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Transmission of yellow fever vaccine virus through blood transfusion and organ transplantation in the USA in 2021: report of an investigation

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JB, JOV, JJL, KAF, EHD, HRH, ACB, LS-F, SR-S, LFW, KAG, SMi, MRW, KSG, ABD, and CYC collected, interpreted, and validated data. CVG, RJF, TLR, MAB, RC-S, RPLK, YAP, AR, DT, JES, AP, JMR, SMo, WRM, and TM collected and interpreted the data; with RAS, JAL, DAK, and EL contributing to data collection; and SVB, MJK, CO, and TD contributing to data interpretation. LS-F, SR-S, JOV, JJL, KAF, EHD, HRH, ACB, JB, MRW, SMi, CYC, and CVG developed the methods. JA, AH, DT, MP, VB, KD, TLR, SMo, MAB, RC-S, RPLK, LFW, TM, AR, RAS, MJK, JAL, SR-S, AP, SVB, JES, CVG, RJF, and CYC conducted the investigation. CYC, JES, CVG, and RAS searched and reviewed the literature. CYC, SR-S, ACB, SVB, JES, CVG, and JB supervised the work. MRW, CYC, CO, JMR, EHD, and HRH prepared the figures. CVG, RJF, JB, and JES wrote the manuscript; all other authors reviewed and edited the manuscript. CVG, RJF, JB, CYC, and JES accessed and verified all data. All authors had full access to all the data in the study and accept responsibility to submit for publication.

Declaration of interests

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Yellow Fever Vaccine Virus Transplant and Transfusion Investigation Team*

Summary

Background—In 2021, four patients who had received solid organ transplants in the USA developed encephalitis beginning 2–6 weeks after transplantation from a common organ donor. We describe an investigation into the cause of encephalitis in these patients.

Methods—From Nov 7, 2021, to Feb 24, 2022, we conducted a public health investigation involving 15 agencies and medical centres in the USA. We tested various specimens (blood, cerebrospinal fluid, intraocular fluid, serum, and tissues) from the organ donor and recipients by serology, RT-PCR, immunohistochemistry, metagenomic next-generation sequencing, and host gene expression, and conducted a traceback of blood transfusions received by the organ donor.

Findings—We identified one read from yellow fever virus in cerebrospinal fluid from the recipient of a kidney using metagenomic next-generation sequencing. Recent infection with yellow fever virus was confirmed in all four organ recipients by identification of yellow fever

virus RNA consistent with the 17D vaccine strain in brain tissue from one recipient and seroconversion after transplantation in three recipients. Two patients recovered and two patients had no neurological recovery and died. 3 days before organ procurement, the organ donor received a blood transfusion from a donor who had received a yellow fever vaccine 6 days before blood donation.

Interpretation—This investigation substantiates the use of metagenomic next-generation sequencing for the broad-based detection of rare or unexpected pathogens. Healthcare workers providing vaccinations should inform patients of the need to defer blood donation for at least 2 weeks after receiving a yellow fever vaccine. Despite mitigation strategies and safety interventions, a low risk of transfusion-transmitted infections remains.

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Introduction

Yellow fever virus is a mosquito-borne flavivirus present in tropical areas of Africa and South America. Although most infections are asymptomatic, clinical disease ranges from a mild febrile illness to a potentially fatal disease with hepatitis and haemorrhagic manifestations.[1] Yellow fever can be prevented by a live, attenuated viral vaccine (17D strain) that is recommended for people aged at least 9 months who are travelling to or living in areas at risk of yellow fever. Like all live vaccines, yellow fever vaccine is contraindicated for immunocompromised individuals, including recipients of solid organ transplants, because of an increased risk of vaccine-associated adverse events.[2]

In October, 2021, four recipients of solid organs from a common, deceased donor in the USA each developed a febrile illness followed by neurological symptoms 2–6 weeks after transplantation. We describe the results of an investigation into the cause of these neurological symptoms.

Methods

Study design and participants

Recognition of neurological disease in four recipients of solid organs (heart, liver, and kidneys) from a common donor was reported to public health officials in the USA, and an investigation involving 15 agencies and medical centres was launched on Nov 7, 2021 to identify the cause. We included all organ and tissue transplant recipients who received their transplant from the same donor.

This public health investigation did not meet the definition of research as determined by the US Centers for Disease Control and Prevention (CDC) Human Research Protections review. Written informed consent was obtained from transplant recipients or their family members to be included in the case report.

Procedures

We reviewed information on medical history, clinical course, and exposures for the organ donor and organ and tissue recipients. Various specimens from transplant recipients underwent testing for infectious, autoimmune, and other diseases at university, commercial, state public health, and CDC laboratories. Cerebrospinal fluid (CSF), frozen tissue, and intraocular fluid specimens were tested for viral, bacterial, fungal, and parasitic pathogens by metagenomic next-generation sequencing at the University of California San Francisco (San Francisco, CA, USA; appendix p 1).[3,4] Formalin-fixed, paraffin-embedded (FFPE) tissue from organ biopsies and autopsy specimens were evaluated by transplant centre pathologists and the CDC Infectious Diseases Pathology Branch (Atlanta, GA, USA) using immunohistochemistry, a yellow fever virus-specific in-situ hybridisation assay targeting the envelope gene,[5] and yellow fever virus-specific and flavivirus-specific conventional RT-PCR assays, followed by Sanger sequencing of PCR amplicons as previously described (appendix p 2).[6–8]

