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Emerging infections in transfusion medicine

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Concerns about transfusion-transmitted infectious diseases (TTIDs) have been raised since the beginning of routine blood administration for therapeutic purposes in the late 1930s. Syphilis was the first transmissible disease for which prospective blood donor testing was implemented in the 1950s [1]. Although infectious post-transfusion hepatitis (PTH) was recognized and described in the early 1940s [2], it was believed to be a transient and generally minor infectious complication, and interventions to reduce PTH did not occur until the discovery of hepatitis B virus and hepatitis C virus decades later. Awareness of infectious disease transmission by transfusion did not slow the dramatic increase in administration of donated blood from the 1950s through 1970s, which paralleled the expansion of medical care and procedural capability during that period.

Given the widespread use of transfusions by the early 1980s, the realization that the then newly-discovered fatal acquired immunodeficiency syndrome could be transmitted by transfusion came as a shock to transfusion medicine specialists and the public. Since then, efforts to protect the blood supply from the viruses causing AIDS (HIV) and PTH have resulted in an unparalleled focus of resources and attention on the characterization, detection, and prevention of TTIDs. This commitment has paid off, and blood safety is now at historically unprecedented levels, at

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least in high-income countries (Table 1) [3,4]. However, public uneasiness and concerns among health professionals about disease transmission by transfusion persist, and these concerns have caused a redirection of attention from the classical TTIDs to “emerging infections.” For the purposes of this review, the authors apply this term broadly to include (1) recently discovered entities, such as variant Creutzfeldt-Jacob Disease and severe acute respiratory syndrome (SARS), for which transmission by transfusion is unproved; (2) longer-known pathogens, such as West Nile virus, that are newly recognized as agents causing TTIDs, and (3) conditions such as bacterial contamination and malaria that have been a concern since the beginning of transfusion therapy but are emerging as more pressing concerns now that classical TTIDs have been nearly eliminated. The authors follow a taxonomic order in reviewing the various pathogens. The article closes with a brief reflection on the challenges posed by new and emerging pathogens and an outline of the general approaches that are pursued to protect the blood supply.

Table 1
Risk of transfusion-transmitted diseases in the United States

| Pathogenic agent | Average estimated risk per unit |
|--|--|
| Hepatitis A | Unknown; presumably <1:1 million |
| Hepatitis B | 1:205,000 ^a |
| Hepatitis C | 1:1,935,000 ^b |
| Human immunodeficiency virus-1 | 1:2,135,000 ^b |
| Human T-lymphotropic virus-I, II | 1:2,993,000 |
| Cytomegalovirus (CMV) | Infrequent with leukocyte-reduced components |
| Parvovirus B19 | Unknown; presumably <1:1 million |
| West Nile and other arboviruses | Regional and seasonal risk; observed incidence of transmissions during 2003 season after implementation of pooled NAT approximately 1:1 million recipients |
| Bacterial contamination associated with symptomatic sepsis | 1:5 million per red blood cell unit ^c 1:100,000 per apheresis or pooled platelet unit ^c |
| Malaria | 1:4,000,000 |
| Babesia | <1:1 million |
| Chagas' disease | Unknown; presumably <1:1 million |
| Creutzfeldt-Jakob Disease (CJD), variant CJD | Single probable case reported in United Kingdom |

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; NAT, nucleic acid testing.

^a Estimates for HBV reflect risk projections prior to implementation of blood donor screening with NAT [3].

^b Estimates for HIV, HCV indicate risk projections following implementation of NAT for these agents in 1999 [3].

^c Risk estimate reflects the experience of a 2-year United States national study from 1998–2000, prior to implementation of standards to detect and limit bacterial contamination. Because of likely underreporting, true risks were probably higher [58].

Emerging issues in established transfusion-transmitted viruses

Hepatitis B to D viruses

Hepatitis B virus (HBV) and hepatitis C virus (HCV), members of the Hepadna and Flavivirus families, respectively, are two of the classically known transfusion-transmitted viruses (TTVs) and are therefore not considered further in this article. Detailed information can be found in recent reviews [5,6]. Hepatitis D, originally called the delta agent, is a defective RNA-containing passenger virus that requires HBV to act as a “helper” for assembly of envelope proteins. Screening for HBV prevents transfusion-associated hepatitis D cases by identifying donors who are co-infected with hepatitis B and D.

Hepatitis A and E viruses

These viruses, which are primarily transmitted via the fecal-oral route, cause frequent epidemics of acute hepatitis in developing countries, with occasional focal outbreaks in the United States and other developed countries. Viremic periods are generally brief, although, at least in the case of hepatitis A virus (HAV), viremia may be present up to 30 days before onset of symptoms, which is longer than previously realized [7]. Because prolonged carrier states have not been described and parenteral transmission is rare, neither HAV nor hepatitis E virus (HEV) is considered a blood-borne pathogen in the strict sense. Animal inoculation studies and one recent report in humans have documented transmission of HEV by transfusion of fresh components, although the risk is undoubtedly very small given the rarity of reports and the high prevalence of this infection in many developing countries [7–9]. On the other hand, cases of transfusion-transmitted HAV have been well documented and are estimated to occur at a rate of less than approximately 1 per million units transfused [6,10,11]. A greater concern is contamination of plasma derivatives, such as albumin, gamma globulin preparations, and clotting factor concentrates, by HAV. Because of the pooling of thousands of plasma donations in the manufacture of plasma derivatives and the relative resistance of HAV to inactivation, even rare instances of donor viremia can lead to widespread contamination of these products. Consequently, HAV has caused several outbreaks of clinical disease from contaminated coagulation factor preparations [12,13]. Albumin, plasma protein fractions, and immune globulin preparations, however, have not been implicated in HAV transmission. The manufacturing process for these products partitions virus into different plasma fractions, and the presence of anti-HAV in immune globulin preparations can be expected to protect against residual infectious HAV, should it enter this product [14]. Newer viral inactivation methods and nucleic acid testing (NAT) on pooled plasma samples promise further protection from HAV transmission by means of plasma products. Because

of the remote risk of HAV transmission from transfusion, screening of blood donors using NAT technology has not been recommended.

Non-A to E hepatitis

The vast majority of post-transfusion hepatitis cases can be attributed to known A to E hepatitis viruses, mostly HBV and HCV. Occasional cases, however, appear to be caused by as yet undiscovered agents, and several novel candidate viruses have been identified. Hepatitis G virus (GBV-C), a member of the Flaviviridae family, is transmitted predominantly by the parenteral route. Infection occurs frequently among those infected with HCV and HIV. Approximately 2% of blood donors and 15% to 20% of injection-drug users in the United States have detectable GBV-C RNA. Additional persons have antibodies against E2 envelopes protein in the absence of GBV-C RNA, suggesting viral clearance. Epidemiologic studies have shown no association of GBV-C infection with liver disease [15,16], but have demonstrated a delay in disease progression for those co-infected with HIV [17]—an intriguing observation that is not yet fully understood.

