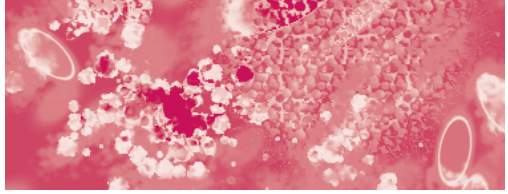

Yellow fever: an update

Thomas P Monath

Yellow fever, the original viral haemorrhagic fever, was one of the most feared lethal diseases before the development of an effective vaccine. Today the disease still affects as many as 200 000 persons annually in tropical regions of Africa and South America, and poses a significant hazard to unvaccinated travellers to these areas. Yellow fever is transmitted in a cycle involving monkeys and mosquitoes, but human beings can also serve as the viraemic host for mosquito infection. Recent increases in the density and distribution of the urban mosquito vector, *Aedes aegypti*, as well as the rise in air travel increase the risk of introduction and spread of yellow fever to North and Central America, the Caribbean and Asia. Here I review the clinical features of the disease, its pathogenesis and pathophysiology. The disease mechanisms are poorly understood and have not been the subject of modern clinical research. Since there is no specific treatment, and management of patients with the disease is extremely problematic, the emphasis is on preventative vaccination. As a zoonosis, yellow fever cannot be eradicated, but reduction of the human disease burden is achievable through routine childhood vaccination in endemic countries, with a low cost for the benefits obtained. The biological characteristics, safety, and efficacy of live attenuated, yellow fever 17D vaccine are reviewed. New applications of yellow fever 17D virus as a vector for foreign genes hold considerable promise as a means of developing new vaccines against other viruses, and possibly against cancers.

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Yellow fever is the original viral haemorrhagic fever (VHF), a pansystemic viral sepsis with viraemia, fever, prostration, hepatic, renal, and myocardial injury, haemorrhage, shock, and high lethality. In recent years, popular attention has been drawn to another VHF—Ebola—as the most frightening emerging infection of humankind. However, patients with yellow fever suffer as terrifying and untreatable a clinical disease, and yellow fever is responsible for 1000-fold more illness and death than Ebola. Yellow fever stands apart from Ebola and other VHFs in its severity of hepatic injury and the universal appearance of jaundice.

Yellow fever virus is the prototype of the genus Flavivirus (family Flaviviridae) which comprises approximately 70 viruses, most of which are arthropod-borne. The earliest description of yellow fever is found in a Mayan manuscript in 1648, but by genome sequence analysis it appears that yellow fever virus evolved from other mosquito-borne viruses about 3000 years ago,[1] probably in Africa from where it was imported to the New World during the slave trade. Yellow fever was a major scourge in the 18th and 19th centuries in colonial settlements in the Americas and west Africa. The discoveries (in 1900) that mosquitoes were responsible for transmission and that the disease was preventable by vector control, as well as the development of vaccines (in the 1930s), have reduced both the fear associated with the disease and its medical impact. However, yellow fever remains an endemic and epidemic disease problem affecting thousands of people in tropical Africa and South America,[2] and is a continued threat to people who travel to these regions without vaccination.

Causative agent

Flaviviruses are positive-sense, single-stranded RNA viruses—obligate intracellular pathogens that replicate in the cytoplasm of infected cells. The yellow fever genome contains a single open-reading frame of 10 233 nucleotides encoding three structural and seven nonstructural (NS) proteins, flanked by short non-coding regions.[3] The structural proteins are incorporated in released mature virus particles, while the NS proteins responsible for replication remain in infected cells. The viral envelope consists of a lipid bilayer derived from the infected cell, with dimers of the envelope (E) protein on the surface anchored at their hydrophobic tails. The E protein is responsible for the initial phases of infection of host cells and is also a principal target for the host's immune response. It contains an Arg-Gly-Asp (RGD) sequence involved in both attachment to glycosaminoglycan receptors (eg, heparan sulfate) on cell membranes and internalisation of the virus by membrane fusion. Antibodies to epitopes on the E protein interfere with these functions. Other viral proteins of major biological significance are NS1 and NS3. These proteins are associated with the infected cell, where they are targets for immune elimination. Antibodies to NS1 bind complement and contribute to protective immunity by lysing infected cells. NS3 is a target for attack by cytotoxic T cells (figure 1).

Yellow fever virus is a single serotype. At the sequence level, five genotypes can be distinguished (three in Africa, and two in South America).

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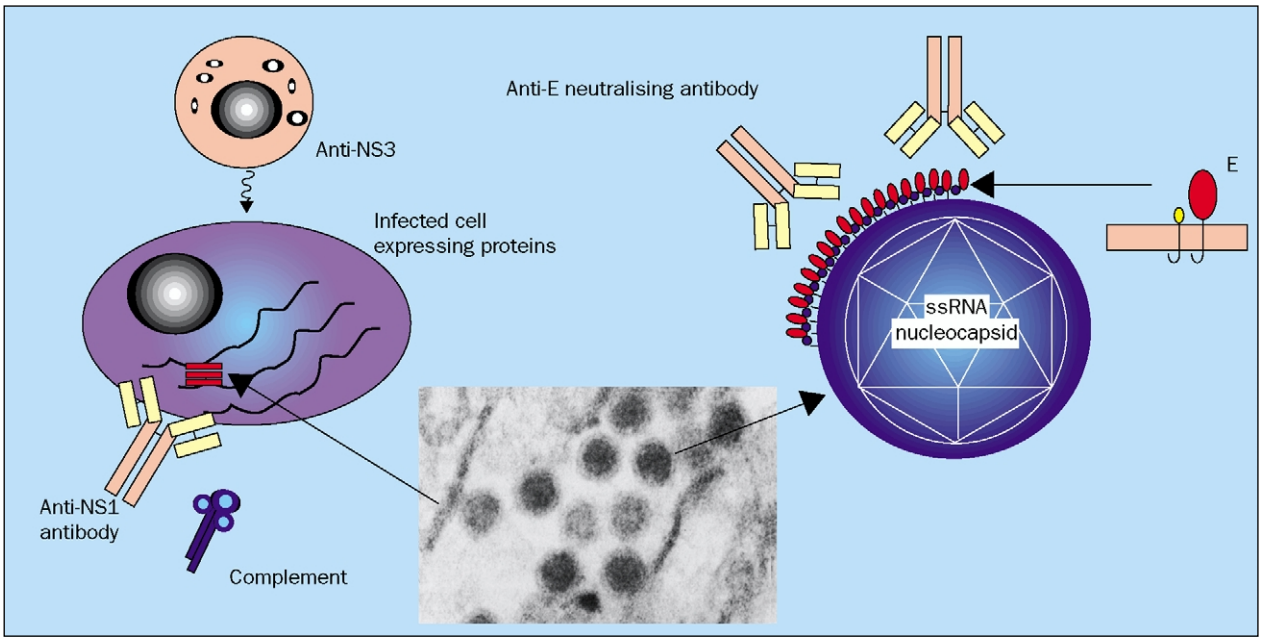