RNA extractions from FFPE autopsy tissue specimens underwent metagenomic next-generation sequencing at the CDC Arboviral Diseases Branch (Fort Collins, CO, USA; appendix pp 2–3).[9] Stored serum from the organ donor and serum and CSF specimens from organ and tissue recipients were tested for RNA and IgM and neutralising antibodies for various arboviruses at multiple laboratories, including the CDC Arboviral Diseases Branch, the Laboratory of Viral Diseases at the Wadsworth Center of the New York State Department of Health (Albany, NY, USA), and commercial laboratories, as previously described.[10,11]

Host RNA sequencing gene expression data generated from the clinical metagenomic next-generation sequencing assay in CSF from three organ recipients and one corneal transplant recipient were compared with gene expression data for patients with viral encephalitis or autoimmune or non-infectious diseases (appendix pp 1–2).[12,13]

We reviewed the blood products received by the organ donor, the other products (known as co-components) that were derived from these blood donations, and the current health and any adverse events after the transfusion of patients who received these transfused co-components. Any available co-components were quarantined. The blood donors involved were contacted by blood bank staff to ask about the presence of symptoms around the time of donation, tick or mosquito exposure, travel, and receipt of vaccines within 30 days before blood donation.

Outcomes

The primary outcome of the investigation was to determine the cause and source of the potential infection in the transplant recipients. Secondary outcomes were to prevent any additional infections, if possible, and to establish whether potential risk mitigation could have prevented the infections and be strengthened to prevent subsequent infections.

Role of the funding source

The funders of this study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

The investigation was conducted from Nov 7, 2021, to Feb 24, 2022, and included six transplant recipients (four who received solid organs [two kidney, one heart, one liver] and two who received corneas), one organ donor, and a blood traceback investigation.

The recipient of the right kidney was a woman in her 60s with a history of type 1 diabetes, hypertension, and a previous kidney and pancreas transplant. She had no history of travel in the month before transplantation or of yellow fever vaccination. For the current kidney transplant she received induction immunosuppression with high-dose steroids and basiliximab, followed by a maintenance regimen of tacrolimus, mycophenolate mofetil, and prednisone. 4 weeks after transplantation she was admitted to hospital with fever, fatigue, and malaise (figure 1). Within 6 days of hospitalisation, the patient's cognitive status rapidly deteriorated and she developed left hemiparesis and unresponsiveness requiring intubation for airway protection. Multiple brain MRI scans showed chronic microvascular changes. Loss of brainstem reflexes was noted 10 days post-onset. A lumbar puncture showed 2 white blood cells per μL, 28 red blood cells per μL, normal glucose concentrations, and increased protein concentrations (table 1). Testing for multiple infectious causes of meningoencephalitis was negative (appendix pp 4–5). The patient's clinical status remained unchanged despite treatment with high-dose steroids, plasmapheresis, and intravenous immunoglobulin G. After being treated in hospital for approximately 1 month, she was transferred to long-term acute care, where she remained ventilator-dependent with no neurological improvement and died 7 months after transplantation.

Clinical metagenomic next-generation sequencing of CSF collected 11 days post-onset was negative for pathogenic organisms, except for the detection of yellow fever virus at sub-threshold reporting levels (one read). The read was 100% identical across 106 base pairs to 17D yellow fever vaccine strain (figure 2). Deeper metagenomic sequencing did not identify any additional reads from yellow fever virus or other pathogens.

The CSF contained detectable yellow fever virus-specific IgM antibodies but not neutralising antibodies, and viral RNA was not detected by RT-PCR. Serum collected 6 days before transplantation had no yellow fever virus-specific antibodies, whereas serum collected 14 weeks after transplantation had yellow fever virus-specific IgM and neutralising antibodies, indicative of seroconversion (table 2). The host gene expression data generated from the metagenomic next-generation sequencing assay of the CSF aligned more closely with CSF transcriptional profiles from patients with viral meningoencephalitis than from patients with autoimmune or non-infectious encephalitis (figure 3).

The recipient of the heart was a man in his 50s with a history of hypertension, type 2 diabetes, ischaemic cardiomyopathy, and congestive heart failure. He had no history of travel in the month before transplantation and his vaccination history was unknown. The patient

received induction immunosuppression with high-dose steroids and basiliximab, followed by a maintenance regimen of tacrolimus, mycophenolate mofetil, and prednisone. 17 days after transplantation he was admitted to hospital with fever, malaise, and lethargy, followed by a progressive decline in cognitive status (figure 1). A lumbar puncture 12 days post-onset showed mild lymphocytic pleocytosis and increased protein concentrations (table 1). Brain MRI showed chronic volume loss. Tacrolimus was switched to cyclosporine and then to sirolimus, owing to concerns of possible calcineurin inhibitor-related encephalopathy. However, the patient progressed to unresponsiveness with no movement of his extremities. A trial of high-dose steroids was initiated, which was followed by a transient increase in responsiveness, tracking, and the following of some commands. Subsequent treatment with plasmapheresis and intravenous immunoglobulin G did not result in further improvement. The patient's family opted to withdraw support, and he died 56 days after transplantation.

Testing of CSF specimens was negative for pathogenic organisms, except for the detection of human polyomavirus 2 (JC virus) by PCR (appendix pp 5–6). CSF collected 19 days post-onset had detectable yellow fever virus-specific IgM antibodies but no detectable neutralising antibodies, and no viral RNA was detected by RT-PCR or metagenomic next-generation sequencing (table 2). The host gene expression data generated from the metagenomic next-generation sequencing assay aligned more closely with profiles from patients with viral meningoencephalitis than from patients with autoimmune or non-infectious encephalitis (figure 3).