The TT virus, a nonenveloped DNA virus discovered in 1997, has a 1% to 2% prevalence in North America. It is transmitted by transfusion, but its presence has not yet been correlated with liver disease. Another entity, the SEN virus (SENV), was identified while investigating patients for possible TT virus agents. It is a member of the Circovirus family, characterized by circular DNA contained in a nonenveloped, round nucleocapsid. Despite an initial report of two patients with transfusion-associated non-A to E hepatitis harboring SENV variants (SENV-D and SENV-H), subsequent reports have failed to link SENV with clinical hepatitis [18,19].

To date, neither GBV-C, the TT virus, nor SENV has borne out initial indications that they represented clinically significant viruses; hence donor screening has not been implemented. It is expected that identification of new putative hepatitis viruses will continue, perhaps closing the perceived gap in the list of known causative agents of infectious hepatitis.

Retroviruses

Since the first reports of HIV transmission by transfusion appeared in the early 1980s, this retrovirus (so named after reverse transcriptase, the enzyme that allows retroviruses to transcribe viral RNA into DNA that is inserted in the host genome) has become the best known TTV and has had a dramatic impact on public perceptions of blood safety. Owing to a series of incrementally effective preventive measures, the risk of HIV-transfusion-transmitted infection has declined remarkably in high-income countries and can now only be estimated through mathematical modeling [4,20]. The most recent estimate projects a residual risk of approximately 1 in 1.9 million transfusions [3]. Blood donor screening with currently available antibody

and nucleic acid assays is highly sensitive and reliable. Unfortunately, low-income nations, many of which are burdened with appallingly high prevalence of HIV, lack the resources and infrastructure to screen their blood supply consistently for contamination with HIV and other TTVs. Because rates of HIV infection are still reaching new populations, particularly in Eastern Europe and Asia, HIV qualifies as an emerging virus in parts of the world. Another cause of concern is the appearance of new subtypes and the overall increasing genetic diversity of HIV [21]. Blood donor screening tests were initially developed and validated to detect the main group (group M), subtype B HIV-1 virus: a reflection of the predominance of this HIV strain in North America and Europe. Worldwide, however, non-subtype B strains of HIV-1 group M predominate. In the past, existing antibody assays did not perform as well with non-subtype B viral strains [22], causing concern that infections with such strains could be missed. Ongoing surveillance studies monitor the emergence of new HIV variants [23] and the effectiveness of routine HIV test kits in detecting these viruses.

Human T-lymphotropic virus I and II (HTLV-I and -II) are closely related retroviruses in the oncovirinae group that were identified as TTVs shortly after the discovery of HIV [24]. As with HIV, the risk of HTLV transmission by transfusion has plummeted in the United States since the late 1980s when blood donor screening began [3]. As a result, these viruses are currently not considered a rising threat from a transfusion-safety perspective.

Human herpes viruses

Human herpes viruses (HHV) are enveloped, structurally complex double-stranded DNA viruses that cause common infectious diseases, usually associated with life-long carrier states and the possibility of recurrent reactivation infections. They are classified in three subfamilies: alpha-, beta-, and gamma-Herpesvirinae. Of these, only the beta herpes virus cytomegalovirus (CMV or HHV-5) figures prominently in current transfusion practice. Use of blood products from CMV seronegative donors for patients at high risk of serious CMV disease (eg, stem cell transplant recipients) has been the major strategy for prevention of CMV transfusion complications over the past 4 decades. Since CMV transmission by transfusion generally requires transfer of infected leukocytes, the overall risk of CMV transmission can be expected to have declined significantly since the transition toward an all-leukocyte-reduced blood supply in high-income nations in recent years [25]. Although Epstein Barr virus (EBV) also can be transmitted by transfusion, EBV is ubiquitous in both donors and recipients, and transfusion acquisition is generally asymptomatic or associated with a benign course.

Among the HHVs, only HHV-8, a gamma herpes virus linked to Kaposi's sarcoma, body-cavity-based lymphoma, and Castleman's disease,

might be considered an emerging pathogen. The virus has tropism to lymphocytes and monocyte-macrophages and has been shown to be transmitted by transplantation. Definitive evidence of transmission by transfusion is lacking, however. The seroprevalence of HHV-8 among United States blood donors has been reported in regional studies to be as high as 20% to 25% [26]. However, a recent multicenter study of 1000 donors from five representative United States regions, involving seven state-of-the-art assays, found a seroprevalence of only 3% to 3.5% and no evidence of donor viremia [27]. These findings are in agreement with epidemiologic studies that suggest that sexual contact is the preferred route of HHV-8 transmission, whereas blood-borne transmission is an unlikely event [28,29]. A greater potential for HHV-8 transmission by transfusion may exist in sub-Saharan Africa, where HHV-8 DNA was reported in approximately 20% of investigated blood donors, and fresh, nonleukoreduced transfusions are common practice [30,31].

Parvovirus B19

Parvovirus B19, a nonenveloped DNA virus, has been known to be transmitted by transfusion for decades. It has recently attracted more attention because of the realizations that viremia is relatively common in blood donors, that inactivation of the virus is not easy and reliable, and that recipients of blood and blood products include patient populations that are increasingly vulnerable to infection and its sequelae. The virus causes an exanthematic illness called fifth disease in infants and a generally asymptomatic acute infection in adults [32]. Most infections occur in those 6 to 15 years of age, such that approximately 50% of adults are seropositive for parvovirus B19 antibodies. The virus is classified as an erythrovirus, reflecting its tropism for erythroid progenitor cells through its receptor, the erythrocyte P antigen [33]. The P antigen is expressed on megakaryocytes, endothelium, placenta, fetal liver, and fetal heart cells, but erythroid precursor cells are the most susceptible. Following infection, neutralizing antibodies appear. Patients with sickle cell anemia, thalassemia, and other conditions associated with shortened red cell survival are at risk for developing acute aplastic or hypoplastic anemia following infection. Others who are at risk for aplasia following parvovirus infection include immunodeficient patients, HIV-infected patients, solid organ transplant recipients, and children with malignancies. Immunocompromised patients may develop severe chronic anemia due to persistent parvovirus infection. Acute parvovirus infection during pregnancy may result in fetal loss, neurologic abnormalities, and congenital infection [34]. Red blood cell aplasia and chronic anemia due to parvovirus infection often respond to infusion with immune globulin preparations [35].

Parvovirus can be expected to be present in 1 in 20,000 to 50,000 blood donors, with significantly higher incidence during epidemic periods.

