Emerging and Exotic Viruses in the Blood Supply

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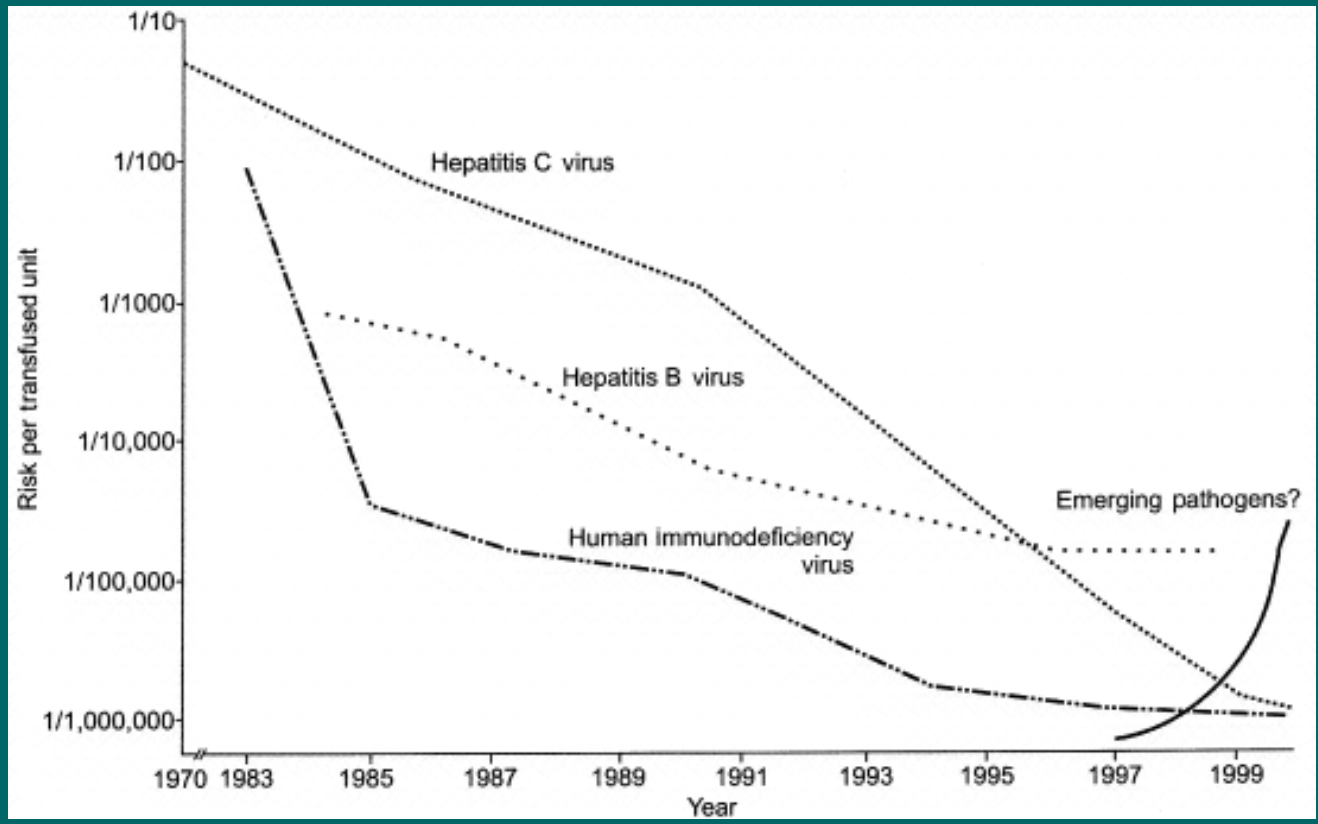
VIDRL

Introduction

- Transfusion safety never greater
- Continued improvements in
  - Donor screening/testing
  - Viral inactivation in plasma products
- Transfusion-transmitted infection risk can never be zero
- Balance between blood supply safety & life-saving blood product availability
- Balance between small margins of benefit and economic factors
- In recent years many emerging infections identified
- Potential risk to the blood supply has needed assessment.

Estimated Risks of Transfusion-Transmitted Disease in the USA 1970-1999



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 Infectious Diseases

"A new, re-emerging or drug resistant infection whose incidence in humans has increased within the last two decades or whose incidence has threatened to increase in the near future."