No yellow fever virus-specific or flavivirus-specific RNA was detected by RT-PCR in FFPE heart biopsy tissue after transplantation (table 2). FFPE brain tissue obtained at autopsy showed encephalitis with multiple foci of perivascular mononuclear inflammation, mild gliosis, and patchy regions of neuronal necrosis (figure 4). Immunohistochemistry results were negative for yellow fever virus; however, viral RNA was detected by RT-PCR in blocks from the dentate, hippocampus, pons, and basal ganglia. Sequence analysis showed shared identities with 17D yellow fever vaccine strain (table 2). An in-situ hybridisation assay found that yellow fever virus RNA was localised in encephalitic lesions with punctate assay labelling, suggesting the presence of viral RNA in degenerating neuronal processes (figure 4). RNA extracted from the brain blocks had 15 sequences aligning with 17D yellow fever vaccine strain identified by metagenomic next-generation sequencing (table 2). In total, there was 8% coverage of the yellow fever virus genome representing several different genes, although the depth of coverage was low and was often a single read. Compared with the canonical 17D yellow fever vaccine strain genome, one amino acid mutation of an arginine to a glycine was identified in a single read in the envelope protein at position 52 (appendix p 10).

The recipient of the liver was a man in his 40s with a history of autoimmune hepatitis and cirrhosis. He had no history of travel in the month before transplantation or of yellow fever vaccination. After induction with basiliximab, the patient was on a maintenance immunosuppressive regimen of tacrolimus and mycophenolate mofetil. 15 days after transplantation, he developed fever, nausea, vomiting, abdominal pain, and malaise (figure 1). A CT scan of the patient's chest, abdomen, and pelvis showed moderate pelvic ascites. 9 days post-onset, the patient had persistent fevers and developed headache, diplopia,

photophobia, multidirectional nystagmus, dysmetria, and nuchal pain. Analysis of the CSF showed lymphocytic pleocytosis and increased protein concentrations (table 1). Brain MRI showed subtle diffusion uptake and T2 hyperintensity in the cerebellum. Extensive testing for infectious diseases was negative except for *Clostridioides difficile* toxin in stool (appendix pp 6–7). The patient declined neurologically with myoclonus, posturing, and unresponsiveness, leading to intubation for airway protection. Because autoimmune encephalitis was suspected he was started on high-dose dexamethasone, which was followed by the rapid resolution of fever. He continued to exhibit hyper-reflexia, nystagmus, and myoclonus, but opened his eyes to stimuli. He subsequently underwent five rounds of plasmapheresis. After the second round he began following commands, opening his eyes spontaneously, and myoclonus and hyper-reflexia were resolved. He was extubated, and the findings of his neurological examinations returned to normal except for residual bilateral symmetrical weakness. The patient became ambulatory and was discharged to home at 31 days post-onset. 1 year after transplantation he was doing well.

FFPE liver tissue from a post-transplantation biopsy showed non-specific changes of steatosis and chronic portal inflammation and was negative for multiple pathogens, including yellow fever virus by immunohistochemistry and flavivirus by RT-PCR (table 2, appendix pp 6–7). Serum collected 13 days post-onset was negative for yellow fever virus RNA by RT-PCR and metagenomic next-generation sequencing, for yellow fever virus-specific IgM antibodies, and for yellow fever virus by viral culture (table 2). CSF collected 14 days post-onset was negative for yellow fever virus RNA by RT-PCR but positive for IgM antibodies; an insufficient quantity was available to test for neutralising antibodies. Serum collected 57 days post-onset contained yellow fever virus-specific IgM antibodies and neutralising antibodies; a serum specimen collected 1 day before transplantation had no detectable yellow fever virus-specific antibodies, indicating seroconversion (table 2).

The recipient of the left kidney was a woman in her 40s with a history of type 2 diabetes, hypertension, coronary artery disease, and peripheral vascular disease. She had no history of travel in the month before transplantation or of yellow fever vaccination. Her induction regimen consisted of high-dose steroids and basiliximab followed by a maintenance regimen of tacrolimus, mycophenolate mofetil, and prednisone. 40 days after transplantation, she was admitted to hospital with fever, nausea, vomiting, and diarrhoea (figure 1). She was noted to have tremors and unsteady gait. CSF analysis showed 7 white blood cells per μL and normal protein concentration (table 1). Brain MRI showed mild dural thickening and enhancement. Extensive testing for infectious disease causes was negative (appendix p 8). The patient's dose of mycophenolate mofetil was discontinued and she showed clinical improvement; she did not receive any treatment with intravenous immunoglobulin G. 1 year after transplantation she was doing well.

Metagenomic next-generation sequencing of CSF collected 4 days post-onset was negative for yellow fever virus RNA (table 2); however, the host gene-expression data generated from the CSF metagenomic next-generation sequencing assay aligned with the transcriptional profiles of the other two organ recipients and with the viral meningoencephalitis cohort (figure 3). Serum collected 31 days post-onset contained yellow fever virus-specific IgM

antibodies and neutralising antibodies. Serum collected 8 months before transplantation had no yellow fever virus-specific antibodies, indicating seroconversion (table 2).