However, protective antibodies are frequently present in the donor, recipient, or both, and only occasional transmissions of parvovirus due to single-donor components have been reported [35]. On the other hand, transmission from plasma-derived products has been a significant concern, because of the near ubiquity of the virus in the large plasma pools that are the source material for plasma products and its resistance to common inactivation methods. Although the presence of neutralizing antibodies in the plasma pool provides some protection, this does not guarantee recipients will not be infected [36]. Prospective studies in previously untreated hemophilic patients who received virus-attenuated factor concentrates demonstrated a persistent 40% risk of parvovirus infection [37]. Fortunately, these patients in general do not suffer serious or long-term hematologic sequelae, regardless of HIV serostatus [37]. Parvovirus DNA has also been detected in albumin preparations, and earlier studies suggested that the routine 10-hour pasteurization step at 60°C in the manufacturing process may not be effective in inactivating the virus. However, a more recent study using a cell culture system for human parvovirus indicates that the virus is rapidly destroyed by heating, consistent with the good safety record of albumin with regard to parvovirus transmission [38]. Recently implemented NAT screening of source plasma to exclude high-level viremic donations from use in manufacturing promises to reduce the risk of parvovirus infection from these products [39]. Further elimination of the virus from manufactured plasma products may be enabled by development of an effective nanofiltration method [40].

New and emerging viruses

Several recent, highly publicized outbreaks of communicable diseases in humans, including SARS [41], monkey pox infection [42], and avian influenza A [43], were caused by viruses usually found in animals, highlighting the problem of an apparent increase in species-to-species transmission of pathogenic viruses. This phenomenon, termed zoonosis, was also responsible for the epidemics of HIV (derived from simian immunodeficiency viruses) and HTLV (derived from simian T-lymphotropic viruses). Zoonotic transmissions and epidemics can be expected to continue and perhaps increase in the future, because of such factors as shrinking natural habitats, increased human activities around wildlife, and more frequent international travel, trade, and migration. When humans are exposed to new viruses in this way, the lack of immunity in the population has the potential to cause devastating pandemics such as the past global outbreaks of influenza and the ongoing AIDS pandemic. With the exception of West Nile virus (discussed in detail later in this article), none of the viruses that may be considered emerging human pathogens are blood-borne organisms in the classical sense, and they are therefore not likely to be

transmitted by transfusion. Nevertheless, episodes of viremia have been documented in SARS [44], prompting temporary prophylactic deferral of blood donors at risk for the disease. SARS is probably not unusual in this respect; it can be assumed that periods of asymptomatic viremia also occur in other zoonotic infections that have caused disease outbreaks in humans. Another unknown but potential threat is that zoonotic viruses might acquire new mutations as they adapt to the human host that could change their mode of transmission and alter other properties, increasing transmissibility and pathogenicity in general [45].

A recent object of concern with regard to emerging viral infections and transfusions is well known human viruses that cause common communicable diseases, such as enteroviruses. Although these may cause symptomatic infection and mild self-limited disease in healthy adults, they can cause severe symptoms in vulnerable populations such as infants, immunosuppressed patients, and the elderly. Researchers in Scotland have identified seasonally fluctuating viremia involving enterovirus species in approximately 1 in 4000 Scottish blood donors (Fig. 1) [46]. The significance of this finding for disease transmission and the need for routine donor screening and other preventive measures to protect the blood supply have not yet been established, but are obvious areas for further investigation.

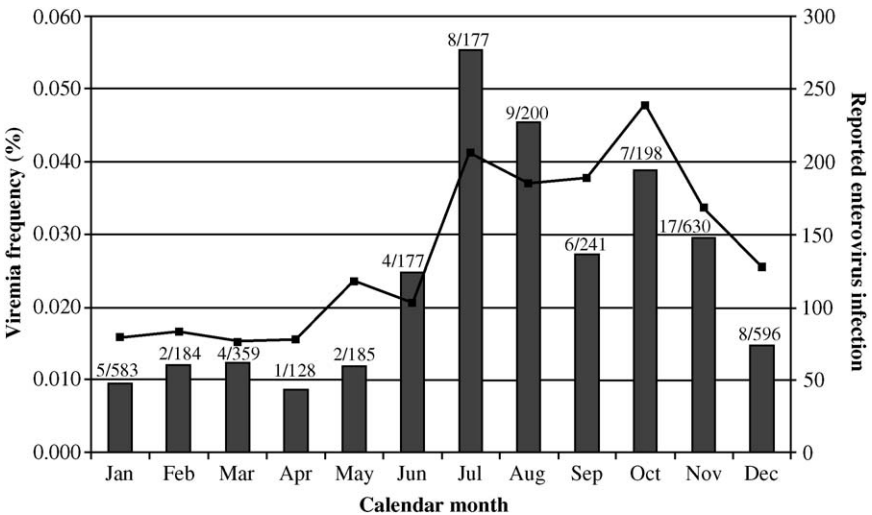


Fig. 1. A comparison of the monthly observed frequency of enterovirus RNA detection in Scottish blood donors (bars) during 1999–2001 with clinical isolation of non-polio-enteroviruses (solid squares) reported to the Scottish Center for Infection and Environmental Health for the same time period. (From Welch J, Maclaran K, Jordan T, Simmonds P. Frequency, viral loads, and serotype identification of enterovirus infections in Scottish blood donors. *Transfusion* 2003;43(8):1063; with permission.)

West Nile and other mosquito-borne viral infections

West Nile virus (WNV) is a single-stranded RNA virus of the Flavivirus family and a member of the Japanese encephalitis virus serocomplex that includes Japanese encephalitis virus and St. Louis encephalitis virus [47]. Viruses in this complex are arthropod-borne or arboviruses (ie, transmitted by mosquitoes and other arthropod vectors) with the potential to cause meningoencephalitis. WNV was first isolated in 1937 in the West Nile District of Northern Uganda and derives its name from that region. Over the next several decades its geographic range was found to extend over eastern and southern Europe, Africa, the Middle East (including Israel), Russia, and western and southern Asia (especially India). The natural life cycle of the virus includes certain species of female mosquitoes as vectors, with birds serving as the primary vertebrate hosts that replicate the virus to high titer (amplifying hosts). Humans and other mammals (particularly horses) are incidental hosts, with transmission occurring through bites of infected mosquitoes. Peak transmission occurs in the late summer and early fall.

There were no cases of WNV infection in North America (or the entire Western Hemisphere) before a WNV outbreak in New York City in summer 1999 [48]. The virus became dormant during the winter months and re-emerged to cause a small number of human cases during the summers of 2000 and 2001. However, a much larger epidemic occurred in the United States in 2002, with significant geographic spread including westward migration (Fig. 2). Over 4000 cases and 284 deaths were reported in 40 states [49].