Institute of Medicine Report 1992

Subclinical Viraemia

- The crux of transfusion risk is circulating virus in the absence of significant clinical symptoms
- Patterns include:
  - Asymptomatic carrier state (HBV)
  - Onset of viraemia prior to symptoms (Parvovirus B19)
  - Low clinical attack rate (West Nile Virus)

"Classic" Transfusion Transmitted Viruses

- Blood-borne viruses: HBV, HIV, HCV, HTLV, (CMV)
- A prolonged asymptomatic carrier state with the infections agent circulating in the donor's blood.
- A significant clinical syndrome.
- Even a moderately prevalent infection could be transmitted quite frequently.
- Blood testing focus predominantly detecting chronic infection markers: antibodies/antigens

Potential Transfusion – Transmissible Emerging and Exotic Viruses

1. New “Hepatitis” Viruses
2. Transmissible spongiform encephalopathies
3. West Nile Virus
4. SARS
5. Pandemic Influenza

Non A-E Hepatitis

- Majority of past transfusion hepatitis attributable to A-E
- Occasional cases suggest yet undiscovered agents.
- Several transfusion transmitted candidates discovered in 1990's
- SEN-Virus, TT-Virus, GBV-C virus (hepatitis G virus)
- Good evidence accumulated for transfusion transmission.
- Studies have not supported initial hepatitis association (or any clinical significance)
- Hence donor screening not implemented

GBV-C/ Hepatitis G Virus

- Discovered in 1995
- Positive stranded RNA virus, HCV-like Flavivirus
- Detection by PCR
- Prevalence varies 1-9%, 1-4% Australian donors viraemic
- Transfusion transmission proven:
  - 15/23 (65%) recipients donations from 2 viraemic donors infected
  - Sequence homology between donors and recipients.
- No illness or LFT abnormality association with infection
- Controversial association with fulminant hepatic failure
- Resolution of 80% of infections, viraemia may persist 10 years/more
- Haematopoietic cells rather than liver probably replication site
- May protect against HIV infection in coinfection

TT - Virus

- Discovered in 1997 by RDA from Japanese Non A-G patients
- Temporal association between TTV-viraemia and elevated ALT in 3/5 patients
- Single-stranded circular DNA virus in family Circovirus
- Global distribution, eg 7-11% USA (cf HCV 0.3%, HBV 0.1%, HGV 1.5%)
- Strong association with blood transfusion:
  - 26% transfused vs. 5% not transfused.
  - Stepwise risk up to 35% in those getting ≥ 13 units
- Clearance 38% year 1, 29% within 5 years, 33% up to 22 years
- Rate of newly acquired TTV identical with hepatitis (23%) or without hepatitis (22%)
- No evidence of impact on severity of co-existent HCV
- No temporal association between ALT & TIV viraemia levels
- Hence, no support for TT-V as a hepatitis virus

Matsumoto et al Hepatology (1999)

SEN Virus

- Discovered in 1999 by Diasorin, Italy
- Circovirus with 55% Na and 37% aa Homology to TTV
- 8 strains with D & H most frequently linked to transfusion
- Marked geographic variation:
  - 1.8% USA
  - 10-22% Japan
  - 8-17% Germany
- Transfused surgical patients 30% viraemic vs. 3% untransfused
- Homology 99% between donor and recipient NA sequence
- Association between transfused volume & SEN-V infection
- 42-68% prevalence in haemophiliacs, and high prevalence in IDU
- SEN-V more frequent in liver disease patients, but causal evidence weak
- No biochemical differences (ALT) between infected/uninfected HAV/HBV/HCV patients.
- SEN-V found in serum of HCC patients, but Japanese study found 76% HCC patients and 75% in controls.
- Undetectable DNA within 6 months in 55%, 39% after 1 year.
- Research on SEN-V diminished in absence of hepatitis causal link

Akiba et al. Transfusion (2005

Human Herpes Viruses

- Only CMV figures prominently in current transfusion practice
- Blood products from CMV seronegative donors for high risk recipients
- Transition toward leukocyte reduced blood supply in developed world
- EBV is transfusion transmittable, but ubiquitous in recipients
- Only HHV-8 (Y- herpesvirus) could be considered an emerging virus
- Transmittable by transplantation
- Likely potential to cause Kaposis sarcoma in immunocompromised recipients
- In developing world, eg sub-Saharan Africa 20%-40% blood donors viraemic and evidence of transfusion transmission
- Multicentre US study found prevalence 3-35% in donors and no viraemia
- Epidemiologic studies have not found blood borne transmission
- Risk factors overlap for HBV, HIV & HCV in developed world.
- Not clear how adequately role has been excluded.