The recipient of the right cornea was a man in his 20s with a history of migraines who underwent penetrating keratoplasty of his cornea following ocular trauma. He was receiving topical but no systemic immunosuppressive medications. He had no history of travel in the month before vaccination or of yellow fever vaccination. 40 days after transplantation he developed intermittent headaches, nausea, and tongue tingling. He had no fever or other neurological symptoms. Testing of the CSF for multiple causes was negative (table 1, appendix pp 8–9). His headaches resolved, but because of the suspicion of infection in recipients of organs from the same donor and the presence of neurological symptoms in the patient, the cornea was removed and replaced. 10 months after transplantation the patient was doing well.

Testing of CSF, corneal explant tissue, and aqueous humour from the anterior chamber was negative for yellow fever virus RNA by metagenomic next-generation sequencing, and the host gene-expression data aligned more closely with that from patients with autoimmune or non-infectious diseases than with the CSF transcriptional profiles of the three organ recipients or the viral meningoencephalitis cohort (figure 3). FFPE corneal explant tissue was negative for yellow fever virus by immunohistochemistry and for flavivirus by RT-PCR. A follow-up serum specimen obtained 76 days after transplantation was negative for yellow fever virus-specific IgM antibodies (table 2).

The recipient of the left cornea was a woman in her 60s who had a partial thickness cornea transplant. The patient had no reported symptoms and was doing well 1 year after transplantation; no post-transplantation testing for yellow fever virus was conducted.

The organ donor was a man in his 50s with no clinically significant medical history who had a traumatic brain injury. The patient had no history of travel in the several months before transplantation or of yellow fever vaccination. During his 9-day treatment in hospital before organ donation, the patient received six blood products—five packed red blood cell units and one unit of platelets—from six donors. He was declared brain dead 5 days after admission. No signs or symptoms of neurological or other infection were present at the time of death.

FFPE tissue from needle biopsies of the liver and kidneys collected at procurement showed no histopathological findings suggestive of infection and were negative for multiple pathogens, including yellow fever virus (table 2). Pre-transplantation serum collected within 1 day of organ procurement and after the patient received all blood-product transfusions had no detectable yellow fever virus RNA or antibodies.

Five of six blood donors were reached and interviewed retrospectively. No donors reported any symptoms, international travel, or exposure to mosquitos within 30 days of donation. However, one donor reported receiving a primary yellow fever vaccine dose (YF-VAX; Sanofi Pasteur, Swiftwater, PA, USA) 6 days before donating blood (figure 1). During pre-donation screening, this blood donor did not report receiving any live vaccines within 8 weeks of donation. The donor provided a split apheresis red blood cell donation resulting in two red blood cell products. One red blood cell unit was transfused into the organ donor 24

days after the blood donation and 3 days before organ procurement (figure 1). The second unit was transfused into a different patient 26 days after the donation; no adverse events attributed to the vaccine were identified on review of this patient's medical record. This second recipient died 10 weeks after the blood transfusion from complications related to cancer; no specimens from this patient were available for testing.

The US Vaccine Adverse Event Reporting System and the vaccine manufacturer were queried about any safety concerns associated with the specific vaccine lot administered to the blood donor; no increase in reported adverse events was noted.

Discussion

Transmission of 17D yellow fever vaccine strain by blood transfusion to an organ donor and subsequent transmission through solid organ transplantation led to neurological disease in four organ recipients. Two recipients recovered and two died after neurological deterioration.

Yellow fever vaccine was originally developed in the 1930s through serial passage of Asibi wild-type yellow fever virus.[1] All currently available vaccines are live, attenuated vaccines using 17D viral substrains. Serious adverse events from the vaccine are rare but include acute anaphylactic reactions, yellow fever vaccine-associated viscerotropic disease (which resembles natural infection), and yellow fever vaccine-associated neurological disease.[2] As with all live vaccines, yellow fever vaccine is contraindicated in people with altered immune function from underlying conditions, such as thymus disorders, or people who are on immunosuppressive or immunomodulatory therapies, including organ transplant recipients.[2,14]

Yellow fever vaccine-associated neurological disease is caused by either direct neuroinvasion of the virus into the CNS (neurotropic disease) or an autoimmune-mediated process triggered by the virus. The most common presentation of neurotropic disease is meningoencephalitis. Autoimmune-mediated neurological disease typically presents as Guillain-Barré syndrome, acute disseminated encephalomyelitis, or transverse myelitis; however, cases of autoimmune-mediated encephalitis have been reported.[15] Symptom onset typically occurs 2 weeks after vaccination, but can range from 2 days to 50 days.[16] Initial symptoms can include fever and headache followed by more specific signs and symptoms related to the clinical syndrome. Diagnosis is based on a combination of clinical, imaging, and laboratory findings and the absence of an alternative diagnosis.[2] Patients with meningoencephalitis typically have a good outcome with complete recovery, whereas older patients and those with other autoimmune manifestations can have more complications. Deaths with the condition are rare, with only three cases reported in the literature.[17–19]

Yellow fever vaccine-associated neurological disease among the transplant recipients in this cluster was notably severe and led to deaths. Because yellow fever vaccination is contraindicated in the recipients of solid organ transplants, data on adverse events of the vaccine are scarce for this group. Although no serious adverse events have been described in organ recipients who inadvertently received the vaccine, most patients had received

transplants several years previously, and the numbers of cases are too small to assess safety.[20–22]