Transmission of WNV by transfusion was considered biologically plausible given that asymptomatic WNV infection is associated with a brief, up to 2-week period of viremia [50]. In late August 2002, a case of WNV transmission from an organ donor to four recipients was documented, and evidence was obtained indicating that the organ donor was likely infected by one of over 60 transfusions given before organ harvest [50,51]. Over the next several months intensive investigations confirmed 23 cases of transfusion-transmitted WNV infection in the United States in the summer and fall of 2002. Twelve of these transfusion recipients developed meningoencephalitis and several died. All types of blood components (red blood cells, platelets, and fresh frozen plasma) transmitted infection. The severity of infection appeared to be to a large degree dependent on the recipient, with more severe outcomes seen in immunocompromised patients.

The Food and Drug Administration took a number of actions to lower the risk of transfusion transmission [50]. Policies for management of donors with proved or suspected WNV infection were implemented, along with policies for product quarantine/retrieval and notification of transfusion recipients. In December 2002, a recommendation was made to voluntarily withdraw from hospital and blood center inventories selected frozen

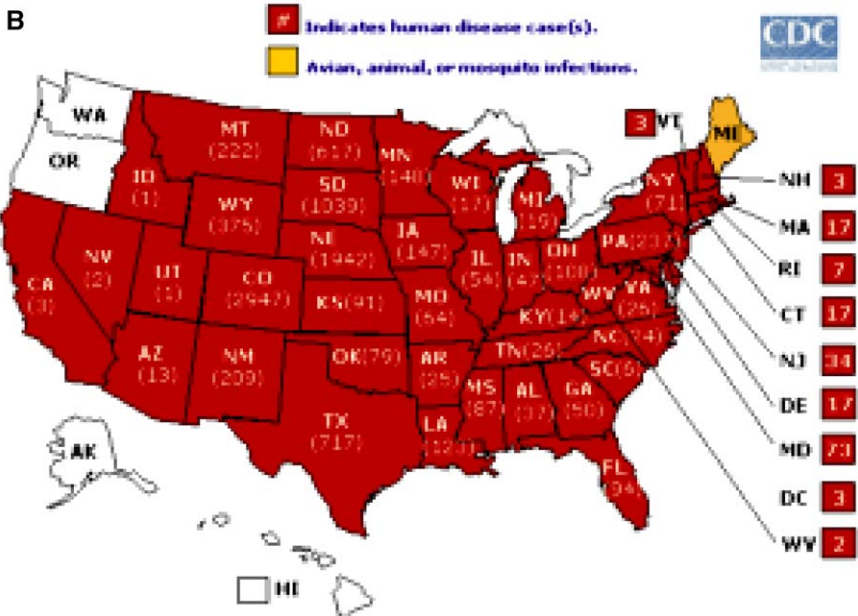
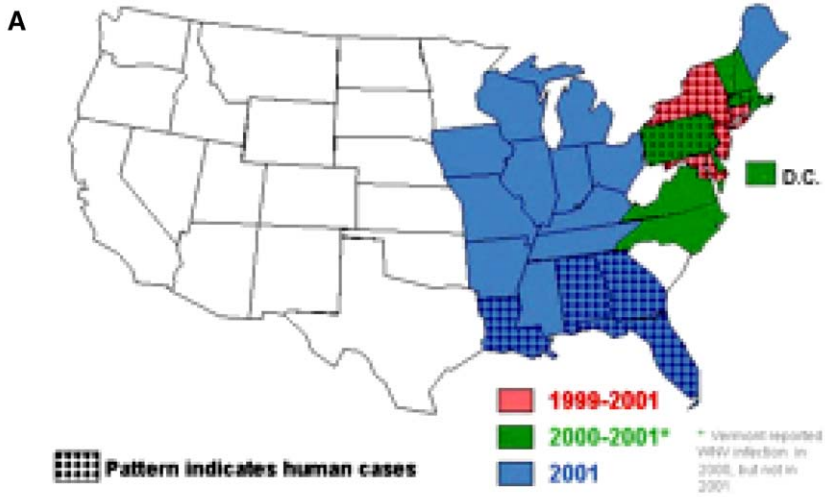


Fig. 2. (A) Geographic distribution of West Nile virus infections, including human cases (checkered pattern) from 1999–2001. This period marks the beginning of the current United States epidemic that flares and spreads during the summer months, coinciding with the mosquito season. (B) The reach of the epidemic in 2003, with the number of human cases shown for each involved state. A total of 9858 cases and 262 WNV-related deaths were reported to the Centers for Disease Control and Prevention in 2003, approximately twice as many cases as in 2002 but with a slightly lower number of deaths (284 in 2002). The majority (85%) of viremic blood donations were observed in the nine central-western states, coinciding with the highest numbers of reported cases. (From CDC, Division of Vector-Borne Infections Diseases, West Nile Virus, <http://www.cdc.gov/ncidod/dvbid/westnile/surv&control03Maps.htm>. Last accessed: June 17, 2004.)

products collected during the 2002 mosquito-borne transmission season in areas of the country that had documented mosquito-borne transmission of WNV to humans.

Blood donor screening with NAT assays was implemented with unprecedented speed and was in place throughout the United States in early summer 2003, just in time for the year's main mosquito season. From late June to December 2003, approximately 6 million donations were screened for WNV, yielding over 800 (0.01%) presumptive viremic donations based on repeated positive NAT results on donor plasma samples (Fig. 3). The majority of WNV-positive blood donations originated in central western areas of the United States, consistent with the predicted westward migration of the outbreak.

As in HIV and HCV blood donation screening, samples were tested for logistical reasons in minipools of 16 to 24 (MP-NAT), a process that lowered the sensitivity of the WNV NAT assay and potentially allowed for breakthrough transmissions by units with low-level viremia. Overall, six cases of confirmed or probable transfusion-transmitted WNV were documented in 2003 [52]. The median age of affected recipients was 63 (range 13 to 82); four had WNV encephalitis, one had West Nile fever, and one critically ill patient did not have discernible WNV-compatible illness despite confirmed WNV infection. Each had received multiple blood components, including single infectious units collected during the summer months of

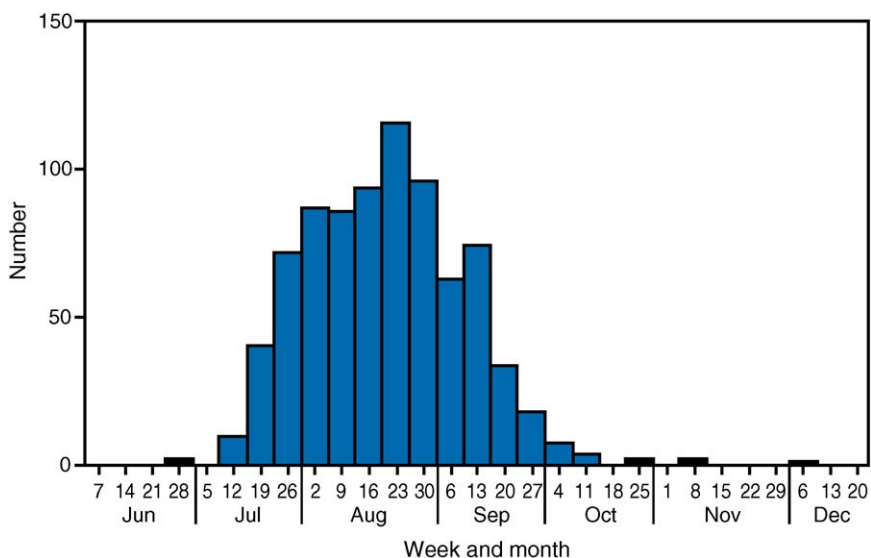


Fig. 3. The number (total $N = 818$) of United States blood donors with presumed viremic WNV infection by week of donation from June to December 2003. (From Centers for Disease Control and Prevention. Update: West Nile virus screening of blood donations and transfusion-associated transmission—United States, 2003. *MMWR Morb Mortal Wkly Rep* 2004;53(13):282.)