Creutzfeldt – Jacob Disease (CJD)

- Human transmissible spongiform encephalopathy (TSE)
- Caused by a transmissible prion protein
- Most cases sporadic, 10-15% familial
- Small (250) number of iatrogenic cases (contaminated instruments, corneal or Dural grafts)
- TSE demonstrated in the blood of rodents during incubation and disease
- Transfusion transmission of TSE in hamsters demonstrated uncommonly
- No confirmed reports of classical CJD by blood products
- 5 case control studies/2500 patients showed transfusion not a CJD risk factor
- Recipients from blood of donors incubating CJD shows no transmission
- Active surveillance of 12,000 haemophiliacs since 1995 with no CJD cases

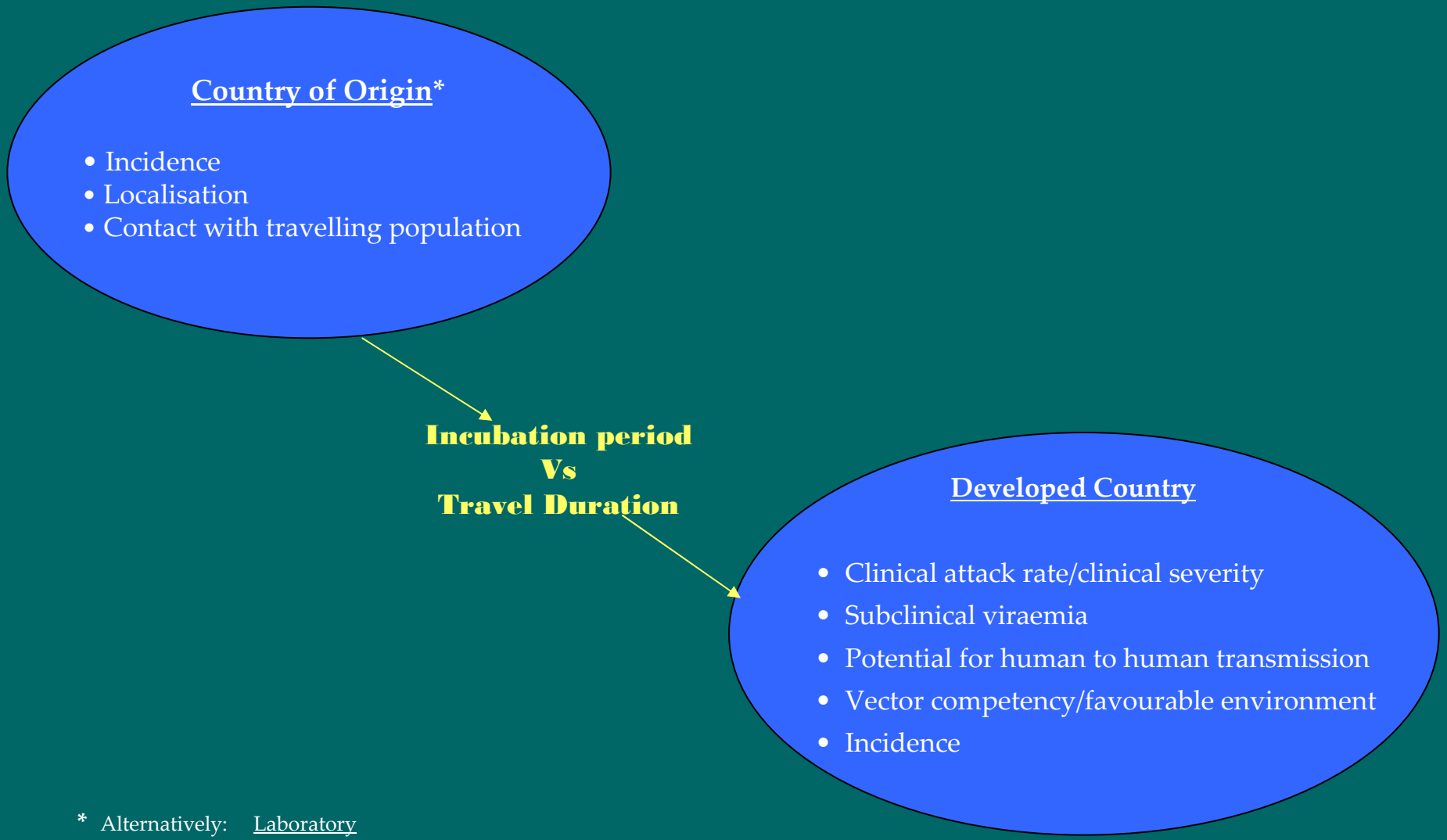
New Variant Creutzfeldt – Jacob Disease (vCJD)