Figure 1. Role of yellow fever proteins in provoking the infected host's immune response and as targets for immunity. Electron micrograph shows yellow fever virus particles in the endoplasmic reticule of an infected cell. The E glycoprotein is the major component of the viral coat and contains epitopes responsible for neutralisation; neutralising antibodies against E are the principal mediators of protection against reinfection. The non-structural (NS) proteins are expressed in infected cells. NS3 (and other NS proteins, as well as the E protein) elicit cytotoxic T cells, which in turn clear infected cells during recovery from infection. The NS1 protein, found both in cells and as a secreted form, stimulates cytotoxic antibodies, which bind complement and are involved in viral clearance and recovery from infection. Photomicrograph shows yellow fever virus particles in endoplasmic reticulum, magnification x280 000.

Incidence and epidemiology

Yellow fever occurs in tropical regions of Africa and South America (figure 2). Fortunately, the virus has never emerged in Asia, and vaccination for travel is not indicated here. Asia is considered vulnerable to the future introduction of the virus, due to the presence of a large susceptible human population and presence of the urban vector, *Aedes aegypti*. Possible explanations for absence of the disease in Asia include cross-protection afforded by hyperendemic dengue, low competence of local populations of *Ae aegypti*, and occurrence of yellow fever in remote areas in people who do not travel by air and are unlikely to spread the infection.

Up to 5000 cases in Africa and 300 in South America are reported annually, but the true incidence is believed to be 10–50 fold higher than the official reports. Between 1990 and 1999, 11 297 cases and 2648 deaths were reported in Africa (figure 2). The largest number of cases was in Nigeria, which suffered a series of epidemics between 1986 and 1994. Epidemics have also occurred in Cameroon (1990), Ghana (1993–1994, 1996), Liberia (1995, 1998), Gabon (1994), Senegal (1995, 1996), Benin (1996), and Kenya (1992). An epidemic is currently occurring along the border of Liberia and Guinea, an area torn by war with disruption of vaccination and medical services. During epidemics in Africa, the incidence of infection may be as high as 20% and the incidence of disease 3%. In South America, yellow fever occurs principally in the Amazon region and contiguous grasslands. Between 1990 and 1999, 1939 cases and 941 deaths were reported. Peru and Bolivia had the highest incidence, reflecting low vaccination coverage.

In Africa, where the human population is seasonally exposed in and around villages, children without naturally acquired immunity are at highest risk of disease and there is a slight excess of cases in males. In South America, where the virus is transmitted in sparsely populated forested areas, it principally affects men engaged in lumbering or clearing land for agriculture.

Transmission cycle

Yellow fever is a zoonotic infection, maintained in nature by wild non-human primates and diurnally active mosquitoes that breed in tree holes in the forest canopy (*Haemagogus* spp in the Americas, and *Aedes* spp in Africa; figure 3). People are sporadically exposed to infected mosquitoes when they encroach on this cycle during occupational or recreational activities ("jungle yellow fever"). In the moist savanna regions of Africa (the so called "zone of emergence"),[4] tree-hole-breeding *Aedes* species mosquitoes reach very high densities and are implicated in endemic and epidemic transmission, transferring virus from monkey to people and between people. *Aedes aegypti*, a domestic mosquito that breeds in man-made containers, may transmit yellow fever virus between human beings ("urban yellow fever"). The virus is maintained over the dry season by vertical transmission in mosquitoes. Ova containing virus survive in dry tree-holes and hatch infectious progeny mosquitoes when the rains resume.

The disease

Despite intense study, relatively little is known about the disease beyond purely descriptive accounts. In part, this is

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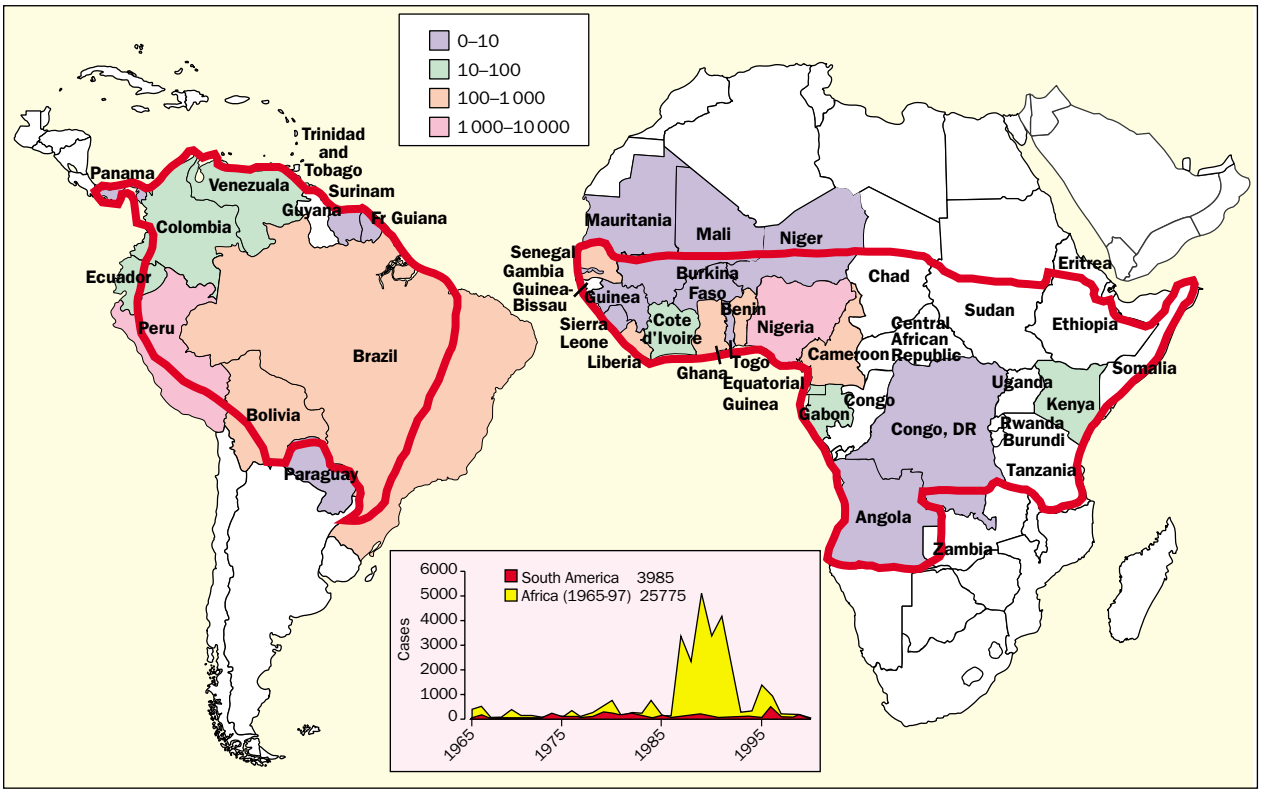