2003. The presumptive transmitting donations were nonreactive by pooled screening but tested positive upon retrospective testing on an archived undiluted sample, demonstrating the capability of WNV to be transmitted by transfusion at very low levels (estimated median viremia from four of the six transmission cases was 0.11 plaque-forming units/mL). None of the donors reported WNV illness before or after donation. The documentation of these breakthrough infections has led to implementation of strategies for conversion from MP-NAT to single donation NAT in collection regions with high WNV activity, as evidenced by clinical case reporting or MP-NAT yield rates.

Another member of the Flaviviridae family, also transmitted to humans by the bite of an infected mosquito, is the dengue fever virus [53]. The clinical syndrome most often caused by dengue is an acute flu-like illness; meningoencephalitis is rare. The rarer, more severe complications of dengue are thought to require an initial infection with one strain of the virus and a second infection with a different strain. Dengue is widespread in tropical regions of the world, including Central and South America and the Caribbean islands, and the number of cases in these regions has increased over the last several decades. Cases of dengue fever are rare in the United States and Canada, and there is no evidence of local mosquito-borne transmission in this part of the continent.

To date, no transfusion-transmitted cases of dengue have been reported anywhere in the world. Nevertheless, transfusion transmission of arboviruses other than WNV may be expected, since there is an acute viremia of a few days to perhaps 2 weeks following exposure. In the United States these arboviruses would most likely be eastern equine encephalitis, western equine encephalitis, St. Louis encephalitis, and La Crosse encephalitis [53].

Bacterial contamination

Bacterial contamination of blood components, which may result in septic reactions, even death, in transfusion recipients, has been a well-recognized threat since the early days of the procedure [54]. There is no indication that bacterial contamination per se is on the rise, but continual increase in the use of platelets, the blood component associated with the vast majority of septic reactions, has resulted in an overall increase in and awareness of bacteria-induced transfusion reactions. Indeed, bacterial sepsis is currently the number one cause of acute transfusion-related mortality linked to an infectious agent. In addition, the declining risk of HIV and hepatitis transmission has played a role in focusing attention on bacterial contamination as a re-emerging infectious risk of transfusion.

As stated, the risk of septic reactions, classically characterized by fever, chills, and rigors that potentially progress to septic shock, is far greater with platelets than with red blood cells. The primary reason is the required room-

temperature storage of platelets, a procedure that, despite restriction of storage length to 5 days, provides far better growth potential for bacteria than 42-day refrigerated red blood cell storage. Notably, autologous red cell transfusion has also been associated with a higher risk of septic reactions than has administration of allogeneic red cells [55], a finding readily explained by the generally poorer health of autologous donors, higher rates of unrecognized bacteremia, and application of less stringent donor selection criteria in autologous donation [56].

The prevalence of bacterial contamination in allogeneic blood components, detectable by culture of components, has been estimated at approximately 1 in 3000 for platelets and 1 in 30,000 for red cells [57]. In a recent 2-year national study in the United States, serious septic reactions were reported in approximately 1 in 100,000 platelet transfusions and 1 in 5 million red cell unit transfusions [58]. The rates for fatal reactions associated with platelet and red cell transfusions were 1 in 500,000 and 1 in 10 million, respectively. Owing to lack of clinical awareness and reporting, these figures undoubtedly represent a low estimate; significantly higher rates have been documented by surveillance studies in single institutions [54].

Microorganisms isolated from contaminated red cell units include *Staphylococcus epidermidis*, *Serratia liquefaciens*, *Pseudomonas* species, and *Yersinia enterocolitica*. The last of these, a gram-negative endotoxin-producing organism, is remarkable for its preference for growth at colder temperatures in iron-enriched environments. Gram-positive skin saprophytes account for most of the organisms contaminating platelet concentrates, with the remaining contaminations attributed to gram-negative organisms associated with occult bacteremia. Most fatal reactions are due to endotoxin reactions associated with gram-negative bacteria. Platelet concentrates contaminated with *Bacillus* species, *Escherichia coli*, *Klebsiella* species, *Staphylococcus aureus*, *S epidermidis*, *Serratia marcescens*, and *Streptococcus* species account for 85% of fatal reactions [59]. Moreover, although the bacterial content in a contaminated component at the time of collection may be exceedingly small, bacteria propagate during component storage (unlike viruses), resulting in a many log increase in concentration and release of endotoxin and other toxic metabolites. The minimum bacterial doses in platelet concentrates needed to produce morbidity or mortality are not known precisely, although bacterial concentrations of 10^8 colony-forming units per mL have consistently resulted in fatalities [60].

Time-honored approaches to preventing bacterial contamination include selection of healthy blood donors and adherence to strict aseptic techniques at likely entry points of bacteria, such as blood donation, component preparation, transport, storage, and infusion. In a determined effort to further reduce bacterial contamination and septic transfusion reactions, US Blood Centers and Transfusion Services were expected to implement systems that detect and limit bacterial contamination in platelets in early 2004 [61]. Most detection systems require sterile sampling of platelet components

24 hours after phlebotomy (to allow viable bacteria to propagate to detectable levels). Approximately 4-mL to 10-mL samples of the platelet component are inoculated into automated culture systems where bacteria are detected through evidence of oxygen consumption or carbon dioxide generation in enriched growth media. Though generally successful, these methods have natural limitations due to slow growth rates and the lack of easily detectable metabolic products in some pathogenic bacteria. Several non-culture bacterial detection systems have also been described [54]. Finally, more effective prevention of bacterial contamination can be achieved by the use of newer skin disinfection solutions during donor phlebotomy and diversion of the first 15 mL of collected blood (which are then used for blood typing and infectious disease screening). These methods alone or in combination may reduce the bacterial load in skin fragments trapped in the phlebotomy needle that subsequently enter blood storage containers [62,63].