All organ recipients in this cluster were confirmed to have recent yellow fever virus infection. Neuroinvasive disease was confirmed in the recipient of the heart by molecular testing of brain tissue. The recipient of the right kidney had initial molecular evidence of yellow fever virus in the CSF by metagenomic next-generation sequencing. The recipients of both kidneys and the liver had evidence of seroconversion in peripheral blood samples. However, the recipients of the heart, liver, and right kidney had yellow fever virus-specific IgM antibodies in the CSF but no neutralising antibodies in the available specimens. Immunosuppression could explain the absence of or delay in neutralising antibody responses in the CSF. Another possible explanation is an autoimmune-mediated process, which was originally suspected in the recipient of the liver, who had autoimmune hepatitis; underlying autoimmune disease can be triggered by vaccines through activation of cellular and immune responses.[15] However, host gene-expression signatures in the CSF of all three organ recipients in whom this was evaluated suggest a predominantly neuroinvasive pathway. Although using human gene-expression data for diagnosing neurological infections is still in its infancy, these results show the potential for host response-based profiling to complement traditional microbiological methods in diagnosing infectious diseases.[23,24]

The pathogenesis leading to neurological rather than viscerotropic disease in all organ recipients in the present cluster is unclear. Early studies suggested that a low dose of vaccine might cause a delayed immune response and increased risk of encephalitis, although this has not been supported by subsequent data.[1] Reversion of the vaccine strain to neurovirulence, either by mutation in a variant virion in the vaccine lot (unlikely given the absence of safety signals identified) or in the blood or organ donor, was also considered. Such reversion was previously seen in a fatal case of yellow fever vaccine-associated encephalitis in which sequencing of virus from the brain revealed a mutation at position 303 of the envelope protein that is associated with increased neurovirulence in mice and monkeys.[17] Sequencing of viral RNA from the brain tissue of the heart recipient identified one amino acid substitution at position 52 of the envelope protein, in which arginine reverted to glycine as in the Asibi wild-type strain. Although this mutation is not known to be associated with neurovirulence, it could cause a confirmational change that affects viral entry into host cells.[1]

Transmission of 17D yellow fever vaccine strain through the transfusion of blood products has previously been reported with no adverse events identified in five recipients.[25] In immunocompetent individuals who have not previously been exposed to yellow fever wild-type or vaccine virus, peak viraemia occurs 3–5 days after vaccination and can last for up to 10 days after vaccination, by which time most people develop neutralising antibodies.[1] The blood donor in this investigation received the vaccine 6 days before donating blood, so a low-level viraemia could have been present at the time of donation. In this case, the standard pre-donation screening questionnaire—which included a question about recent vaccinations—was administered but failed to elicit vaccination history to prevent donation, showing the limitations of obtaining accurate information through the administration of donor history questionnaires. A previous cognitive evaluation of the uniform donor history

questionnaire used in the USA revealed that donors often answer questions through the perspective of “is my blood safe?” rather than providing objective answers to questions.[26] As a result, individuals who would otherwise be deferred from donation might continue to donate blood, resulting in risk to recipients. Risk-mitigation strategies, such as pathogen reduction technology for apheresis platelets and plasma, are increasingly adopted by blood collection organisations and could be available for red blood cell products in the future, should such products be approved for use.[27,28]

This investigation was limited by the lack of availability of crucial specimens. We were not able to test for yellow fever virus in the implicated donated blood because blood products were no longer available at the time of the investigation. Yellow fever virus was not detected in the organ donor, possibly related to dilutional effects of resuscitation measures, low-level viraemia below the detection threshold, or viraemia resolution and viral sequestration in the organs during procurement.[29] Low volumes of tissue from organ biopsies might have prevented the detection of yellow fever virus. These limitations were overcome for some recipients, in whom seroconversion was revealed by the testing of stored serum collected before transplantation and post-transplant samples. However, this investigation also highlights the need to ensure sample retention—both before transplantation and from the acute illness—to facilitate confirmatory testing.

The transmission of 17D yellow fever vaccine virus through solid organ transplantation illustrates the value of clinicians recognising the possibility of donor-derived infection and collaborating with public health authorities to complete a comprehensive evaluation. This finding also substantiates the clinical use of metagenomic next-generation sequencing for the broad-based detection of pathogens if a transplant-transmitted infection is suspected and traditional diagnostic testing has not identified a cause.[23] This cluster of cases occurred because a blood donation was made soon after receipt of a live virus vaccine, and the associated blood product was transfused into an organ donor. Although blood transfusion continues to become safer over time, transfusion-transmitted infection of an organ donor can rarely result in transplant-associated infections.[30,31] Continued efforts at haemovigilance, adoption of risk-mitigation strategies, and implementation of new safety interventions should reduce the risk of transfusion-transmitted infections.[32] Healthcare workers providing yellow fever vaccination should inform patients of the need to defer blood donation for at least 2 weeks after vaccination.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data sharing

Individual participant data will not be made available.

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Research in context

Evidence before this study

Yellow fever vaccine is a live, attenuated viral vaccine (17D strain). Like all live vaccines, yellow fever vaccine is contraindicated for people who are immunocompromised—such as the recipients of solid organ transplants—because of an increased risk of adverse events, including yellow fever vaccine-associated neurological disease and viscerotropic disease. Transmission of 17D yellow fever vaccine strain through blood transfusion was previously reported in 2009 in the USA; no adverse events were identified in five recipients of blood products. Transmission of yellow fever vaccine strain through solid organ transplantation has not been described previously.

