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Adam Finn • Andrew J. Pollard
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Hot Topics in Infection and Immunity in Children IV

 Springer

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Preface

This book contains chapters based on lectures given by speakers at the “Infection and Immunity in Children” course, held in June 2006 at Keble College, Oxford. It was the fourth annual course of this name and this is the fourth book in the series as well. Together the courses and books have become important components of the training available to paediatricians with an interest in this specialty and we are especially pleased that so many young doctors from all over Europe as well as further afield have attended each year.

The fifth Oxford course was held on 25–27 June 2007 and we expect to produce another edition of this book thereafter, covering another set of important and rapidly developing topics.

Paediatric Infectious Diseases is now a recognised specialty in Europe with established training programmes and training centres, overseen by the European Society for Paediatric Infectious Diseases. We hope that this book and the course that precedes each edition will continue to play a useful part in the development and knowledge of specialists throughout the continent and beyond.

Adam Finn and Andrew J. Pollard

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1

The Enteroviruses: An Emerging Infectious Disease?

The Real, the Speculative and the Really Speculative

Mark J. Abzug

1 Introduction

The human enteroviruses (EVs), which include the polioviruses, Coxsackie A and B viruses, ECHO viruses, and numbered EVs, produce a well-recognized list of clinical manifestations, ranging from mild febrile illness, respiratory infections, and characteristic exanthematous and enanthematous diseases such as hand-foot-mouth disease and herpangina, to potentially severe illnesses such as myocarditis, encephalitis, and neonatal sepsis (Table 1). The EVs have also been linked to a variety of other conditions, including chronic autoimmune diseases, cardiovascular diseases, and neurologic diseases, although definitive proof of causation is lacking (Table 2) (Palacios and Oberste 2005).

As global efforts to eradicate poliovirus-associated poliomyelitis proceed, another EV pathogen, EV 71, has emerged as a major cause of encephalitis and acute flaccid paralysis. In recent years, evidence has been presented that suggest that EVs may be a more common cause of in utero infection than previously recognized and that EVs may possibly be implicated in Chronic Fatigue Syndrome (CFS). This review will describe the emergence of EV 71 as a significant cause of neurologic disease and will examine the evidence for and against an expanded role of EVs in congenital infections and the speculative role of EVs in CFS.

2 Enterovirus 71: A “New” Neurologic Threat

2.1 Modern Epidemiology and Clinical Presentations

EV 71 emerged in the late 1990s as an important neurologic threat in regional outbreaks in East Asia, with an especially large epidemic occurring in Taiwan. These outbreaks predominantly affected children <5 years of age; frequent findings included hand-foot-mouth disease and herpangina (Ho et al. 1999; Lin et al. 2002; Shah et al. 2003). Of greatest concern, a subset of affected children developed severe neurologic disease, most commonly brainstem encephalitis and/

Table 1 Clinical manifestations of enterovirus infections and common associations

Non-specific febrile illness
Non-specific exanthems – Echovirus 9
Respiratory illness
URI
Pleurodynia
Herpangina – Coxsackie A viruses
Hand-foot-mouth disease
Coxsackievirus A16; Enterovirus 71 pandemics
Hemorrhagic conjunctivitis
Enterovirus 70, Coxsackievirus A24; pandemics (tropics, Far East, Europe)
Neurologic signs
Myocarditis/Pericarditis
Coxsackie B viruses (~25–35% cases with proven etiology)
Infections in immunocompromised hosts
Chronic central nervous system infection, disseminated infection
Neurologic diseases
Meningitis, encephalitis
Enterovirus 71 brainstem encephalitis and acute flaccid paralysis
Perinatal infections
Neonatal infection
In utero infection

Table 2 Diseases in which enteroviruses have been implicated

Autoimmune disease
Diabetes mellitus (Type I)
Polymyositis/dermatomyositis
Sjögren's syndrome
Cardiovascular disease
Chronic/dilated cardiomyopathy
Atherosclerosis
Sudden cardiac death/ventricular arrhythmias
Transplant rejection
Neurologic disease
Amyotrophic lateral sclerosis
Sudden infant death syndrome
Chronic fatigue syndrome

or acute flaccid paralysis and, occasionally, other manifestations such as Guillain-Barré Syndrome, transverse myelitis, and cerebellar ataxia. Some children with brainstem encephalitis rapidly developed pulmonary edema and/or hemorrhage with subsequent cardiopulmonary collapse (Ho et al. 1999; Huang et al. 1999; McMinn et al. 2001; McMinn 2002; Chang et al. 2004a; Hsia et al. 2005). This complication had not previously been observed with EV 71 infection, although it had been described in cases of poliovirus-associated bulbar paralysis. Mortality was high in these EV 71 outbreaks, particularly in infants and young children, with deaths occurring within 1–2 days after presentation (Ho et al. 1999; Lin et al. 2002;