The outlined measures aimed at reducing bacterial contamination of blood components are likely to have a significant positive impact on the number of septic transfusion reactions. Concerns are being raised, however, about unwelcome consequences of these efforts. These consequences could include significant increases in costs and more frequent platelet shortages due to higher discard rates and shorter windows during which platelets are available for distribution. It is to be hoped that evidence of lower bacterial contamination rates attributable to the new safeguards will permit return to a 7-day platelet storage period, which was standard before rising rates of septic reactions in the 1980s forced implementation of the current 5-day storage period. A longer distribution period for platelets could at least partially offset financial losses and raise the availability of this essential blood component [64].

Tick-borne bacterial infections

Ticks are an increasingly recognized vector of bacterial zoonoses; examples of infections they transmit to humans include Lyme disease, ehrlichioses, and rickettsioses [65]. Reports of tick-borne diseases, which also include viral and parasitic infections (among them babesiosis, which is discussed under parasites), have risen sharply in the United States in recent years [66,67]. This increase may be in part due to newly instituted reporting requirements of tick-borne infections to state health agencies, but one may speculate that there is also a true increase caused by factors such as the widening geographic range of tick vectors in the United States, the spread of residential areas to tick habitats, and the increasing popularity of outdoor sports and leisure activities that bring humans in contact with the vector.

Lyme disease, caused by the spirochete *Borrelia burgdorferi*, is the most common vector-borne illness in the United States, with more than 23,000 infections in 2002 [68]. The disease is transmitted to humans by bites from

infected ticks of the species *Ixodes scapularis* or *pacificus*. Spirochetemia probably occurs postinfection and may be present in asymptomatic persons. Nonetheless, no clinical or serologic evidence of transfusion-transmitted Lyme disease was demonstrated in cardiothoracic surgery patients receiving blood collected in New England during the peak deer tick season [69], and to date there have been no confirmed reports of transfusion-acquired Lyme disease [67].

Other tick-borne pathogens that may be transmitted by transfusion include *Rickettsia rickettsii*, the causative organism of Rocky Mountain spotted fever (RMSF), and *Anaplasma phagocytophilum* (formerly *Ehrlichia* species), the agent responsible for human granulocytic ehrlichiosis (HGE), an acute febrile illness similar to RMSF but without the characteristic petechial rash. Single cases of transfusion-transmitted RMSF and HGE were reported in the late 1970s and 1990s, respectively [67]. In both cases the transmitting blood units had not been leukocyte-reduced. Because the causative agents are obligatory intracellular bacteria, recent widespread adoption of leukocyte reduction of cellular blood components can be expected to lower the risk of transfusion transmission of these organisms [70].

Parasites

A variety of parasitic infections are transmissible by transfusion, most commonly malaria, Chagas' disease, and babesiosis, which are discussed in detail in this section. Rare reports of human transfusion-transmitted trypanosomiasis (African Sleep sickness), leishmaniasis, toxoplasmosis, and microfilariasis have also been reported, primarily in areas endemic for these organisms [71,72]. In accordance with the natural geographic distribution of the major blood-borne parasites—with the exception of babesiosis, which is found in areas with temperate climates—transfusion-transmitted parasitic infections are primarily a concern of tropical and subtropical countries, where a significant proportion of prospective blood donors are afflicted. The problem is compounded by the lack of resources and infrastructure in many of these nations, which interferes with effective preventive measures [73]. However, increasing international travel, tourism, and human migration from countries where transfusion-transmissible parasitic infections are endemic raise concern about an increase in transfusion-transmitted parasitic disease in areas that are not naturally affected.

Malaria

In the United States, malaria occurs in travelers, military personnel, and immigrants from endemic countries. Occasional cases result from mosquito transmission, blood transfusion, or organ transplantation. Approximately

three transfusion-associated malaria cases occur per year in the United States, with a reported incidence of 0 to 0.2 cases per million units transfused during the 5-year period from 1993 to 1998 [74]. Malarial parasites *Plasmodium falciparum*, *P vivax*, *P ovale*, and *P malariae* maintain viability in red blood cells (RBC) stored at 4°C, in platelet concentrates stored at room temperature, and following RBC cryopreservation and thawing. Malaria is not transmitted by RBC-free components such as fresh frozen plasma and cryoprecipitate.

The incubation period following transfusion ranges from 7 to 50 days (average 20 days). The clinical presentation includes chills, fever, and splenomegaly. Fatigue, nausea, vomiting, headache, and diarrhea may also occur. Anemia may be severe, with associated hemoglobinemia and hemoglobinuria, especially with *P falciparum* infection. Glucose-6-phosphate dehydrogenase-deficient RBC are resistant to malaria. Persons lacking Duffy RBC antigens are refractory to *P vivax* infection [75]. Patients with sickle cell trait have partial resistance to *P falciparum*. Babesiosis (see next section) should be considered in the differential diagnosis.

In endemic countries, where the vast majority of donors and recipients have been previously infected, all recipients are administered inexpensive prophylactic medication before transfusion. Prevention of transfusion-transmitted malaria in nonendemic countries currently relies on deferral of blood donors emigrating or returning from malaria-endemic regions, an imperfect strategy that misses some infected donors and excludes many noninfected people from donation. Currently available laboratory tests are not helpful in most blood donor screening settings, as they are impractical, insensitive, or too nonspecific. Experimental assays for combined detection of antimalarial antibodies and antigens [76] and a NAT-based assay [77] are under evaluation in several countries (eg, Great Britain) with large immigrant populations from malaria-endemic countries. Preliminary data suggest that these assays may prove useful in the future.

Babesiosis

Babesiosis is a malaria-like zoonosis in which humans are infected incidentally, usually through the bite of an infected tick of the genus *Ixodes*. Most cases of human babesiosis in North America are caused by *Babesia microti*; the pre-eminent species in Europe is *B divergens*. Babesia infections are usually asymptomatic or associated with mild flu-like symptoms. However, immunocompromised individuals are at risk for life-threatening disease [78]. Endemic to the Northeast and upper Midwest of the United States, the disease has more recently been recognized in eastern and western regions of the nation as well [79,80]. More than 40 cases of transfusion-transmitted babesiosis associated with either RBC or platelet transfusions have been reported in the United States since 1980 [81]. Persons with

a history of babesiosis are deferred from donating blood, but no blood donor screening test or other effective means of detecting asymptomatic carriers of the parasite currently exists [67,82].

Chagas' disease

American trypanosomiasis or Chagas' disease, named after the Brazilian physician Carlos Chagas who rendered the first description in 1909, consists of a generally asymptomatic or mild, self-limiting acute illness that resolves within 4 to 8 weeks and a chronic phase marked by cardiac disease, megacolon, or achalasia that occurs in up to 30% to 40% of infected patients after a long latency period [83].