Added value of this study

To our knowledge, we identified the first known transmission of live, attenuated yellow fever vaccine virus through solid organ transplantation to four organ recipients, causing severe neurological disease and two deaths. Because the source of infection in the organ donor was a blood product obtained from a recently vaccinated blood donor, the investigation highlights the risk of transmission of live vaccine virus strains through blood transfusions.

Implications of all the available evidence

This investigation illustrates the importance of the continued adoption and implementation of new safety interventions to reduce the risk of transfusion-transmitted infections. Healthcare workers providing yellow fever vaccination should inform patients of the need to defer blood donation for at least 2 weeks after vaccination. The investigation substantiates the clinical use of metagenomic next-generation sequencing coupled with traditional diagnostic testing to confirm the source of the infection, and also highlights the potential for host response-based profiling to complement methods in diagnosing infectious diseases.

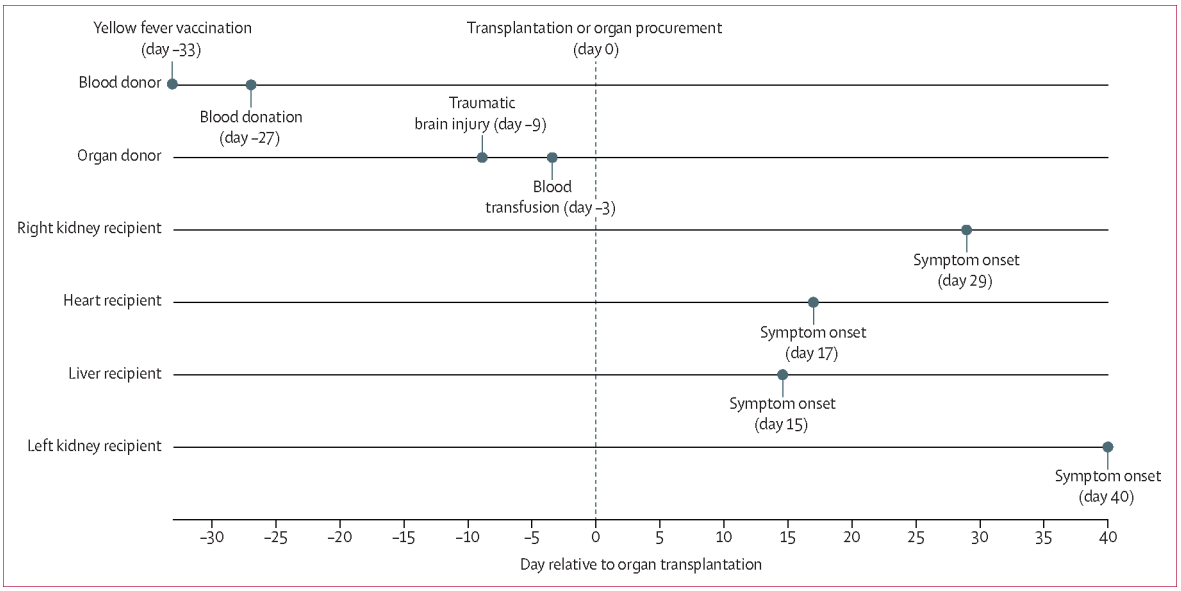

Figure 1:

Timeline of events related to the transmission of yellow fever vaccine virus through blood transfusion and solid organ transplantation

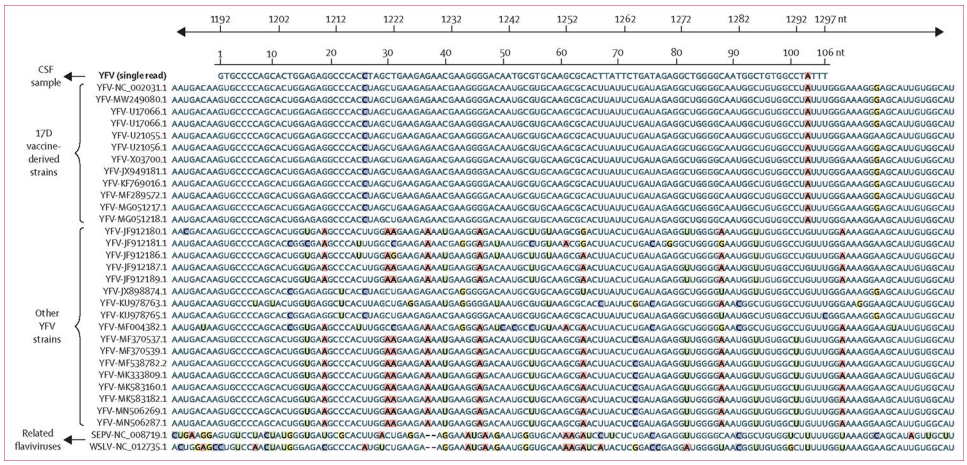

Figure 2: Initial detection of yellow fever virus by clinical metagenomic next-generation sequencing of CSF from a kidney recipient

One yellow fever virus read out of 14 608 788 reads in the RNA metagenomic library was detected. Multiple sequence alignment of the read from the recipient of the right kidney (YFV-single-read) along with 29 representative strains of yellow fever virus and two related flaviviruses (Wesselsbron virus and Sepik virus) was done using multiple alignment using fast Fourier transform.[33] The alignment shows that the read was identical across 106 base pairs to the live attenuated 17D yellow fever vaccine strain. CSF=cerebrospinal fluid. SEPV=Sepik virus. WSLV=Wesselsbron virus. YFV=yellow fever virus.