Figure 2. Yellow fever endemic regions (outlined in red), based on serological surveys, field studies, and previous reports of human disease. The range of number of cases of yellow fever officially reported to the World Health Organization, 1990–99, is shown by country. White indicates that no cases were reported. Inset: incidence of yellow fever in South America and Africa over the past 35 years, and the marked increase during the late 1980s–mid 1990s. Regions of the world outside the yellow fever endemic zone infested with Ae aegypti and thus receptive to the introduction and spread of the disease include coastal areas of South America, Central America, the Caribbean, the southern USA, South Africa, India, southeast Asia, Australia (Queensland), southern China, Taiwan, and the Pacific islands.

due to the occurrence of the disease in remote areas without access to sophisticated medical care. Although the human disease can be modelled quite precisely in non-human primates, virtually no research on its pathogenesis has been conducted in the past 20 years.

The clinical disease varies from non-specific, abortive illness to fatal haemorrhagic fever.[5] The incubation period after the bite of an infected mosquito is 3–6 days. Disease onset is typically abrupt, with fever, chills, malaise, headache, lower back pain, generalised myalgia, nausea, and dizziness (figure 4). On physical examination the patient is febrile and appears acutely ill, with congestion of the conjunctivae and face (figure 5) and a relative bradycardia with respect to the height of fever (Faget's sign). Virus is present in blood at titres up to 10^5–10^6 infectious particles/mL, and the patient may thus serve as a source of infection for mosquitoes. The average fever is 39° C and lasts 3·3 days. Young children may experience febrile convulsions. Laboratory abnormalities include leukopenia ($1·5$–$2·5\times10^9$/L) with a relative neutropenia. Between 48 and 72 h after onset and before the appearance of jaundice, serum transaminase levels may rise. This so-called "period of infection" lasts several days and may be followed by a "period of remission", with the disappearance of fever and symptoms lasting up to 24 h (figure 4). During the period of remission, virus is cleared by antibodies and the cellular immune response. The blood may contain non-infectious immune complexes detectable by immunoassays or PCR. Patients with abortive infections may recover at this stage, without further signs or symptoms.

In approximately 15–25% of people affected, the illness reappears in a more severe form (the so-called "period of intoxication") with fever, vomiting, epigastric pain, jaundice, renal failure, and a haemorrhagic diathesis (figure 4). Serum transaminase levels rise and jaundice deepens, the direct bilirubin concentrations reaching 171–257 μmol/L. Interestingly, serum aspartate aminotransferase (AST) concentrations often exceed alanine aminotransferase (ALT), presumably due to direct viral injury to myocardium and skeletal muscle. Serum transaminase levels reflect disease severity. In one study, AST and ALT concentrations were 2766 IU/L and 660 IU/L, respectively, in fatal cases, while in survivors with jaundice the mean concentrations were 929 IU/L and 351 IU/L, respectively.[6] Protein concentrations in urine range between 3 and 20 g/L, urine volume diminishes, and serum creatinine rises to 265–1061 μmol/L. Haemorrhagic manifestations include petechiae, ecchymoses, epistaxis, and oozing of blood from the gums and at needle puncture sites. In many cases there is major bleeding, coffee-grounds haematemesis, melaena, or metrorrhagia. Laboratory abnormalities include thrombocytopenia, prolonged clotting and prothrombin times, reduced fibrinogen and factors II, V, VII, VIII, IX, and X, and the presence of fibrin split products.[7] These abnormalities suggest

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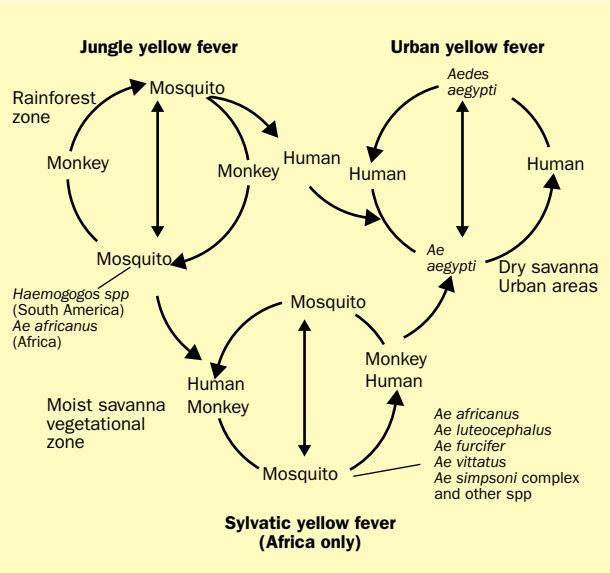

Figure 3. The transmission cycles of yellow fever. The virus is maintained by transmission between monkeys and tree-hole breeding mosquitoes. Human beings acquire "jungle yellow fever" when exposed to the bite of mosquitoes that have previously fed on an infected monkey. The vectors and ecology differ in Africa and South America. In Africa, tree-hole breeding Aedes spp reach high densities in the moist savanna vegetational zone and transmit the virus between people. In both continents, Ae aegypti, which breeds in and around houses in man-made containers, is responsible for interhuman transmission of "urban" yellow fever virus.

a multifactorial bleeding disorder caused by reduced synthesis of clotting factors and consumption coagulopathy. Platelet dysfunction, demonstrated by collagen and ADP stimulated aggregation, has been demonstrated in the monkey model. Myocardial injury is manifest by ST-T wave abnormalities on the electrocardiogram, and occasionally by acute cardiac enlargement.