The causative agent is the flagellate protozoan parasite *Trypanosoma cruzi*. The disorder is limited to the Western Hemisphere, where it is widespread in Latin America from Mexico to the lower half of the South American continent. The parasite is transmitted to humans through bites from *T cruzi*-infected insects of the Reduviidae family. Efforts to eradicate the vector have resulted in a decrease in new infections in endemic areas. Infected persons maintain a low-level, intermittent parasitemia that usually persists for life; treatment is only effective in eradicating the parasite when rendered during the initial acute stage. Vector transmission is unlikely outside endemic areas, but 50,000 to 100,000 infected Latin American immigrants reside in the United States, and congenital transmission may contribute to the reservoir of infected persons among immigrant communities [84]. At least six transfusion-associated cases of Chagas' disease have been reported in immunocompromised patients in the United States and Canada since 1989. In all cases the implicated donors were *T cruzi*-infected Latin American immigrants, and in five of the six cases platelets appeared to be the transmitting component [85]. The low number of confirmed cases of transfusion-transmitted Chagas' disease in North America may be misleading, because they involved fulminant disease in immunosuppressed patients; transmissions in immunocompetent recipients with asymptomatic or mild acute disease may be overlooked.

The prevalence of *T cruzi* infection among United States blood donors varies widely. Nationwide estimates suggest a rate of 1 in 25,000 donors, whereas communities with a large Latin American immigrant population have three to four times higher rates [85,86]. The parasite may survive 2 to 3 weeks of cold storage and cryopreservation in blood components [71], but the risk of transmission of *T cruzi* from donors residing in the United States remains unclear. In a survey of 18 recipients who received blood from a seropositive donor and were available for testing, none had evidence of infection [85]. Nevertheless, transfusion-transmitted Chagas' disease is seen as a rising concern in the United States, and universal screening for infected blood donations may be implemented once suitable screening and confirmatory assays are licensed.

Experience from South America, where blood donors are routinely screened with tests for antibodies against *T cruzi* antigens, demonstrates the insufficient sensitivity and specificity of current antibody tests but suggests that improved performance may be achieved with newer multiantigen assays [87]. Pathogen reduction methods with psoralens [88] or other agents and, to a lesser degree, leukocyte reduction [88,89] offer alternative approaches to reducing transfusion transmission of the parasite. Leukocyte reduction, which appears to be only 40% to 50% effective, has been widely adopted in North America and Europe for reasons unrelated to its effect on transmission of *T cruzi*. Reliable pathogen reduction methods are still essentially limited to plasma products, with methods for treatment of platelet and red cell components currently in clinical trials [90,91].

Classical and variant Creutzfeldt-Jacob disease

Classical Creutzfeldt-Jacob disease (CJD) is a rare, fatal, degenerative neurologic disease with a long asymptomatic latent period that was first described in 1920. The causative agent of CJD is thought by most experts to be a prion protein (PrP^{sc}), an abnormal conformation of a normal cellular protein (PrP^c) that can induce conformational transformation (recruitment) of additional PrP^c to PrP^{sc}, resulting in deposition of insoluble precipitates in neural tissue and progressive dementia [92]. CJD is one of a variety of prion diseases of humans that occur spontaneously at a rate of approximately 1 per million throughout the world and can be transmitted vertically in familial conditions such as Gerstmann-Straussler-Scheinker syndrome or horizontally through ritualistic cannibalism (kuru).

There are no reported cases of transmission of classical CJD by blood transfusion. Nevertheless, because of the long incubation phase of the disease (as demonstrated from growth hormone transmissions), concern arose in the mid-1990s that CJD transmission could occur from asymptomatic donors to blood transfusion recipients [93]. This theoretic risk led to the establishment of enhanced donor deferral policies (based on iatrogenic exposure or family history of the disease) for potential CJD carriers. Several recent epidemiologic studies have confirmed earlier studies in failing to establish a link between transfusion and transmission of CJD [93]. Although it is still regarded as a theoretic risk, there is an emerging consensus that classical CJD is not transmitted by transfusion.

Like classical CJD, variant (v) CJD is a fatal, degenerative neurologic disease, although it occurs in younger persons and has distinctive clinical, histopathologic, and biochemical features, including the presence of readily detectable prion protein in non-central nervous system lymphoreticular tissues such as appendix, spleen, tonsil, and lymph nodes. In contrast to classical CJD, vCJD disease is new, first reported in the United Kingdom in 1996 [94]. Over the first several years of investigation, it was proved that the

causative agent of vCJD (probably also a prion) is the same agent that causes bovine spongiform encephalopathy (BSE). A massive epidemic of BSE occurred in Great Britain in the 1980s and early 1990s as a result of the recycling and processing of material (offal) from dead sheep and cattle into food meal for cattle. Although this practice was stopped in the mid-1990s following appreciation of the BSE epidemic, an estimated 250,000 cattle had already been infected with BSE. Transmission of the BSE prions to humans occurred by oral consumption of beef and other cattle products containing reticular endothelial or neural tissue, resulting in a delayed outbreak of vCJD in the United Kingdom (Fig. 4).

Several observations raised the theoretic concern that vCJD could be spread by blood transfusion. These included (1) the unknown but

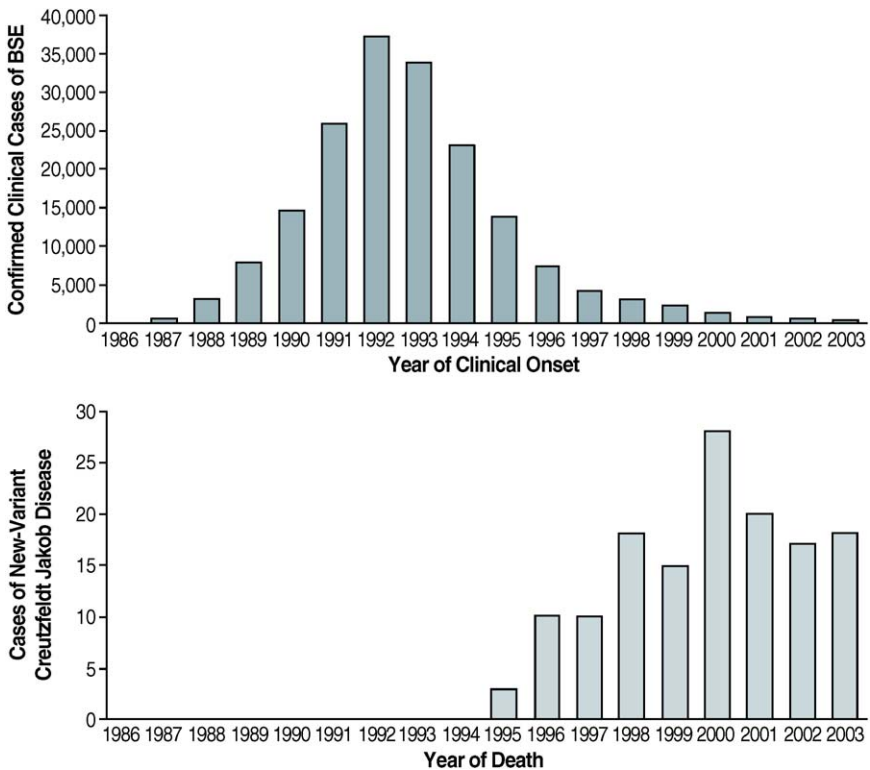


Fig. 4. Numbers of confirmed cases of BSE (*top*) vis-à-vis numbers of clinical cases of vCJD (*bottom*) from 1986–2003 in Great Britain. The epidemic of BSE in Britain peaked in 1992, 4 years after the introduction of the ban on feeding tissues of one ruminant animal to other ruminant animals. First cases of the associated human disease, vCJD, appeared several years after the onset of the BSE epidemic. The incidence of vCJD cases appears to be leveling off after reaching a maximum in 2000, and it can be hoped that they will follow the same trend as BSE cases in Britain. If so, the concern about transfusion-transmitted vCJD should decline as well. (From Donnelly CA. Bovine spongiform encephalopathy in the United States—an epidemiologist’s view. N Engl J Med 2004;350(6):540; with permission.)

