Ayacucho and the town of San Francisco (see Figure 2) is merely 150 km, but it takes nearly 12 hours to travel between them by land. Aerial travel to this area is irregular, and local traffic generally uses boats or dirt trails.

Figure 2. A map of the Lower Apurímac River Valley area in Ayacucho and Cuzco departments showing where reported yellow fever cases occurred in 1978.



The natural vegetation of the high forest in this area is exuberant. It consists principally of trees belonging to the families Moracea (*Secropia tessmennii, Ficus anthelmintica, Artrocarpus incisa*), Bombacacea (*Ochroma lagopus* and *piscatoria*), and Euforbiacia (*Croton erythrochilus* and *Cedrela* sp.), and of legumes and other plants and bushes typical of mountainous habitats.

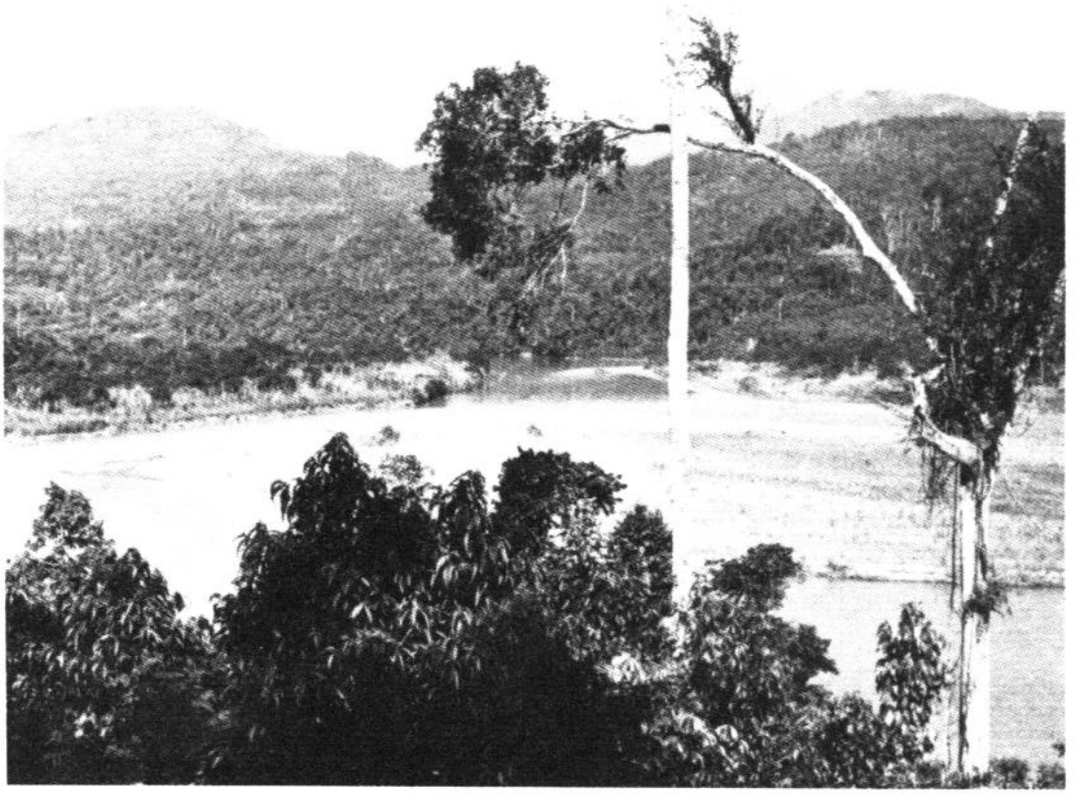
The region's fauna includes monkeys of the genera *Alouatta* and *Aotus* as well as small deer (*Mazama americana*), collared peccary (*Tayassu tajacu*), capybara (*Hydrochoerus hydrochaeris*), nine-banded armadillos (*Dasypus novemcinctus*), porcupines (*Coendou* sp.), agoutis (*Dasiprocta* sp.), and others.

The occurrence of the first cases of YF in this area is not documented, but local clinical-epidemiologic reports since 1963 and viscerotomy reports since 1977 confirm the presence of the disease *(9)*. In 1976 a YF control program was initiated in Peru, and virologic, serologic, and histopathologic studies were made of individuals with hemorrhagic and other illnesses compatible with YF.

Materials and Methods

During the period 16–24 February 1978 we observed patients attending the hospital at San Francisco, which we used as a temporary base of operations. The people of San Francisco and neighboring villages (all 650–750 m above sea level) are treated at this hospital. In the course of this nine-day period we observed four individuals with illnesses compatible with YF; two were febrile and two were gravely ill.