20–50% of patients with hepatorenal disease die, typically 7–10 days after onset. Events preceding death include hypotension—an increasingly difficult symptom to manage with fluids and vasopressors. Patients also experience agitated delirium, stupor, coma, Cheyne-Stokes respirations, metabolic acidosis, hyperkalaemia, hypoglycaemia, and hypothermia. The cerebrospinal fluid is under increased pressure, with raised albumin but no increase in white blood cells, which is consistent with cerebral oedema. Pathological changes in the brain include microscopic perivascular haemorrhages and oedema.[8] True yellow fever viral encephalitis due to viral infection of brain tissue (as opposed to encephalopathy) is very rare.

In a study of 103 patients, the average stay in hospital for surviving patients was 14 days (range 5–42 days) and the average duration of acute illness was 17·8 days.[9] Patients surviving the acute illness may have superimposed bacterial sepsis or pneumonia, or require dialysis to manage renal tubular necrosis. The convalescent phase is characterised by prolonged weakness and fatigue lasting several weeks. Deaths occurring weeks after recovery have been described, possibly caused by cardiac arrhythmia, but this complication is not well documented. The duration of jaundice in survivors is unknown, but abnormal liver function tests have been found 2 months or more after the onset of recovery. Healing of the liver and kidneys is complete without post-necrotic scarring. Chronic hepatitis B, which is widely prevalent in the yellow fever endemic zone, does not appear to modify the illness or the recovery from yellow fever.

Host range, pathogenesis, and pathophysiology

Current knowledge about the pathogenesis of human yellow fever is derived principally from studies of experimentally-induced disease in non-human primates. Monkeys display the "viscerotropic" properties of yellow fever virus, including replication in and injury to liver, spleen, heart, and kidneys. The only non-primate species that develop viscerotropic infection after experimental infection are the European hedgehog (an insectivore) and laboratory hamsters given virus that has been adapted by serial passage in this species.[10] In rodents (mice, hamsters, and guineapigs), the unadapted, wild-type virus causes encephalitis. Indeed, the wild-type virus must have a relatively low inherent neuroinvasiveness and neurovirulence in human beings, since encephalitis is not a complication of viraemic infection without the hepatic syndrome. The neurotropism of yellow fever is, however, manifest in very young human infants who occasionally develop encephalitis after vaccination with yellow fever vaccine.

The extreme lethality of yellow fever virus is evident when one considers that the 50% lethal dose for monkeys is less than 1 plaque forming unit. Fixed macrophages (Kupffer cells) in the liver are infected 24 hours after inoculation. Infection of the kidney, bone marrow, spleen and lymph nodes follow.[11,12] Infection and degeneration of hepatocytes is a relatively late event, occurring in the last 24–48 h before death in the monkey[12,13] and in the last phase of infection in people (figure 6). A unique feature of yellow fever hepatic injury is its mid-zonal distribution, with sparing of cells around the central vein and portal tracts (figure 7). This distribution of hepatic lesions in yellow fever might simply reflect low-flow hypoxia due to terminal shock. However, yellow fever virus antigen and RNA have been observed principally in hepatocytes in the midzone,[14] indicating that these cells are most susceptible to virus replication. The infected hepatocyte undergoes eosinophilic degeneration with condensed nuclear chromatin (Councilman bodies) typical of apoptotic cell death and distinct from the ballooning and rarefaction necrosis seen in viral hepatitis. Apoptosis has been documented as a late event in human hepatoma cells infected with yellow fever virus.[15] Hepatic injury from apoptosis rather than necrosis explains the virtual absence of inflammatory cells in yellow fever, preservation of the reticulin framework, and healing without fibrosis.

The renal pathology is characterised by eosinophilic degeneration and fatty change of tubular epithelium, once again without inflammation. Yellow fever antigen is found in renal tubular cells of fatal human cases, suggesting that direct viral injury has a role. However, in the monkey model, renal tubular function was maintained during the course of the illness. Oliguria was shown to be caused by pre-renal

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failure associated with hypotension, and acute tubular necrosis was a terminal event.[13] The marked albuminuria in yellow fever may be due to alteration of glomerular function, since histological changes are observed in basement membrane and cells lining Bowman's capsule and yellow fever antigen is present in glomerulae 2–3 days after infection of monkeys. Necrosis of germinal centres of spleen, lymph nodes, tonsils, and Peyer's patches has been noted in monkeys and human beings,[13] but it is uncertain whether this is due to viral injury or depletion by corticoid-induced stress.

Hypotension and shock in the late stage of illness are probably mediated by cytokine dysregulation, as in other VHFs and bacterial sepsis. Tumour necrosis factor (TNF)α and other cytokines produced by infected/activated Kupffer cells and splenic macrophages in response to direct virus injury and cytotoxic T cells involved in viral clearance might be responsible for cell injury, oxygen free radical formation, endothelial damage and microthrombosis, disseminated intravascular coagulation, tissue anoxia, oliguria, and shock. It is noteworthy that the dramatic pathophysiological events are initiated at the stage when immune clearance of yellow fever virus infected cells—ie, at the inception of the "period of intoxication". Future studies of patients or experimentally infected monkeys are required to define the cytokine mediators of the shock syndrome. Direct viral injury to myocardial fibres, which contain viral antigen and show changes consistent with apoptosis, may contribute to shock.