Hsia et al. 2005). In one report from Taiwan, of 129,106 cases of hand-foot-mouth or herpangina reported in 1998 by a sentinel physician network, there were 405 cases of severe disease, most of which occurred in children <5 years of age and were caused by EV 71. Nineteen percent of severe cases were fatal; 91% of deaths were in children <5 years and the highest case fatality rate occurred in 7–12-month-old infants (Ho et al. 1999; Lin et al. 2002). Morbidity among survivors included hypoventilation, dysphagia, and paresis (Huang et al. 1999; Hsia et al. 2005). High rates of household and preschool spread and of viral isolation from throat specimens suggested the possibility of respiratory droplet transmission in these outbreaks (Chang et al. 2004b).

2.2 EV 71 Brainstem Encephalitis

EV 71 brainstem encephalitis presents clinically with a constellation of findings that include myoclonic jerks, tremors, ataxia, limb weakness, and cranial nerve palsies and can progress to include seizures, altered consciousness, and increased intracranial pressure. Rapid development of pulmonary edema, hemorrhage, and/or interstitial pneumonitis, followed by cardiopulmonary collapse and shock may ensue. Brain pathology reveals destruction and inflammation in the diencephalon, pons, cerebellum and medulla, including brainstem respiratory and vasomotor centers. Similarly, magnetic resonance imaging demonstrates high intensity lesions in the brainstem; affected areas may progress to cavitation (Huang et al. 1999; Wu et al. 2002).

2.3 EV 71 Acute Flaccid Paralysis

EV 71 acute flaccid paralysis may occur with concomitant encephalitis or as an isolated entity. Motor weakness without associated sensory loss is typical, with the area of weakness correlating with the site of spinal cord involvement. Although paralysis is frequently asymmetric, symmetric disease is also described. The pathology is typically limited to the spinal cord central gray matter, with an affinity for anterior horn cells, as in poliovirus-associated poliomyelitis. Magnetic resonance imaging may identify high intensity lesions involving the anterior horn cells and/or the ventral spinal nerve roots (Huang et al. 1999; Chen et al. 2001).

2.4 EV 71 Diagnosis

Direct detection of virus is the mainstay of diagnosis. EV 71 is isolated primarily from throat and stool specimens, and occasionally from skin vesicle fluid, whereas isolation from cerebrospinal fluid is rare (although virus has been detected in brain

tissue) (Shah et al. 2003). The low yield from cerebrospinal fluid is similar to that seen with poliovirus. Polymerase chain reaction (PCR) and microchip probe technology using both generic EV 5' non-translated region and EV 71 viral protein 1 (VP1)-specific primers and probes have also been useful, generally with greater sensitivity than culture for sites such as throat and stool, although still with relatively low yield from cerebrospinal fluid (Perez-Velez et al. 2004; Tan et al. 2006; Carlos Perez, personal communication). EV 71-specific serologic assays have also been developed.

2.5 EV 71 Pathogenesis

A combination of brainstem damage and viral sepsis underlies the proposed pathogenic mechanisms of EV 71 brainstem encephalitis and cardiopulmonary collapse (Fig. 1) (Lin et al. 2003a; Shekhar et al. 2005). Destruction of vasomotor and respiratory centers in the brainstem, similar to that observed in bulbar poliomyelitis, produces sympathetic hyperactivity, with excess catecholamine and cortisol release, and autonomic dysfunction (Lin et al. 2002; Fu et al. 2003; Kao et al. 2004). These factors, in addition to increased activity of inducible nitric oxide synthase and enhanced production of nitric oxide in the pulmonary vascular bed, are thought to create a state of systemic hypertension and vasoconstriction coupled with increased pulmonary vascular permeability, resulting in shifting of blood from