A view of the Apurímac River Valley in La Mar Province, Ayacucho Department, on the border of Cuzco Department.



Attempts were made in 1977, 1978, and 1981 to isolate virus from whole blood specimens obtained from these patients, from six patients ill in 1977 in Ayacucho, and from four patients ill in 1981 in Cuzco.[7] These virus isolation attempts were made by inoculating suckling mice intracranially with 0.03 ml of whole blood from the patient, and also by inoculating suckling mouse, $LLCMK_2$, Vero, and C6/36 (*Aedes albopictus* mosquito) cell cultures and examining the cultures for plaques or cytopathic effects. Isolated virus strains were identified by means of a serum dilution-plaque reduction neutralization (N) test *(10)* in Vero cells, or by a combination of this test and complement fixation *(11)*.

In addition, serum specimens were collected from 262 healthy individuals in areas where 29 cases of YF had been reported in 1977, and where cases were being reported in 1978. Of these subjects, 120 had been vaccinated with YF (17D strain) the previous year or before, 92 had not been vaccinated, and 50 had no known history of vaccination. These 262 sera were tested for neutralizing antibody to the 17D strain.

Results

During the 1978 outbreak, a total of 27 cases were diagnosed clinically, histopathologically, serologically, or by virus isolation. Clinically, the patients later shown by histopathologic lesions or serologic conversion[8] to have been infected with YF virus had a similar presentation and course of illness: sudden onset with a fever of about 39°C, severe headache, and icteric conjunctiva. Later, the symptoms became more acute with abdominal pain, marked

[7] From 1977 through 1981, local authorities reported a continuous seasonal occurrence of yellow fever in the departments of Cuzco and Ayacucho. Such occurrences were reported to reach epidemic proportions from time to time.

[8] At least a four-fold rise in the antibody titer found by the hemagglutination-inhibition or complement fixation test.

jaundice of the skin and mucous membranes, choluria, hypothermia, epistaxis, restlessness, and often petechiae on the abdomen and thorax. Terminally, the patients experienced agitation and disorientation, generalized hemorrhagic manifestations, melena, severe convulsions, and death.

Histopathologic observations were made on liver specimens obtained at autopsy from 17 cases. Findings from 14 of these can be described as classical for YF infection—with mid-zonal necrosis, microvacuolar fatty degeneration, the presence of Councilman bodies, conservation of lobular architecture, and moderate inflammatory infiltration of portal spaces. However, liver specimens from the three other patients showed diffuse necrosis, with normal cells (intercalated between necrotic cells and Councilman bodies) occurring into the periportal spaces and around the centrolobular vein.

Also, a bone marrow aspirate from one patient contained normal cells as well as many megakaryocytes showing hyaline changes with loss of the nucleus, simulating Councilman bodies. (This megakaryocyte necrosis was noteworthy, because the observed changes could relate to the thrombocytopenia associated with YF infection.)

None of the 27 patients had been vaccinated; all but one were males; and, with the exception of a seven-year-old student, all were agricultural workers from outside the immediate area of the outbreak. The 27 ranged in age from seven to 53 years, but most were between 15 and 40 years old (their average age was 26.9 years). The months of onset of their cases were January (two cases), February (nine cases), March (eight cases), April (two cases), May (one case), June (three cases), and July (two cases). Only two patients survived. Of the 25 who died, 23 died three to 10 days after the onset of their illnesses, the average survival time being 6.4 days.

Regarding virus isolations, YF virus was isolated from blood samples provided by three of the six 1977 Ayacucho patients. (These samples had been stored at – 70°C.) YF virus was also isolated from blood samples provided by one of the four 1978 Ayacucho patients and two of the four 1981 Cuzco patients, whose samples were also kept at – 70°C. In addition, as Table 1 indicates, the virus was isolated from sentinel mice exposed near Cuzco in 1981.

During primary virus isolation attempts, all seven of the above isolates caused cytopathic effects on days 6–8 in C6/36 cells, but only five of the seven killed suckling mice. Therefore, supernatant fluids from infected C6/36 cells were supplemented with 20% heat-inactivated fetal bovine serum, stored at – 70°C, and used as a source of seed viruses. These preparations were subsequently titrated by plaque assay in $LLCMK_2$ cells, using a double agar overlay technique, and were tested for virus identification with a serum dilution-plaque reduction neutralization test in $LLCMK_2$ cells against (1) hyperimmune mouse ascitic fluids for 10 flaviviruses, (2) a grouped mouse ascitic

Table 1. Isolations of yellow fever virus from human and sentinel mouse blood samples collected in Ayacucho and Cuzco, Peru, 1977–1981.