- New variant of CJD (v CJD) recognised in UK 1996
- Same agent responsible for BSE outbreak in cattle
- Cattle fed scrapie contaminated meat/bone meal in 1980s
- vCJD patients younger, & psychiatric manifestations prominent
- 166 cases of vCJD in UK
- Estimates of 4000 individuals harbouring vCJD based on tonsil/appendix screening
- In vCJD widespread replication of agent of PrP^{sc} deposition in lymphoreticular tissues: tonsils, spleen, lymph nodes cf CJD
- 66 individuals received blood products from 18 donors subsequently developing vCJD
- 3/66 subsequently developed proven vCJD 6.5-8.5 years later and 1/66 had PrP^{sc} in lymphoid tissue at time of unrelated death
- Represent 6% total recipients and 12.5% recipients surviving $\geq$ 5 years
- In North America deferral of donors resident in UK 6/12 1980-1996

New Variant Creutzfeldt – Jacob Disease (vCJD) *(continued)*

- BSE and scrapie recently shown to transmit by transfusion in sheep model
- 36% for BSE and 43% for scrapie
- Clear increase in infectivity of blood with time from nil at <20% incubation period to 80% beyond 50% incubation period
- Leucodepletion already introduced in UK in 1999
- Rodent studies had showed infectivity concentrated in buffy coat
- Leucodepletion of blood from scrapie-infected hamsters removed $\leq$ 72% infectivity

Factors Influencing Blood-Borne Transmission of Exotic Virus



* Alternatively: Laboratory
- Biocontainment
- Contact with travelling population

Some Exotic Viruses With Blood Transmission Potential

	Incidence	Geography	Transmission Human to Human	Incubation Period (d)	Clinical Attack Rate	Clinical Severity	Viraemia/ Symptoms
Filoviruses (Ebola, Marburg)	Rare	E. Africa	Inefficient	5-7	High	High	Coincident
Arenaviruses (Lassa)	Locally quite common	W. Africa	Inefficient	5-16	High	High	Coincident
Bunyaviruses (Crimean Congo)	Uncommon	Africa, Middle East Asia	Inefficient	2-12	High	High	Coincident
(Rift Valley)	Endemic with outbreaks	Sub-S. Africa	Mosquito/ Zoonotic	2-6	Low	Low in ≥95%	
Coronaviruses (HCOV-SARS	Rare	S. China	Variable (superspreading)	4-6	High	High	Peaks 10 days post symptom onset.
Flaviviruses (West Nile Virus)	Common	Africa, Middle East Europe, India, Indonesia, (Australia) USA	Mosquito	2-14	Low	Low in majority	

Transfusion Transmission Potential of SARS-CoV

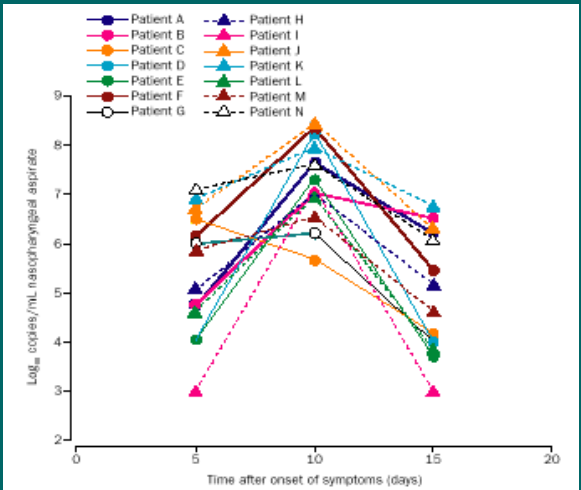


Figure 4: **Sequential quantitative RT-PCR for SARS-associated coronavirus in nasopharyngeal aspirates of 14 SARS patients**

- Transfusion transmission potential of SARS-CoV is unknown
- No Transfusion associated cases among 8422 probable cases
- Typically viraemia peaks 10 days after symptom onset
- Subclinical infection has been described
- Viraemia onset prior to symptoms in some cases not excluded.
- Theoretical risk in Shenzhen calculated to be between:
    - Max 23.57/million
    - Min 14.11/million
    - Highly localised in time to outbreak peak and zero outside this period

Shang et al Transfusion & Apheresis Science 2007

West Nile Virus: Background

- Member of Flavivirus JE serocomplex
- First isolated 1937 Uganda
- Geographic range Africa, Middle East, Russia, W & S Asia, E & S Europe
- Prevalence largely unknown: Nile Delta 6% school children and 4% young adults
- Can cause explosive outbreaks: 55% incidence in Cape Province SA in 1974
- Birds primary hosts, with incidental human and other mammal (horses) hosts
- Peak transmission late summer/early autumn
- Approximately 1% infections cause clinical disease.
- 1-6 day incubation, self limited 3-6 day Dengue-like illness, CNS infection in small %
- No cases in Western hemisphere before 1999