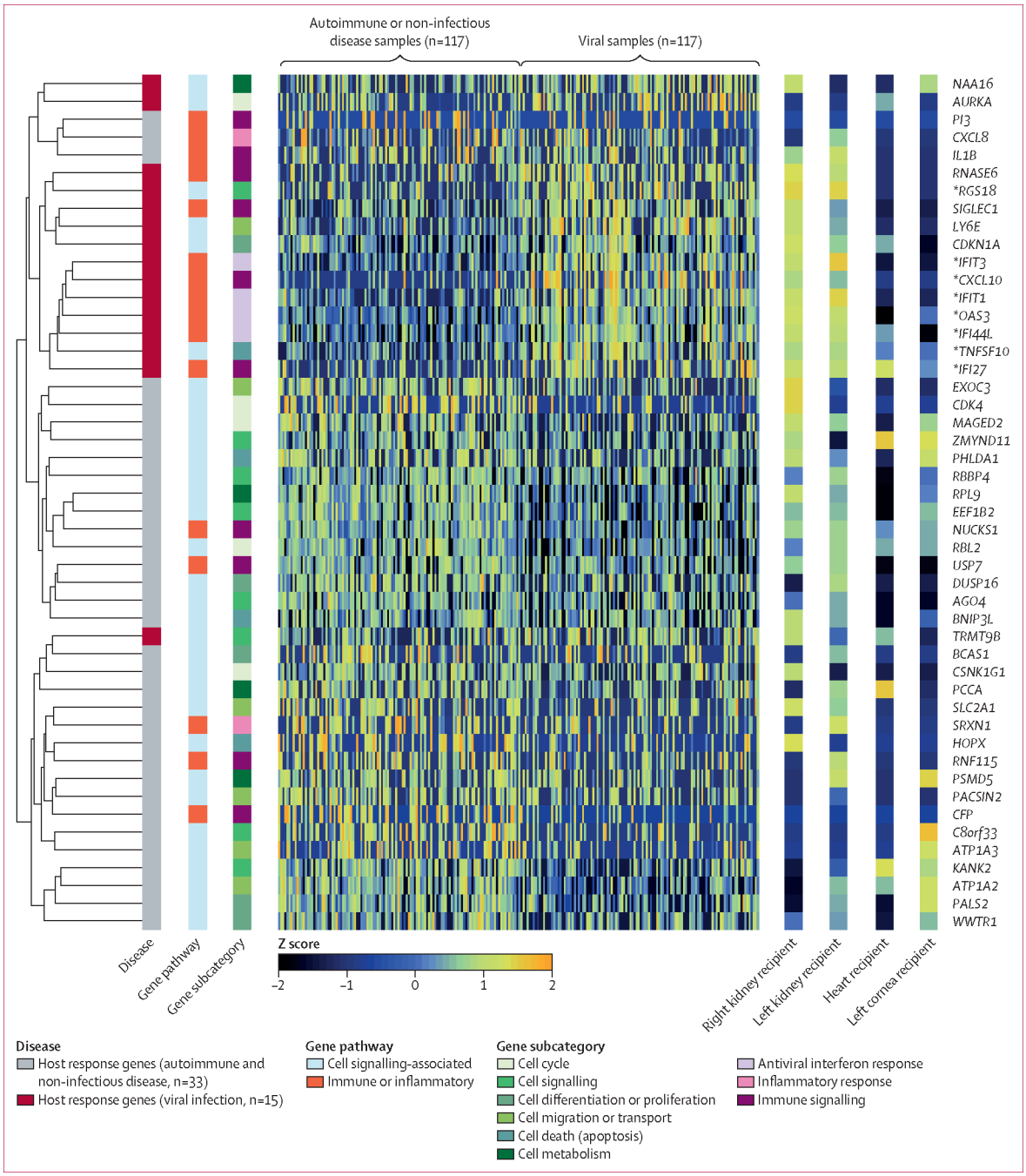

Figure 3: Comparison of gene expression profiles from solid organ and tissue recipients with those from patients with autoimmune or non-infectious encephalitis or viral meningoencephalitis

Heat map showing hierarchical clustering of differentially expressed genes (y axis) by sample (x axis). Normalised RNA gene expression levels, shown as Z scores and visualised using a colour bar gradient, reflect gene overexpression (yellow or orange) and underexpression (blue or black). Data from a cohort of 117 patients with autoimmune or non-infectious encephalitis and 117 patients with viral meningoencephalitis were used. Differentially expressed genes specific for autoimmune or non-infectious encephalitis (n=33) or viral meningoencephalitis (n=12) were selected for use in a machine learning-

based classifier model. The genes shown (n=48) were selected using a classifier model and corresponding to either a cell signalling-associated pathway or an immune or inflammatory pathway, with subcategories assigned on the basis of known function. The asterisks denote genes that have been previously reported to be overexpressed in association with yellow fever vaccination or infection with non-yellow-fever flaviviruses.