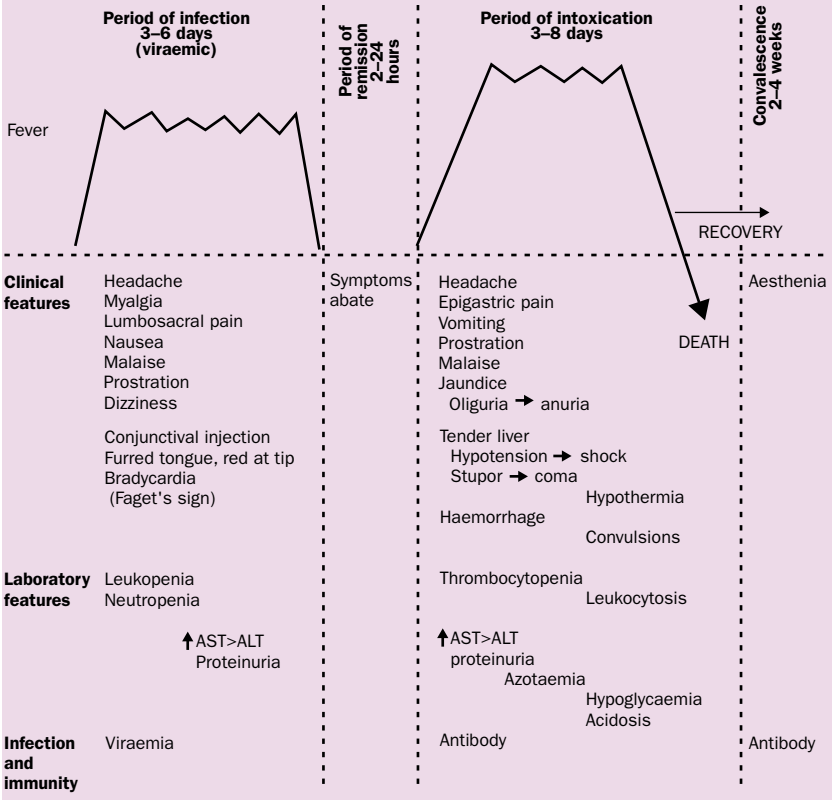

Figure 4. Stages of yellow fever infection, showing the major clinical and laboratory features of the disease.

Immune response

Infection with yellow fever virus is followed by a rapid specific immune response. Only the humoral response has been characterised. IgM antibodies measured by ELISA appear during the first week of illness, peak during the second week, and generally decline rapidly over several months. Neutralising antibodies appear rapidly, towards the end of the first week after onset day of illness and persist for many years. Neutralising antibodies are the principal mediators of protection against disease on re-exposure to the virus. No documented second case of clinical yellow fever infection has been reported. Previous infection with certain heterologous flaviviruses appears to modulate disease expression and severity of yellow fever. This effect is dependent on the specific virus causing prior infection. The evidence suggests that dengue infection in particular, but also some African flaviviruses (eg, Zika and Wesselsbron), may partly cross-protect against yellow fever.

Diagnosis

The full-blown disease in an unvaccinated patient with a history of exposure in the yellow fever endemic zone, presents little difficulty in clinical differentiation. Diseases most closely mimicking yellow fever are leptospirosis and louse-borne relapsing fever (*Borrelia recurrentis*), which are also characterised by jaundice, haemorrhage, disseminated intravascular coagulation, and a high case-fatality rate. Other diseases that must be differentiated include viral hepatitis (especially severe hepatitis E in pregnancy and delta hepatitis), and severe malaria (blackwater fever). Other VHFs are not usually associated

Figure 5. Yellow fever patient during the period of infection. The patient is febrile and acutely ill, with prominent conjunctival congestion. During this pre-icteric phase, the illness is difficult to differentiate from many other infectious diseases. Virus is present in the blood and the patient is infectious for blood-feeding mosquitoes.

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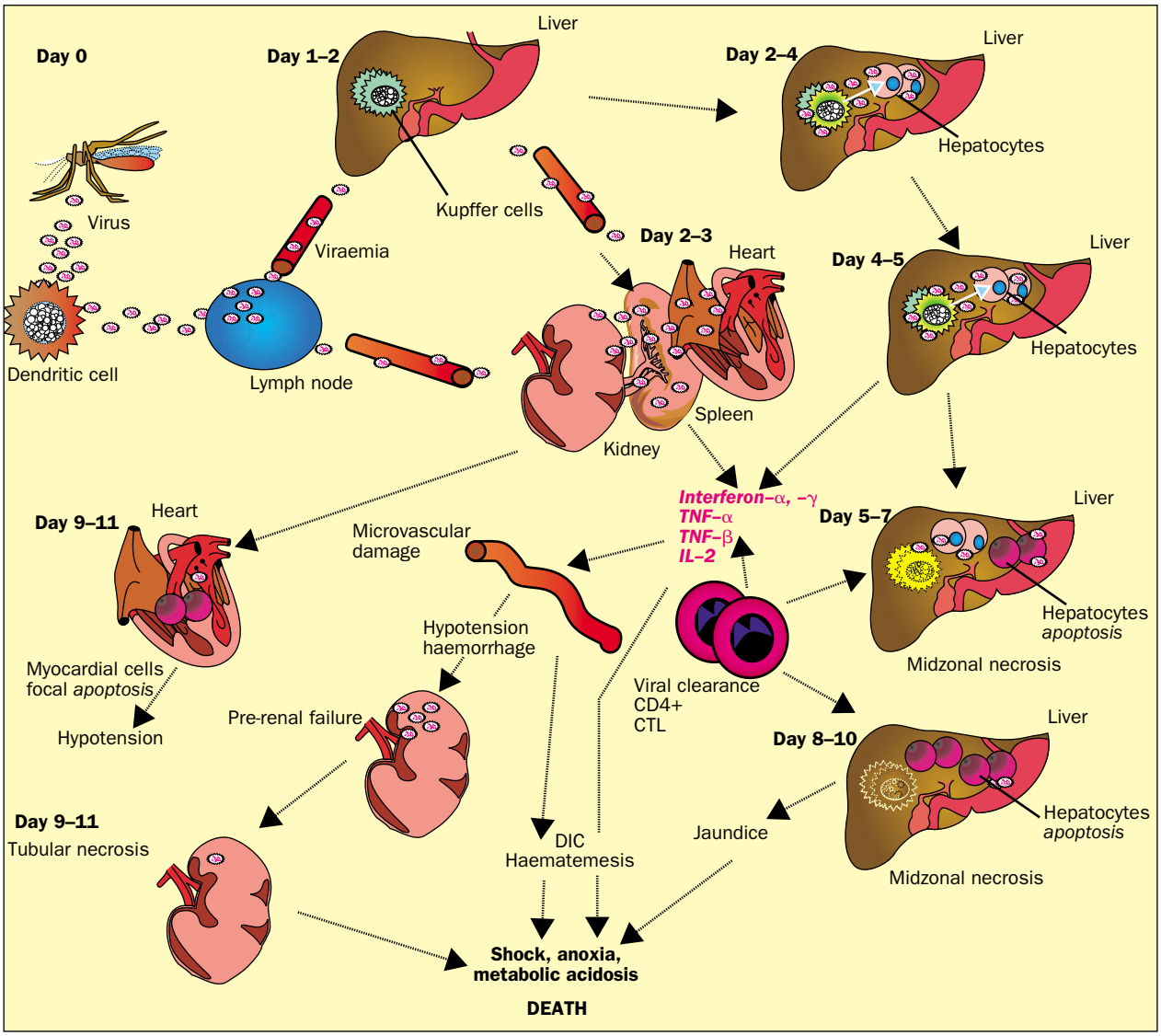