Source	Location	Age/sex	Time of illness onset or exposure	Blood sample collection date	Outcome
Ill patient	Ayacucho	26/M	6/11/77	6/13/77	Death
"	Ayacucho	20/M	6/13/77	6/13/77	"
"	Ayacucho	35/F	6/13/77	6/14/77	"
"	Ayacucho	19/M	2/22/78	2/22/78	"
"	Cuzco	19/M	5/01/81	5/03/81	Recovery
"	Cuzco	17/M	6/05/81	6/09/81	Recovery
Sentinel mice	Cuzco		6/02/81–6/08/81	6/12/81	

fluid for this group, and (3) suitable controls. The results of these tests are presented in Table 2.

Interestingly, when reisolation was attempted, the same observations were made with suckling mice and C6/36 cells, but no consistent pattern of cytopathic effects was observed with Vero or $LLCMK_2$ cells. Thus, C6/36 cell cultures were shown to be reliable indicators of the presence of the YF virus, but other systems were not. This supports and extends the findings of Varma et al. *(12)* using AP-61 (*Aedes pseudoscutellaris*) cells. After a single additional passage of the virus in suckling mice, however, patterns of virus replication were uniform.

Of the 262 individuals tested in the serologic survey, neutralizing (N) antibody ($\geq 1:10$) was detected in 106 of 120 vaccinated subjects, 61 of 92 unvaccinated subjects, and 42 of 50 subjects with an unknown vaccination history. Results of these serologic tests, by ten-year age group, are shown in Table 3. The observed antibody prevalence rates were high in all three groups, irrespective of the subjects' ages, but were of course slightly higher in the vaccinated group. The relatively high rates seen in the group with unknown vaccination status

Table 2. Results of serum dilution–plaque reduction neutralization (N) tests with six virus isolates from humans and one from sentinel mice. These viruses were tested against antibody preparations (hyperimmune mouse ascitic fluids) for the following flaviviruses: yellow fever (strain 788379), St. Louis encephalitis (TBH-28), Bussuquara (BeAn 4073), Naranjal (25008), Ilheus (original), Rocio (SPH 34675), Aroa (VENA-1809), dengue-1 (Hawaii), dengue-2 (TR 1751), and dengue-3 (H-87). Grouped mouse ascitic fluid and suitable controls were also tested. The Bussuquara, Ilheus, Aroa, dengue-1, and dengue-3 ascitic fluids yielded titers of 640, 640, ≥10, 240, 640, and 1,280, respectively, when tested with homologous virus; however, all the tests conducted with our sera yielded titers below 20.

Virus isolate	Source	Date of serum collection	Primary isolation in:		Titer of N antibody to:				
			Suckling mice	C6/36 cells	YF	SLE	Naranjal	Rocio	Dengue-2
1362/77	Ill patient	6/13/77	+	+	5,120	20	<20	40	<20
1368/77	"	6/13/77	–	+	≥2,560	80	<20	<20	<20
1371/77	"	6/14/77	–	+	1,280	40	20	40	20
287/78	"	2/22/78	+	+	≥2,560	40	<20	<20	<20
1896/81	"	5/03/81	+	+	5,120	40	<20	<20	<20
1899/81	"	6/09/81	+	+	≥2,560	20	<20	<20	<20
1914/81 (Homologous)	Sentinel mice	6/12/81	+	+	≥2,560 (10,240)	40 (640)	<20 (640)	20 (640)	<20 (640)

Table 3. Results of serum dilution–plaque reduction neutralization tests for YF antibody in 262 individuals, by history of vaccination with yellow fever (17D strain) and age group.

Age of subjects (in years)	Vaccinated			Unvaccinated			Vaccination status unknown		
	Antibody present	% positive	No. tested	Antibody present	% positive	No. tested	Antibody present	% positive	No. tested
0–10	5	83	6	2	100	2	3	100	3
11–20	29	83	35	27	59	46	15	79	19
21–30	27	90	30	14	78	18	9	82	11
31–40	18	90	20	8	67	12	7	100	7
41–50	13	87	15	6	86	7	3	100	3
51–60	5	100	5	4	67	6	2	67	3
≥61	7	100	7	0	0	1	2	67	3
Unknown	2	100	2	0	-	0	1	100	3
									1
Total	106	88.3	120	61	66.3	92	42	84.0	50

suggest that most of this group's members had been vaccinated, since the prevalence rates were comparable to those found in the vaccinated group.