West Nile in the USA

- Emerged 1999 New York causing 62 cases and 7 deaths
- 2.6% Queens residents infected, mostly asymptomatically
- Virus most closely related to 1998 Israeli isolate
- Re-emerged in New York in 2000 (18 cases and 2 deaths) & 2001
- Much larger outbreak in 2002, with significant westward spread:
  (4200 cases and 284 deaths in 40 states)
- Transmission through blood transfusion first documented in 2002
- Transfusion infected organ donor infected 4 organ recipients
- Total of 23 cases of TT transmitted WNV in US 2002.
- 12/23 developed CNS involvement, and several deaths.
- All blood components (RBC, platelets & FFP) transmitted infection

Spread of West Nile Virus USA 1999-2004

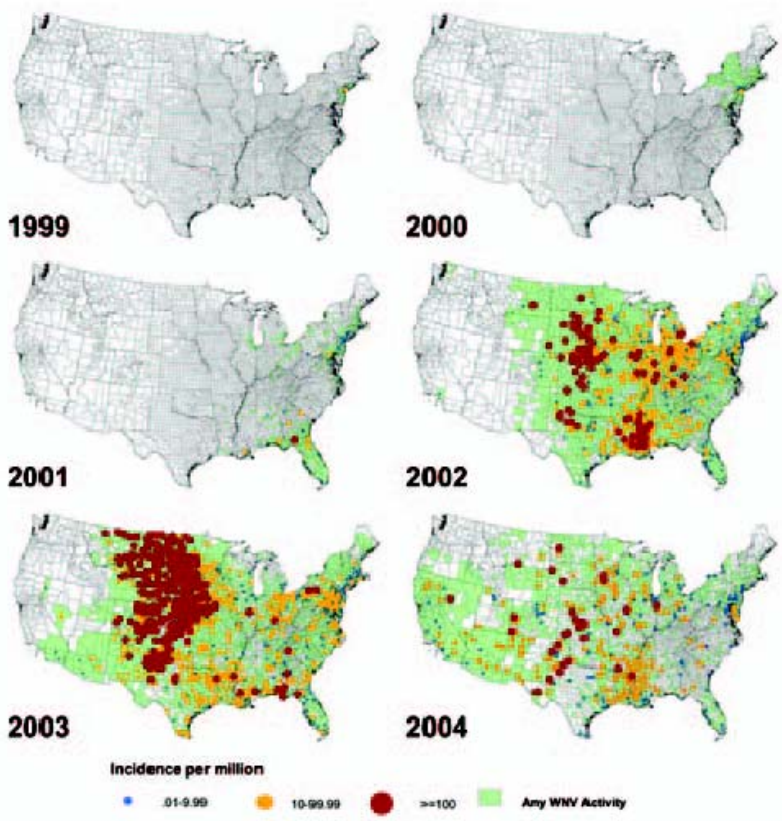


Figure 1. Reported incidence of neuroinvasive West Nile virus disease by county, United States, 1999–2004. Reported to Centers for Disease Control and Prevention by states through April 21, 2005.

Transfusion Transmitted West Nile Infection

- Initially WNV deemed low/no threat to blood supply
  - Slow emergence in USA 1999-2001
  - Absence of chronic infection
  - Brief viraemia
- After recognition of initial TT case, CDC calculated:
  - ≤ 10-15/10,000 units in most seriously affected metro areas
  - If 5% of WNV infected had donated, then ˆ 380 viraemic donations
- Donor screening with experimental NAT assays implemented mid-2003
- July 2003 – June 2005:
  - 27.2 million donations screened
  - 1038 viraemic donors detected
  - Yield 1/26,200
- Platte River area Nebraska August 2003: 1/45 donations reactive.