Figure 6. Pathogenesis of yellow fever based on studies in experimentally infected monkeys and human case reports (bold). Speculative mechanisms shown in italics are drawn from in-vitro data or reports on other flavivirus infections. CTL=cytotoxic T lymphocyte, DIC=disseminated intravascular coagulation, IL=interleukin.

with jaundice, but dengue, and Congo-Crimean haemorhagic fever may occasionally present with features resembling yellow fever.

Specific laboratory diagnosis relies on detection of virus or viral antigen in blood during the pre-icteric phase or by serology. No commercial test is available and diagnostic capabilities reside solely in specialised and referral laboratories. The virus may be isolated by intracerebral inoculation of suckling mice, intrathoracic inoculation of mosquitoes, or inoculation of cell cultures. *Ae pseudoscutellaris* (AP61) cells are most sensitive, but mammalian cells (eg, Vero, SW13, BHK-21) may be used, particularly if combined with PCR or detection of viral antigen by immunostaining. PCR has been used to detect viral genome in clinical samples that were negative by virus isolation[16] and may be the method of choice. Rapid, early diagnosis is also possible by measurement of yellow fever antigen in serum by a monoclonal ELISA. The sensitivity of the PCR and ELISA assays for detection of virus in clinical samples is approximately 2–3 $\log_{10}$ plaque forming units (PFU)/0·1 mL. Under field conditions, antigen-detection ELISA had a sensitivity of 69% and specificity of 100% compared with virus isolation in AP61 cell culture.[17]

Serologic diagnosis is accomplished principally by measurement of IgM antibodies by ELISA. Other serological tests may also be used, including hemagglutination-inhibition and neutralisation. The presence of IgM antibodies in a single serum taken in the late acute or early convalescent phase provides a presumptive diagnosis, and demonstration of a rising titre in paired sera is confirmatory. Cross-reactions between yellow fever and other flaviviruses may complicate serological diagnosis, particularly in individuals who inhabit regions where multiple flaviviruses are endemic.

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Examination of liver reveals the typical midzonal necrosis (figure 7) and viral antigen by immunohistochemistry. Liver biopsy premortem should never be performed, as fatal haemorrhage may ensue.

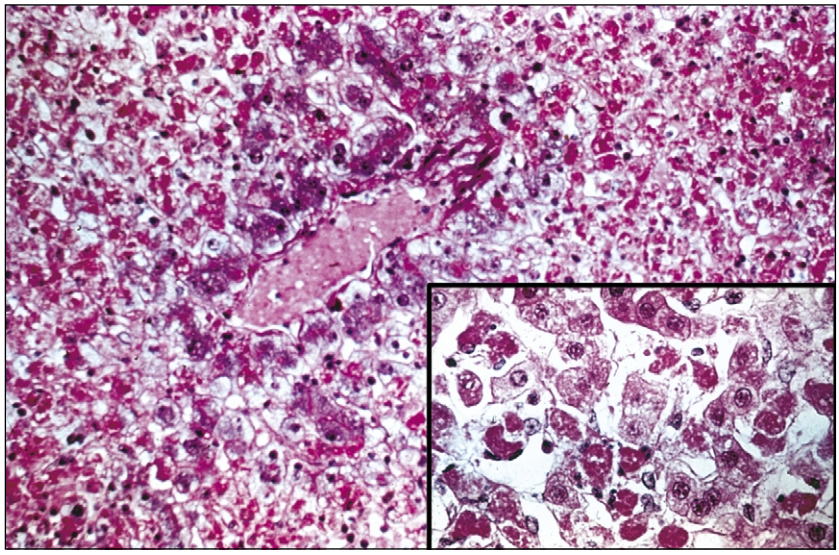

Figure 7. Histopathological features of yellow fever infection of the liver. The lesion is mid-zonal in distribution with sparing of cells around the central vein. There are microvesicular fatty changes but little inflammatory response. Inset: eosinophilic degeneration of hepatocytes (Councilman bodies) reflects apoptotic cell death.

Treatment

There is no specific antiviral treatment. Ribavirin failed in several studies in the monkey model. Passive antibody, the interferon inducer poly(I-poly(C), or interferon-γ are effective only before or within hours after infection, and these have no value except for early post-exposure prophylaxis in an individual (eg, laboratory worker) with known exposure. Corticosteroids have not been evaluated in treatment of yellow fever. Intensive supportive care may not rescue the patient with yellow fever from the inexorable course of fatal infection. Some years ago, an expert panel[18] recommended the following: maintenance of nutrition and prevention of hypoglycaemia; nasogastric suction to prevent gastric distension and aspiration; intravenous cimetidine to prevent gastric bleeding; treatment of hypotension by fluid replacement and vasoactive drugs (dopamine); administration of oxygen; correction of metabolic acidosis; treatment of bleeding with fresh-frozen plasma; dialysis if indicated by renal failure; and treatment of secondary infections with antibiotics. These common-sense recommendations have not been subsequently modified, and no clinical studies to assess their value have been performed. It is noteworthy that, unlike dengue haemorrhagic fever, patients with yellow fever do not respond dramatically to fluid replacement, indicating the irreversible nature of pathophysiological perturbations.