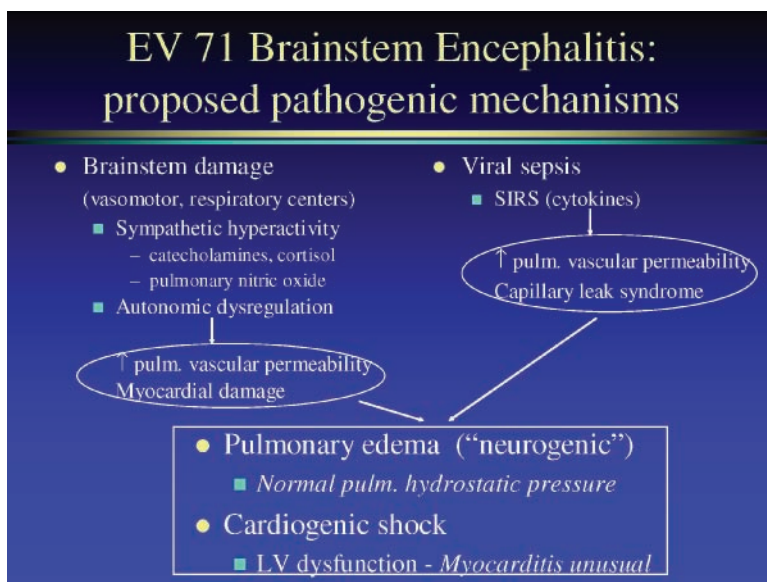


Fig. 1 Proposed pathogenesis of enterovirus 71 brainstem encephalitis and cardiopulmonary collapse. SIRS = systemic inflammatory response syndrome; pulm. = pulmonary; LV = left ventricle

the systemic to the pulmonary circulation and in myocardial damage (Kao et al. 2004). Concomitantly, infection induces the SIRS (system inflammatory response syndrome), characterized by high levels of cytokines, as documented during severe EV 71 disease in the systemic circulation and in cerebrospinal fluid (Wang et al. 2003; Lin et al. 2003a). This further increases pulmonary vascular permeability and induces capillary leak. The final common pathway is neurogenic pulmonary edema, with normal pulmonary hydrostatic pressure, and cardiogenic shock, with evidence of left ventricular dysfunction but generally without inflammatory myocarditis (Chang et al. 2002; Huang et al. 2002; Lin et al. 2002; Wu et al. 2002; Fu et al. 2003; Shekhar et al. 2005).

2.6 EV 71 History and the Emergence of Severe Epidemic Disease

Although EV 71 outbreaks of the late 1990s and early 2000s have brought worldwide attention to this pathogen, EV 71 was actually first described in 1969, in California. Subsequently, outbreaks were reported during the 1970s, 1980s, and early 1990s in the United States, Australia, Japan, Sweden, Bulgaria, Hungary, France, Hong Kong, Taiwan, and Brazil (Palacios and Oberste 2005). These outbreaks included central nervous system manifestations such as meningitis, bulbar and cerebellar encephalitis, and acute flaccid paralysis, but did *not* include the mucocutaneous manifestations of hand-foot-mouth disease or herpangina, *nor* did they include the devastating complications of pulmonary edema/hemorrhage and cardiac compromise.

The epidemiology of EV 71 exploded in the late 1990s and early 2000s, with outbreaks in Malaysia, Japan, Taiwan, Brazil, Australia, Singapore, Korea, India, and Kenya (Palacios and Oberste 2005). Some of these outbreaks have been very large and have extended for multi-year periods. Additionally, these more modern outbreaks have been remarkable for the *new findings* of hand-foot-mouth disease, herpangina, cardiopulmonary distress, and high mortality. Two clusters of cases in Denver, Colorado in 2003 and 2005 were notable for similar findings, including the presence of rash and oral ulcerations in some cases and severe disease, including encephalitis, cardiac dysfunction, pulmonary edema, and/or acute flaccid paralysis, in slightly more than half of identified cases (Perez-Velez et al. 2004; Carlos Perez, personal communication).