Transfusion – Transmitted WNV 2002

- 23 infected recipients identified on follow-up (probable underestimate)
- 10 (43%) immunocompromised
- RBC (13), PLT (8) & FFP (3) implicated
- 16 viraemic donors identified (7 linked to 2-3 recipients)
- 2 of 25 recipients not available for follow up
- Donation samples from implicated donors low titre (< 80pfu)
- All implicated donors WNV IgM negative
- Transmission efficient: all recipients of co-components infected
- 9 donors recalled symptomatic illness around time of donation

Donor WNV Screening 2003

- Routine WNV NAT screening of 16 donor minipools from June 2003
- WNV Transcription-mediated amplification system (Procleix)
- Analytical sensitivity on minipools 45 copies/ml
- Individual testing of reactive pools + donor follow up.
- NAT, serology (Focus) + virus load
- Analytical sensitivity on individual donations 4 copies/ml
- Reactive specimens confirmed by Taqman PCR or Bayer

Reviews of WNV NAT Test Data

(i) California

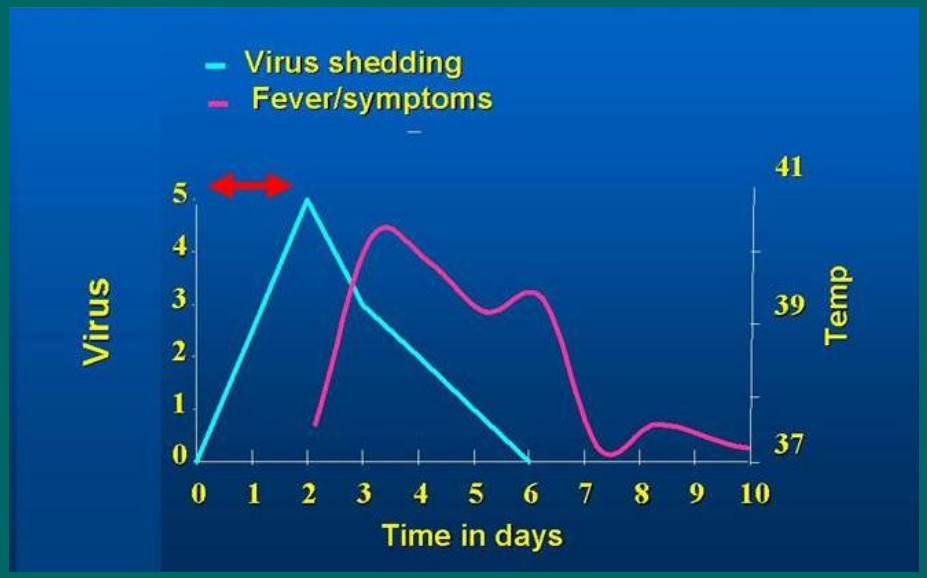
• Retrospective Review of 2003 NAT testing Data			
Period	Donations Tested	Minipool positive	Individual NAT
July-October (inclusive)	677,603	183 (0.027%)	-
September	23,088	0	30
Prospective Testing			
September	3964	3	14

• Serology & Virus loads		
Minipool positive:	167 seronegative 16 IgM positive	5325 copies/ml <50 copies/ml
Individual NAT positive:	31/41 seropositive	At limit of detection
• Follow up of recipients (17 recipients of 14 donations)		
– 2 infections (from seronegative donations) – 2 uninfected (from seropositive donations) – 13 inconclusive		

(ii) Red Cross

- 4 positive minipools + 1/1000 detection frequency individual NAT
- 7 days without NAT positive donations revert to minipools
- In 2003 and 2004 no transfusion associated WNV

Viraemia in Seasonal Influenza



- Acute viral infection viraemias generally short and precede/concurrent with symptom onset
- Influenza incubation period short (1-4 days)
- Symptom onset rapid (over 1 day)
- Shedding brief ≤ 6 days
- Generally no viraemia
- Several reports of viraemia since 1960's
- Presymptomatic/asymptomatic viraemia not documented

Studies of Influenza Viraemia (1)

1962 Influenza H2

- 24 fresh blood pools from 7 acute influenza cases
- 15 pools of frozen samples from 4 cases
- Egg inoculation and culture
- All negative

Minuse et al J Lab Clin Med 59:1016(1962)

1968 Influenza H3

- 21 patients with ILI and 29 healthy contacts
- 12/21 throat washings influenza positive
- No viraemia detected
- 4/29 throat washings influenza positive
- 1 influenza isolation from blood at symptom onset
- Follow-up blood specimens t_{12} and t_{24} negative

Khakpour et al BMJ 420891969)

Studies of Influenza Viraemia (2)

1963

- Low titres of influenza cultured from blood of a patient on day 4 post onset

Nacify K NEJM 269:964 (1963)

1970 Influenza H3

- 5 patients with influenza pneumonia
- 2/5 influenza pos culture from plasma and PBMC

Lehman and Gust Med J Aust 2:1166 (1971)

Studies of Influenza Viraemia (3)