potentially large reservoir of asymptomatic carriers of vCJD in the United Kingdom and other countries with significant imports of United Kingdom beef products; (2) the possibility of a very long incubation period such as has been observed with other transmissible spongiform encephalopathies; (3) the detection of the vCJD prion protein in lymphoid and other reticuloendothelial cells (dendritic cells); (4) the biologic differences between the prions and pathogenesis of vCJD and classical CJD, which make it unreasonable to extrapolate epidemiologic data about the lack of transfusion transmission of classical CJD to vCJD, and (5) studies on rodent and primate animal models that demonstrated infectivity in blood and implicated transfusion as a possible mode of transmission [95].

Based on the theoretic concern that vCJD could be transmitted by blood transfusion and the initial finding that all known cases of vCJD could be traced to time spent in the United Kingdom, a policy was adopted in 1999 of indefinite deferral of persons who spent more than 6 months in the United Kingdom from 1980 to 1996 [96]. This measure was predicted to reduce the number of donors potentially infected with vCJD by approximately 70% and to result in the deferral of approximately 2.5% of otherwise eligible donors. Following discovery of vCJD cases in continental Europe, the deferral policy was revised to include donors who had spent more than 3 months in the United Kingdom or 5 years in Europe since 1980 [96].

As of April 2004, 146 definite or probable cases of vCJD have been reported in the United Kingdom [97], with an additional six cases originating in France and one in Italy. Four additional reported cases have occurred elsewhere (one each in Canada, the United States [98], Hong Kong, and Ireland) but were likely acquired in the United Kingdom. There have been no significant changes in vCJD epidemiology besides the fact that the potential rate increase of vCJD in the United Kingdom that had been predicted by early worst-case models did not occur.

The possibility that vCJD may be transmitted by transfusion received fresh attention after the recent report of the disease in a transfusion recipient who had received one red cell component from a blood donor who died of vCJD 3 years after the donation [99]. The implicated donor was one of 15 identified in late 2003 from a total of then 145 vCJD cases on Britain's national CJD surveillance unit register. The recipient became ill 6 years after the transfusion and died of vCJD 13 months from onset of symptoms. The diagnosis was confirmed by examination of brain tissue. While statistical analysis suggests it is unlikely that the recipient acquired vCJD unrelated to the transfusion, this possibility cannot be ruled out entirely, and definitive proof of vCJD transmission through blood products in humans is still lacking.

In estimating the risk of transmissibility of vCJD by transfusion, it should be emphasized that the aforementioned report is so far the only one that suggests a direct link. To date, none of the other 47 recipients of blood components donated by the 15 individuals with vCJD in the United

Kingdom registry has been diagnosed with the disease, and the donors who provided blood for four transfusion recipients who were later diagnosed with vCJD are all in good health [99]. It also is encouraging to see that the eventual total number of vCJD cases is declining, with recent reports projecting a range of 205 to several thousand total cases. However, such estimates are critically dependent on the nonquantifiable assumptions that underlie them [100].

Summary

Despite the recent dramatic decline in the risk posed by TTVs, particularly HIV and HCV, concerns remain about contamination of the blood supply with novel and emerging viruses, bacteria, parasites, and infectious proteins (prions). Immediate improvement in blood safety can be expected from the adoption of more effective methods to prevent bacteria from entering blood donations and from mandatory implementation of bacterial detection systems. The overall threat from novel and emerging pathogens will be more difficult to address, because multiple agents are involved and the risks of transmission by transfusion and adverse outcomes in recipients are generally not well defined. Discussions about appropriate measures to protect the blood supply from agents like SARS, vCJD, and West Nile virus are ongoing, illustrating the challenge of finding the right balance between the desire and mandate for safety and the need to maintain an adequate and affordable blood supply. Past experience has shown, for example, that closure of the infectious window periods through implementation of new molecular assays can result in significantly enhanced safety, but at a very high cost. Whereas serologic screening of donors for HBV, HIV, and HCV was essentially cost-neutral (ie, the cost of testing was offset by the savings in prevented infections/disease), the costs for NAT testing exceed \$1 million per infection prevented or per quality-adjusted life year saved [101]. Pathogen-reduction methods currently under development promise effective protection from most TTVs and other pathogenic organisms (with the exception of prions), but these methods could nearly double the cost of platelet and RBC components and are likely to raise new safety concerns when they are moved from the trial stage to clinical practice [90,91,102].

Besides measures that are aimed directly at keeping pathogens from entering the blood supply, such as adoption of more restrictive donor acceptance criteria, expanded blood donation screening, and pathogen reduction methods, better knowledge and understanding of established and potential infectious risks are a prerequisite for a rational protective strategy. Essential tools are surveillance studies capable of analyzing large blood donor populations for emerging pathogens [103] and national programs that provide the resources and infrastructures needed to monitor blood safety and rapidly assess whether and to what extent a pathogen is transmitted by

transfusion [104–106]. It is important that surveillance activities be conducted on a global scale. Outbreaks of new viral diseases often start in developing nations but, by means of international commerce and travel, can spread to any region of the world overnight. Proactive surveillance through collaboration with blood collection programs in developing countries is a critical barrier to such an event, as are efforts to assist local agencies with the establishment of sustainable blood collection, processing, and transfusion systems [73,107]. These measures will both protect recipients who require transfusions in developing countries and, in the long run, enhance transfusion safety in high-income nations.

Assistance in the surveillance effort can be expected from technological advances such as multiplexed nucleic acid testing systems [108], microarray-based detection and genotyping systems for virus discovery and identification (“viral gene chips”) [109,110], and integrated, automated surveillance systems for detecting viruses and other infectious agents in human blood [111]. Given the number, variety, and flexibility of biologic systems, it is safe to predict that we will continue to encounter new and changing infectious agents that will need to be assessed as potential threats to transfusion recipients and prevented from entering the blood supply.

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