Prevention of yellow fever

A certificate of vaccination is required under the International Health Regulations for entry into yellow fever endemic countries or travel from endemic countries to *Ae aegypti*-infested countries at risk of introduction. Information on vaccination requirements can be obtained from travel clinics and the CDC website (www.cdc.gov/travel/reference.htm). Despite the legal requirement for a valid certificate, enforcement is lax in many countries.

Yellow fever 17D is a highly effective, well-tolerated live, attenuated vaccine that has been used for over 60 years in approximately 400 million people. Routine use of the vaccine in children in endemic countries has a favourable cost-benefit ratio.[19]

Yellow fever vaccine was developed by empirical passage principally in chicken embryo tissue, resulting in multiple mutations in the viral structural and non-structural genes.[20,21] The vaccine is produced from embryonated eggs, and egg-allergic patients should not be immunised or should be skin tested and desensitised. There are eight vaccine manufacturers (located in the UK, Germany, France, USA, Brazil, Senegal, Russia, and Colombia). Vaccine coverage is better in endemic regions of South America (80–90%) than in Africa, where coverage is 1–40% in most countries. Nevertheless, demands for the vaccine have increased as it is introduced into routine childhood immunisation programmes. Current vaccine supplies are marginal, emphasising the vulnerability to an unexpected emergency such as the occurrence of urban yellow fever in coastal Brazil, the USA, or Asia.

Protective levels of neutralising antibody are found in 90% of vaccinees within 10 days and in 99% within 30 days. Immunity is very durable, probably providing lifelong protection after a single dose; to be conservative, revaccination after 10 years is required under International Health Regulations for a valid travel certificate. The vaccine may be simultaneously administered with measles, polio, DPT, hepatitis B, hepatitis A, oral cholera, and oral or parenteral typhoid.[21] The vaccine is very well tolerated; in practice few patients complain of side effects. In clinical trials, where symptoms have been solicited, common adverse events include injection site pain or redness, headache, malaise, and myalgia within a few days of vaccination in approximately 20–25% of vaccinees. These symptoms are mild and do not interfere with activities. The systemic symptoms may be due to interferon or TNFα[22] provoked by viral replication. During the first few days after vaccination, low titres of virus (not exceeding 2 $\log_{10}$ PFU) are found in blood, and markers of immune stimulation (neopterin, β2 microglobulin, circulating CD8+ cells) appear.[23] Vaccinated people cannot serve as a source of infection for mosquitoes, both because the attenuated 17D virus has lost the capacity to infect vectors and, in any event, because the viraemia is low

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Severe or serious adverse reactions to 17D vaccine are extremely rare. Post-vaccinal encephalitis (due to invasion of the brain by the vaccine virus) has long been recognised as a rare complication related to use of the vaccine in very young infants. 18 of the 21 reported encephalitis cases were in children, of whom 16 were under 7 months. Virus recovered from the brain of the single reported fatal case contained two aminoacid changes in the E gene and exhibited increased neurovirulence in animals.[24] It is unknown whether the other cases were due to mutations in the vaccine virus. Because of the vulnerability of very young infants, yellow fever vaccine is not recommended for infants under 9 months and is contraindicated under 6 months of age. Anaphylactic reactions to yellow fever vaccine occur at a frequency of approximately 1/58 000 and may be due to sensitivity to gelatin used to stabilise the vaccine.[25]

A recent study in the USA found a higher frequency of severe adverse events in elderly people, with those older 75 years having a risk 12 times higher than young adults.[26] The adverse experiences included multisystem and neurological incidents. Four deaths were recognised with liver and kidney dysfunction and shock. In Brazil in 1999–2000, two deaths in a child and a young adult were linked to yellow fever vaccination.[27] These deaths occurred during a period of mass immunisation in which 34 million people were vaccinated. Two other suspect cases in Brazil and another case in Australia[28] have been described. In these patients, the clinical presentation and liver histopathology resembled that caused by wild-type yellow fever virus. There was no evidence for immune suppression or other known risk factors for enhanced infection. Sequencing of virus isolates failed to show mutations that could explain the virulent presentation. Thus, it appears that yellow fever 17D vaccine can cause viscerotropic lethal infection similar to yellow fever disease. This seems to reflect atypical host response rather than genome instability of the vaccine. The frequency of this complication remains uncertain, but may be less than one in a million.

Immunisation is contraindicated during pregnancy on theoretical grounds. Congenital infection appears to occur at a low rate (probably 1–2%) and has not been associated with fetal abnormalities.[29,30] Pregnant women inadvertently vaccinated should be reassured that there is no risk to themselves and very low (if any) risk to the fetus, but should be followed to determine the outcome of pregnancy. If a fetal abnormality is noted, IgM testing of cord blood should be done to determine whether congenital infection occurred. The mother's immune response to yellow fever vaccine is diminished during pregnancy, and revaccination is indicated after parturition if there is a continued risk of exposure. On theoretical grounds the vaccine is also contraindicated in patients with immunodeficiency due to cancers, HIV/AIDS, or treatment with immunosuppressive, since prolonged viraemia may increase the risk of encephalitis. In the USA it is recommended that people with a risk of exposure to yellow fever who have symptomless HIV infection without immune suppression (CD4+ cell counts >200/μL) should be immunised, whereas in the UK vaccination is contraindicated. There is no evidence that adverse events are more frequent in HIV-infected people, although immune responses to yellow fever vaccine may be impaired. If an immunosuppressed individual must travel, consideration may be given to passive prophylaxis with immune globulin. Commercial globulins produced in the USA contain high titres of yellow fever antibodies because plasma donations are included from persons vaccinated during military service. There are no data on globulin preparations manufactured elsewhere. If passive immunisation is considered, advance testing of the globulin preparation and determination of the passive titre in the patient are advised. The level of antibody required for protection is considered to be a log neutralisation index of 0·7.[21]

Risks to the traveller

Between 1996 and 1999, four fatal cases occurred in unvaccinated travellers from the USA and Europe to Brazil (two cases), Venezuela, and Côte d'Ivoire. These unfortunate events were completely avoidable by preventative vaccination. In one case (a US citizen infected in Brazil), the patient had not been immunised because the nearest vaccinating centre was inconveniently located, 25 miles from his home in Tennessee. A geographic analysis of vaccinating centres in the USA showed that they were indeed sparsely distributed in rural regions.[31] By international regulation, yellow fever vaccine can only be distributed by centres approved by the World Health Organization or by designated national health authorities.