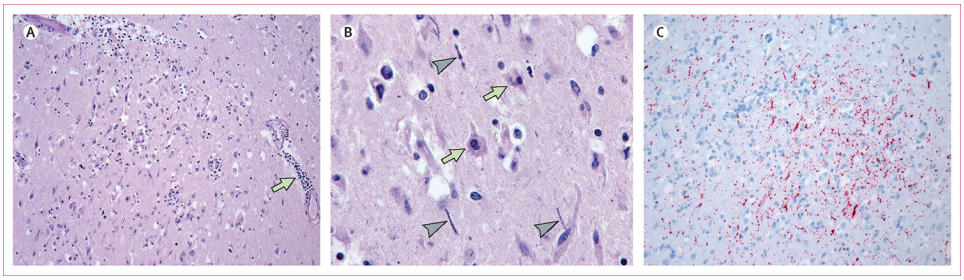

Figure 4: Evidence of yellow fever viral encephalitis in the brain tissue of the heart transplant recipient on autopsy

Left: perivascular lymphocytic inflammation (arrow) and glial proliferation and activation in an area of neuronal degeneration, 100×; middle: degenerating neurons (arrows) and neuronal processes (arrowheads), 400×; right: yellow fever virus RNA staining (in red) within inflammatory focus by in-situ hybridisation, 100×.

Table 1:

CSF profiles of recipients of solid organ and tissue transplants

	Time after transplantation (days)	White blood cells (cells per μL)	Lymphocytes (%)	PMN (%)	Mono/macro (%)	Red blood cells (cells per μL)	Protein concentration (mg/dL)	Glucose concentration (mg/dL)
Right kidney	36	2	36%	30%	34%	28	56	89
Heart	25, 36	14, 16	88%, 86%	2%, 12%	10%, 2%	0, 9	132, 70	106, 70
Liver	24, 29	63, 44	77%, 85%	7%, 0	16%, 15%	87, 4	133, 130	50, 117
Left kidney	44	7	37%	46%	17%	9950	42	98
Right cornea	45	1*	..	..	..	0	31	64

Reference ranges in CSF: total protein 15–60 mg/dL; glucose 50–80 mg/dL (or more than two-thirds of blood glucose concentration). CSF=cerebrospinal fluid. Mono=monocytes. Macro=macrophages. PMN=polymorphonuclear neutrophils. *Differential cell count was not done because fewer than 25 cells were seen. Few lymphocytes and one monocyte/macrophage were seen.

Table 2:

Results of testing specimens from the organ donor and organ and tissue recipients for yellow fever virus

	Day of collection relative to transplantation	Yellow fever virus RT-PCR*	Flavivirus RT-PCR*	mNGS†	Yellow fever virus IHC	Yellow fever virus IgM EIA	Yellow fever virus PRNT titre‡
Organ donor							
Serum	0	Negative	..	Negative§	..	Negative	<10
Liver tissue	0	Negative	Negative	..	Negative	..	..
Right kidney tissue	0	Negative	Negative	..	Negative	..	..
Left kidney tissue	0	Negative	Negative	..	Negative	..	..
Right kidney recipient							
Serum	−5	..	..	..	..	Negative	<10
CSF	36	Negative	..	..	..	Positive	..
CSF	40	Negative	Negative	Yellow fever virus¶	..	Positive	<2
Serum	40	Negative	Negative	..	..	..	..
Serum	98	..	..	..	..	Positive	160
Heart recipient							
Heart tissue	9, 17, 23	Negative	Negative	..	Negative	..	..
CSF	36	Negative	..	Negative	..	Positive	<2
Brain tissue	57	Positive	..	Yellow fever virus‖	Negative	..	..
Liver recipient							
Serum	−1	..	..	..	..	Negative	Negative
Liver tissue	0	..	Negative	..	Negative	..	..
Serum	28	Negative	..	Negative **	..	Negative	..
CSF	29	Negative	..	..	..	Positive	..
Serum	72	..	..	..	..	Positive	10
Left kidney recipient							
Serum	−243	..	..	..	..	Negative	<10
CSF	44	..	..	Negative	..	..	..
Serum	71	..	..	..	..	Positive	320
Right cornea recipient							
CSF	45	..	..	Negative	..	..	..

	Day of collection relative to transplantation	Yellow fever virus RT-PCR*	Flavivirus RT-PCR*	mNGS†	Yellow fever virus IHC	Yellow fever virus IgM EIA	Yellow fever virus PRNT titre‡
Corneal tissue	48	..	Negative	Negative	Negative	..	..
Aqueous humour	48	..	..	Negative	..	..	..
Serum	76	..	..	..	..	Negative	..

CSF=cerebrospinal fluid. EIA=enzyme immunoassay. FFPE=formalin-fixed, paraffin-embedded. IHC=immunohistochemistry. mNGS=metagenomic next-generation sequencing. PRNT=plaque reduction neutralisation test.

*Real-time RT-PCR conducted on serum and CSF specimens; conventional RT-PCR conducted on FFPE tissue specimens.

†Of the specimens listed, mNGS is validated for clinical testing on CSF (University of California San Francisco, San Francisco, CA, USA).

‡90% PRNT result <10 for serum or <2 for CSF is considered negative.

§>500 μL serum concentrated into 10 μL of RNA through enrichment; 900 reads obtained and zero aligned with yellow fever virus.

¶One read detected in RNA library from yellow fever virus (threshold reporting cutoff ≥3 non-overlapping virus reads from individual species or genus), which was 100% identical across 106 base pairs to 17D yellow fever vaccine strain.

//mNGS of RNA extracted from the four tissue blocks identified 15 sequences that aligned with the 17D yellow fever vaccine strain. At least one sequence read was found in each RNA specimen.

**>500 μL serum concentrated into 10 μL of RNA through enrichment; 2·5 million reads obtained and zero aligned with yellow fever virus.