Several pieces of information suggest that circulation of EV 71 may be more common than appreciated, even outside settings of regional disease outbreaks. A serosurvey in New York State in 1972 found that 26% of adults were EV 71 antibody-positive (Palacios and Oberste 2005). In Taiwan, a seroepidemiologic study demonstrated that approximately 50% of adults were EV 71-seropositive before the onset of the 1998 epidemic and that EV 71 appeared to have been circulating for at least the previous 16 years (Lin et al. 2003b; Palacios and Oberste 2005). A serologic study in the city of Belem, Brazil found that 53% of children 0–15 years old had EV 71 antibody,

despite a low incidence of disease (Castro et al. 2005). A survey of blood donations in Scotland found that 1 in 4,000 was EV PCR-positive; EV 71 was among the top four serotypes detected (Welch et al. 2003). Passive EV surveillance in the U.S. conducted by the Centers for Disease Control and Prevention indicated that EV 71 was among the most common 15 EV serotypes (ranking between 9th and 14th per year) identified during 2002–2004 (CDC 2006).

If EV 71 has been circulating for decades, why has the occurrence of outbreaks, particularly ones marked by new disease manifestations and severe complications, accelerated in the past decade? Suggested possibilities include changes in viral neurovirulence, alterations in virus transmissibility, changes in host susceptibility, and an accumulation of susceptibles (Lin et al. 2003b). Multiple genetic lineages of EV 71, representing at least 5–6 subgenogroups, have been shown to be responsible for the recent severe epidemics in Asia. There is evidence of both sequential circulation of different subgenogroups and co-circulation of different subgenogroups and of rapid viral evolutionary change (McMinn 2002; Mizuta et al. 2005; Lin et al. 2006; Sanders et al. 2006).

2.7 Therapy of EV 71 Infection

Multiple therapies have been tried for severe EV 71 disease. Intravenous immune globulin appeared to lack benefit in a retrospective study of an outbreak in Australia, but was used in the Taiwan epidemic (Nolan et al. 2003; Chang et al. 2004a). Corticosteroids and interferon-alpha were also utilized in some cases (Nolan et al. 2003). Pleconaril, an antiviral compound that binds to VP1 on the EV capsid and inhibits viral adsorption and/or uncoating, was used in a modest number of cases, but has been shown to have relatively poor activity against EV 71 in vitro and is not currently available (McMinn 2002; Nolan et al. 2003). It should be noted that none of these agents has been proven clinically effective.

The mainstay of treatment for severe EV 71 disease is supportive management. Modalities frequently used include fluid restriction and vasodilators (Nolan et al. 2003; Chang et al. 2004a). Pure inotropic agents have been associated with worse outcome, whereas milrinone, a phosphodiesterase inhibitor that is a combination inotrope and vasodilator has been associated with reduced mortality (Wang et al. 2005; Barnard 2006). Mechanical ventilation, left ventricular assist device, and extracorporeal membrane oxygenation are used when necessary for significant cardiopulmonary compromise (Huang et al. 2002; Nolan et al. 2003; Hsia et al. 2005).

Antiviral compounds with in vitro activity against EV 71 are in development. Agents being evaluated include pyridazinyl oxime ethers, pyrazolo [3,4-d] pyrimidines, and pyridyl imidazolidinones. Like pleconaril, these agents bind to VP1 on the viral capsid and inhibit adsorption and or uncoating of viral RNA, but have been selected based on activity against EV 71 (Barnard et al. 2004; Chern et al. 2004; Shih et al. 2004). Preclinical work on EV 71 vaccine candidates, including virus-like particle vaccines, inactivated virus vaccines, and VP1 subunit vaccines, is also in progress.

3 Congenital EV Infection

3.1 *Previous Evidence of In Utero EV Infection*

EVs have long been known to be an important cause of neonatal infection, with manifestations ranging from asymptomatic to benign, febrile illness to severe illness consisting of variable combinations of sepsis, hepatitis, coagulopathy, myocarditis, pneumonitis, and meningoencephalitis. Cases of neonatal illness occurring in the first 1–2 days of life, some with viremia detected within hours after delivery, suggest that a subset of neonatal EV disease follows in utero infection. Reports of neonatal disease in which EV was grown from amniotic fluid or umbilical blood also indicate in utero infection. Further evidence of congenital infection comes from cases of spontaneous abortion and stillbirths in which multi-organ disease including myocarditis or pancarditis has occurred and EV infection was detected by maternal serologic tests or viral isolation from amniotic fluid, placenta, and/or fetal tissues. “Softer” evidence of congenital EV infection includes maternal and neonatal serologic studies linking EV infection with fetal anomalies of the cardiovascular, gastrointestinal, genitourinary, and central nervous systems; case reports in which positive PCR of amniotic fluid was associated with central nervous system and cardiovascular abnormalities; and serologic studies indicating that children born to women who had EV infections during pregnancy had an increased risk of developing type I diabetes mellitus subsequently (Abzug 1995).