Travellers may wrongly consider yellow fever an "extinct" disease, and may not obtain accurate information about the risk of infection. In part this is because the indigenous population in Africa and South America is immune and virus transmission occurs in the virtual absence of reported cases. During the rainy season and early dry season all rural areas present a danger. In such areas, the risk of infection during a non-epidemic period approximates 1/1000 per month of exposure, but may increase to 1/15 per month during an epidemic. Immunisation for travel is imperative.

Novel application of yellow fever 17D virus

Given the unique properties of yellow fever vaccine virus (lifelong immunity after a single dose), it is not surprising that there has been considerable interest in harnessing the vaccine as a vector for foreign genes. It is possible to clone the full-length genome as cDNA into bacterial plasmids, manipulate the genetic material, transcribe back to messenger-sense RNA, and reconstitute live viral progeny from cells transfected with the full-length RNA. With this "infectious clone" technology, the envelope genes of yellow fever 17D have been replaced by the corresponding genes of other flaviviruses, including Japanese encephalitis, dengue and West Nile.[32,33] This approach holds great promise for the development of recombinant, live vaccines against an array of other viruses. In addition, recombinant yellow fever vaccine virus has been successfully used to generate cytotoxic cell responses to an expressed tumour antigen.[34]

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Search strategy and selection criteria

Data for this review were identified by searches of Medline, references from relevant articles and book chapters, and unpublished reports from research laboratories and public health agencies; numerous articles were identified through searches of the extensive files of the author. Search terms were "yellow fever", "arthropod-borne virus", "arbovirus", "flavivirus", and "viral haemorrhagic fever". English, Spanish, Portuguese, and French language papers were reviewed.

Future changes in the epidemiology of yellow fever

In the last 30 years, dramatic changes in the distribution of the urban vector, *Ae aegypti*, have occurred in the western hemisphere. Whereas this mosquito was previously eradicated from most yellow fever endemic countries, it has reinfested these areas and is now widely distributed. This fact, together with rapid human population growth and the rapid expansion of air travel, make the return of urban yellow fever in South America inevitable and increase the risk of introduction to receptive (*Ae aegypti*-infested) regions, including coastal regions of the continent where the human population is not vaccinated, the Caribbean, USA, or Asia (figure 2). In 1998, a small number of cases of urban yellow fever in the Americas was reported in Santa Cruz, Bolivia[35]—the first such episode since 1954. Fortunately, there appear to be barriers to urbanisation, which are currently not well understood, but probably include the relatively low viraemia induced by yellow fever infection in human beings (peak titre of 10^5–10^6 PFU/mL), cross-protection afforded by dengue (and other flavivirus) immunity which may reduce yellow fever viraemia levels, relatively low vector competence of *Ae aegypti*, and low vector density. These barriers are not absolute; for example, very high vector densities can overcome restrictions imposed by the other factors.[36] *Ae albopictus*, an import from Asia to Brazil and Nigeria, has been suggested as a possible vector bridging the sylvatic and urban cycles. However, experimental studies indicate that it is a relatively incompetent vector for transmission of yellow fever virus.

Should the virus be introduced to a receptive area, it is likely that cases would be recognised quite early due to the dramatic clinical presentation of yellow fever. However, the response required (surveillance, vector control, and vaccination) would severely test even the most sophisticated health department. If one or more large metropolises were affected, it is uncertain whether sufficient vaccine approved by the national authority would be available.

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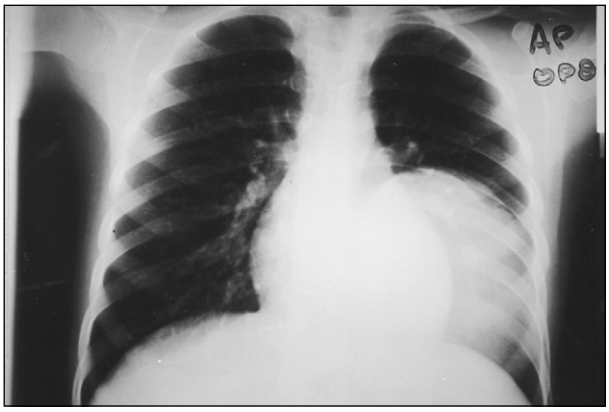
Clinical picture

An unusual cause of mild urticaria



A previously well 8-year-old boy was admitted with urticaria on head, neck, and trunk, and presented with diminished breath sounds over the left lower pulmonary field. No similar episode was recalled and the family history was negative for allergies. Respiratory and heart rates and blood pressure were normal. Abdominal examination did not reveal organomegaly. White blood cell count was $12{\cdot}8 \times 10^9$ cells/L; neutrophil count $7{\cdot}4 \times 10^9$/L; lymphocyte count $4{\cdot}8 \times 10^9$/L; eosinophil count $0{\cdot}37 \times 10^9$/L; and haematocrit 40·7%. The erythrocyte sedimentation rate was 15 mm/h. C-reactive protein was negative and first-line biochemistry, liver, and renal tests, and urinalysis were normal. Chest radiography revealed a round homogeneous consolidation in the left lower lobe (figure). Computed chest tomography confirmed the presence of a cyst compatible with hydatid disease. Serology for *Echinococcus* species was negative by indirect haemagglutination and marginally positive by ELISA, with a titre of 1/100. The rash and the pruritus subsided after administration of intravenous hydrocortisone. The boy was transferred in a good condition to a surgery department where the cyst was removed and diagnosis of pulmonary hydatid disease was confirmed. A 3-year follow-up period was uneventful and free of urticaria episodes.

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