- Influenza A/PR/8 mouse model
  - NP gene nested PCR

Time course study of mice intranasally infected with influenza virus A/PR/8/34

		Days post-inoculation							
Sample	Control	1	2	3	4	5	6	7	14
Lung[b]	- - -	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + -
PBMC	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -
RBC[c]	- - -	- - +	- - +	- + +	- + +	- + +	- - -	- - -	- - -
Plasma	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -	- - -

Detection in mice inoculated with UV-inactivated virus

		Days post-inoculation			
Sample	Control	1	3	5	7
Lung[a]	- - -	- + +	- + +	+ - -	- - -
PBMC	- - -	- - -	- - -	- - -	- - -
RBC	- - -	- - -	- - -	- - -	- - -
Plasma	- - -	- - -	- - -	- - -	- - -

- RBC fraction positive 1-5 days post inoculation
- Abolished with UV pretreatment of virus
- Abolished with pretreatment with hyperimmune serum

Mori et al Microb Pathogen 19:237(1995)

Studies of Influenza Viraemia (4)

- 18 Japanese children 1-14 yrs with influenza
- NP and H3 RT-PCR on PBMC
- 3/18 PBMC PCR pos
- 0/9 influenza H3 patients RBC/WBC culture pos
- 5/17 influenza B patients RBC/WBC culture pos

Tsurnoka et al Nippon Rinsho 10:2714 (1997)

(from abstract of Japanese language paper)

- 4 year old Vietnamese boy with H5 influenza
- Virus isolated from CSF, faeces, throat, serum

de Jong et al NEJM 352:686(2005)

Pandemic Influenza and the Blood Supply

Safety

- Risk of infectious donations?

Supply/ Demand

- Impact on donor availability?
- Impact on the workforce?
- Offsetting effect on demand?

Pathogenesis of reconstructed 1918 Spanish influenza virus (1)

- Genomic RNA from 1918 virus recovered from archived fixed tissues and from victim in Alaskan permafrost
- Complete 1918 H1N1virus created by reverse genetics
- 1918 virus exceptionally virulent in all study systems
- 1918 virus caused necrotising bronchitis/ bronchiolitis and alveolitis with oedema and haemorrhage
- 1918 virus did not spread systematically
- 1918 virus almost exclusively apically released form polarised Calu-3 cells

Tumpey Science 310(2005)

Influenza H5N1

- H5N1 viraemia has been demonstrated in humans and animals
- Viraemia predominantly associated with symptoms
- More study of H5N1 and viraemia needed
- In humans multiple extra pulmonary tissues involved:
  - GI tract, liver, kidneys, (CNS)
- Suggestive of viraemia
- Several reports of virus detected in plasma/serum
- Viral load of 85,000 copies/ml in one
- H5N1 RNA in 9/16 Vietnamese patients in another
- Hence symptomatic H5N1 infection may be associated with viraemia
- Viraemia risk during incubation period/asymptomatic infection incertain

de Jong et al NEJM 2005
Chutinimitkul et al Emerg Inf Diseases 2006
de Jong et al Nat Med 2006

Potential Impact of Pandemic Influenza on the Blood Supply (1)

Impact on Availability

Based on the CDC model (Meltzer, Cox & Fukuda, EID 1999;5:659-71)

- Assume a clinical attack rate of 15% to 35%
- Assume no intervention
- Assume no self deferral by potential blood donors, no reduction in donation due to panic and unwillingness to come out and donate, and no additional deferral by FDA
- Apply to current American Red Cross Blood Service donor population
- 8% to 19% of blood donors could be infected

Zou S Tranf Med Rev 20:181-9(2006)

Potential Impact of Pandemic Influenza on the Blood Supply (2)

Impact on Workforce

- Attack rate of 15% to 35%, no intervention, no reduction in reporting
- Applied to ARCBS employee data
- 8% to 18% of blood supply workers could be infected and not report to duty

Zou S Tranf Med Rev 20:181-9(2006)

Potential Impact of Pandemic Influenza on the Blood Supply (3)

Additional Social Factors

- Some blood donors and blood supply workers may stay home to care for sick family members

- Additional donors and workers may be unable to, afraid to, or unwilling to attend