Intriguing recent reports suggest that EVs may be a more common cause of congenital infection than the evidence summarized above suggests. This possibility is supported by two sets of studies, one focusing on examination of amniotic fluid and the other on placental tissue.

3.2 *New Evidence for Congenital EV Infection: Amniotic Fluid Studies*

Two reports from the same group of investigators implicated EVs in an expanded spectrum of congenital infections. In the first of these reports, a broad viral PCR panel detecting EVs, cytomegalovirus, herpes simplex virus, parvovirus, adenovirus, Epstein–Barr virus, and respiratory syncytial virus was applied to amniotic fluid and other specimens (fetal blood, pleural fluid, tissues) from 303 pregnancies that had one of a variety of abnormalities and 154 control, normal pregnancies. Forty-one percent of specimens from abnormal pregnancies were PCR-positive, compared with 3% of 154 control pregnancies. The abnormal pregnancy specimens were PCR-positive for adenovirus in 24%, cytomegalovirus in 10%, and EV in 7%; only adenovirus (2%) and cytomegalovirus (1%) were found in control specimens (Van den Veyver et al. 1998).

In the second of these reports, 423 consecutive amniotic fluid specimens with normal karyotypes were tested with the same viral PCR panel. Fourteen percent of specimens were positive, including adenovirus in 10%, *EV* in 2%, cytomegalovirus in 1%, and parvovirus in 1%. A comparison of pregnancies in which ultrasounds identified an abnormality, i.e., structural anomaly, intrauterine growth retardation, or hydrops, with pregnancies having normal ultrasounds revealed positive PCR assays in 24% of the abnormal pregnancy group versus 8% of the normal pregnancy group (Reddy et al. 2005).

These two series suggested that gestational infection with EVs and other viruses may be more common than previously recognized and may be associated with abnormal pregnancy outcome. However, other similar studies from the same group of investigators did not show a clear association between positive amniotic fluid viral PCR assays and pregnancy abnormalities (Wenstrom et al. 1998; Baschat et al. 2003a, b).

3.3 New Evidence for Congenital EV Infection: Placental Studies

A series of four reports, from a different investigative group, focused on placental tissue and also implicated EVs in a broader array of congenital infections. These investigators applied several techniques including in situ PCR, in situ hybridization, and immunohistochemistry to placentas, and, in some cases, to fetal and maternal tissues, to detect a wide variety of pathogens, including EVs (detected by in situ PCR and immunohistochemistry using a monoclonal antibody targeting VP1), influenza virus, parvovirus, rotavirus, respiratory syncytial virus, varicella-zoster virus, adenovirus, cytomegalovirus, Epstein–Barr virus, herpes simplex virus, and polyomavirus; probes and monoclonal antibodies to identify bacterial rRNA and tumor necrosis factor- α were also used. Pregnancies yielding idiopathic spontaneous abortions, stillbirths, neonatal deaths, and unexplained neonatal illness (respiratory distress, systemic illness, and/or neurodevelopmental abnormality) were studied. Combined results from these four studies demonstrated that 50/121 (41%) placentas were positive for EVs by in situ PCR and 45/121 (37%) were positive for EVs by immunohistochemistry. Virus was generally identified in Hofbauer cells, macrophages/monocytes, and trophoblast cells, but specific histopathologic abnormalities were not seen. Other pathogens identified less frequently included cytomegalovirus, herpes simplex virus, parvovirus, rotavirus, and bacteria. None of the combined 69 negative control placentas was positive by the assays used. Additionally, EVs were detected in a subset of neonatal tissues (spleen, heart, brain, and lungs) and maternal cardiac tissue from pregnancies with EV-positive placentas. Finally, increased expression of tumor necrosis factor was detected in EV-positive placentas and neonatal spleens. The investigators concluded that congenital placental and/or fetal EV infection and associated cytokine upregulation lead to a variety of adverse outcomes, including fetal death and neonatal illness and neurodevelopmental sequelae (Euscher et al. 2001; Genen et al. 2004; Satosar et al. 2004; Nuovo et al. 2005).