Discussion and Conclusions

Tabulations of reported sylvatic YF cases in Peru during 1976–1980 (Table 4) suggest that, while this disease may occur periodically in epidemic form, its presence in the country is endemic. Since essentially all individuals with YF in Ayacucho in 1978 were newly arrived migrants, and since the prevalence of neutralizing antibody in permanent residents was high, it appears that the endemic presence of YF combined with an active vaccination campaign may have excluded nearly all but recent arrivals from the population at risk.

The migrant workers in this region generally come from high mountain areas where YF virus is not known to occur. Morales-Ayala *(5)* has suggested that immigration of susceptible agricultural workers from the high sierra to the high forest areas of Peru endemic for YF may be responsible for the annual occurrence of the disease in humans, if not for periodic outbreaks. Also, the focus of YF activity in Ayacucho Department may have been associated epidemiologically with endemic areas in neighboring valleys.

Working with population data from the Peruvian Ministry of Health's Office of Statistics, it appears that there were 74.55 YF cases per 100,000 population (29 reported cases and 29 deaths) in villages of the Apurímac River Valley in 1977, 68.47 cases per 100,000 in 1978 (27 reported cases and 25 deaths), and 15 cases per 100,000 in 1979 (six reported cases and six deaths). However, if only the migrant workers (with an estimated yearly population of 10,000) are considered to constitute the group at risk, then the case rates are much higher (290, 270, and 60 cases per 100,000 in 1977, 1978, and 1979, respectively); and the average migrant's chance of becoming ill and dying of yellow fever in those years was one in 345, one in 400, and one in 1,667, respectively.

Table 4. Reported cases of human yellow fever in rural Peru, 1976–1980.

Departments	No. of cases					
	1976	1977	1978	1979	1980	Total
Loreto			1	2	1	4
San Martín	1	19	8	43	8	79
Junín		24	57	31	10	122
Apurimac		29	27	6	11	73
Madre de Dios and Puno		10		15		25
Total reported cases	1	82	93	97	30	303

Source Records of the National Institute of Health, Lima.

Fortunately, the Peruvian Government has begun to take steps to prevent such occurrences in the future by initiating mass vaccination campaigns in the affected areas. However, the difficulties involved in recognizing mild YF cases tend to result in underreporting and complicate prevention. Indeed, the exceedingly high mortality reported here may be a reflection of underdiagnosis.

Although no attempts were made to isolate virus from mosquitoes in the area at the time of the outbreak, a wide variety of mosquitoes—including *Haemagogus capricornii*, a recognized YF vector in Amazonia *(13)*—were reported present in areas neighboring local coffee plantations in 1977 *(14)*. Using humans as attractants, we were able to collect four species of *Anopheles*, three of *Aedes*, two of *Psorophora*, and one each of *Mansonia*, *Sabethes*, and *Culex* in the hills neighboring the coffee plantations of four towns.

Finally, this is the first report of YF virus being isolated in Peru. Evidence pointing to the presence of the virus has existed for more than 40 years, however, and many other viruses—including Group A (Venezuelan equine encephalitis and eastern equine encephalitis), Group B (St. Louis encephalitis), Guama serogroup (Bimiti), and Group C (Marituba) arboviruses—have been isolated in Peru from equine animals, humans, mosquitoes, and sentinel hamsters *(15, 16)*. It is therefore hoped

that the study reported here, in combination with earlier works, will help call attention to a serious and continuing disease problem in rural Peru.

SUMMARY

Beginning in 1977, an extensive outbreak of hemorrhagic disease compatible with yellow fever (YF) occurred in southern Peru; and despite a massive vaccination effort, recurrent outbreaks occurred in subsequent years. Epidemiologic circumstances associated with the outbreaks suggest that a major contributing factor was annual reintroduction of susceptible migrant workers into an enzootic focus of the disease.

In the course of studying these outbreaks, the authors attempted to isolate YF virus from blood specimens provided by four 1977 patients, six 1978 patients, and four 1981 patients from the endemic zone. For this purpose, whole blood from the patients was inoculated into suckling mice (intracranially) and also into C6/36 (*Aedes albopictus*), suckling mouse, $LLCMK_2$, and Vero cell cultures. Virus strains isolated from six of these patients were subsequently identified as YF, the first time this virus has been isolated in Peru.

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