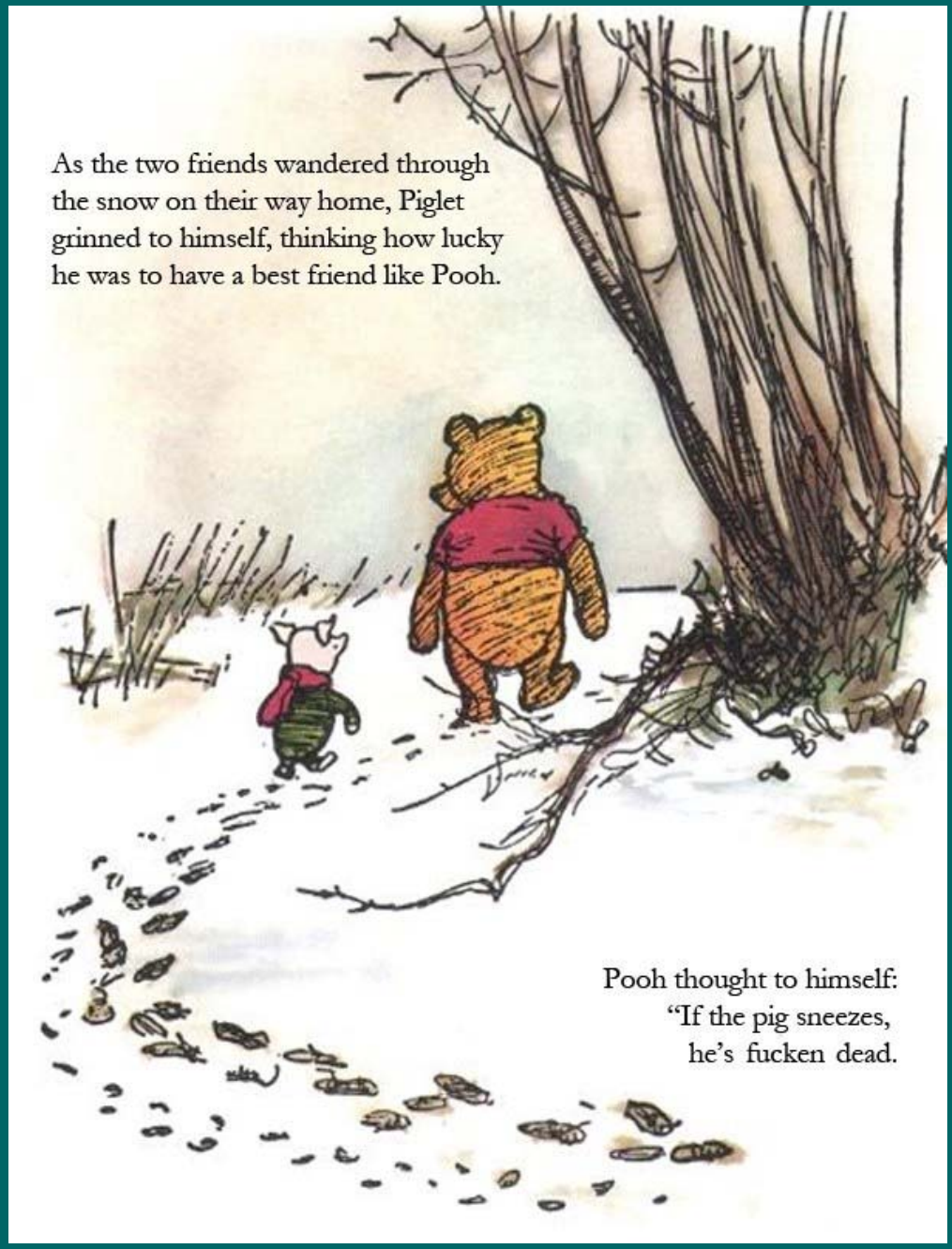
Potential Impact on Blood Demand

- Likely significant decrease in elective procedures
- Likely additional reduction in workforce capacity
- Associated reduction in transfusions
- Balance between supply reduction and demand reduction unquantified
- Differential decrease in demand: whole blood/ platelets possible
- Additional study needed

Proposed Evaluation of Emerging Infection

- Emerging infection identified by surveillance
- Transfusion-transmission biologically plausible
  - Asymptomatic phase with agent present in blood
  - Agent survives in stored blood
  - Susceptible patient population exists
- Investigation of transfusion-transmission potential
  - Determination of prevalence/incidence in population/donors
  - Establish transmission probability
    - Animal model inoculation studies
    - Testing linked donor/recipient and haemophiliac repository samples
- Study of disease consequences in exposed/infected population
- Evaluation of potential interventions
  - Donor deferral via questions/tests
  - Evaluation of removal/inactivation methods in blood components

M. Busch – Transfusion (2006)
Dodd & Lieby – Annual Rev Med (2004)


As the two friends wandered through the snow on their way home, Piglet grinned to himself, thinking how lucky he was to have a best friend like Pooh.
Pooh thought to himself:
"If the pig sneezes,
he's fucken dead.