3.4 New Evidence of Congenital EV Infection: What Does it Mean?

These recent studies of amniotic fluid specimens and placental tissue implicate EVs as a more frequent cause of congenital infection, with a broader spectrum of clinical ramifications, than preexisting literature indicated. Several conclusions could be drawn based on this new information. It is possible that EVs do play a greater role in causing in utero disease than previously recognized. This would be consistent with animal models that demonstrate in utero infection of the placenta and fetus following maternal EV infection (Abzug 1995). It also is biologically consistent with the demonstration that the Coxsackievirus-adenovirus receptor, one of the major EV receptors, is expressed on human trophoblast cells during the first trimester. It also consistent with the hypothesis that cytokine upregulation due to placental infection, even in the absence of fetal infection, may be deleterious to fetal outcome. Another reasonable conclusion is that EV is sometimes present in amniotic fluid and placenta, but is not causal of fetal or neonatal abnormalities; this possibility is suggested by the absence of specific histologic abnormalities associated with the detection of EVs in the placental studies. Similarly, perhaps the state of pregnancy, with its relative immune deficiency, is permissive of EV infection or allows activation of preexisting, persistent EV infection, without disease causation. It is also conceivable that cross-reactive moieties in otherwise abnormal tissue, rather than EV RNA or EV antigens, are actually being detected with EV probes and antibodies. Finally, reports that conflict with the results described above raise the possibility of methodologic errors. One study that used multiplex PCR, including PCR for EVs, to evaluate 191 amniotic fluid specimens found none to be virus-positive (McIver et al. 2005). A recent study of placentas from pregnancies yielding newborns with neurologic abnormalities and of placentas with chronic villitis found evidence of EV infection in only a limited number of placentas by immunohistochemistry and in none by PCR (Cover et al. 2005).

4 Chronic Fatigue Syndrome

4.1 Evidence Implicating EVs: The European Experience

Infectious etiologies of CFS are suggested by the characteristic history of a flu-like illness at the onset of what then becomes persistent illness. Unifying hypotheses include that CFS represents an immune disturbance triggered by an infection or that immune alterations in CFS permit reactivation of persisting pathogens. A variety of infections have been reported as possible causes of CFS (Ablashi 1994). Among them, an extensive literature has implicated EVs as one possible cause of CFS.

Studies supporting the possibility that EVs are a cause of CFS, primarily conducted in Europe, have used multiple methods of viral detection. Some serologic studies associated cases of CFS with EV infection and with EV outbreaks. In a study using stool viral culture and a serum assay for EV VP1 antigen, 22% of CFS patients were found to be stool culture positive for EV, compared with 7% of healthy controls, and 20% and 51% of two groups of CFS patients were positive for VP1 antigen in serum versus none of the healthy controls. Stool culture positivity persisted among CFS patients for up to 12 months, and serum antigen positivity persisted for up to 4–12 months (Yousef et al. 1988). Other studies demonstrated EV RNA in serum by PCR in 41–42% of CFS patients versus 2–9% of healthy controls, with identical EV RNA sequences found ≥ 5 months apart in some CFS subjects (Clements et al. 1995; Nairn et al. 1995; Galbraith et al. 1997). Multiple reports described higher rates of detection of EV RNA in muscle biopsies from CFS patients compared with specimens from healthy controls. In combined results from several groups of investigators, EV RNA was found by nucleic acid hybridization in muscle from 21 to 26% of CFS patients, 26% of patients with polymyositis or dermatomyositis (inflammatory muscle diseases in which EVs have also been implicated) and 1% of healthy controls and by PCR in muscle from 13 to 53% of CFS patients, 20% of inflammatory muscle disease patients, and 0–15% of healthy controls (Archard et al. 1988; Cunningham et al. 1991; Gow and Behan 1991; Gow et al. 1991; Bowles et al. 1993; Douche-Aourik et al. 2003; Lane et al. 2003). Hybridization studies detected an abnormal 1:1 distribution of positive and negative strands of EV RNA, rather than the 100:1 ratio of positive to negative strands typical of a productive, lytic infection (Cunningham et al. 1990, 1991). This observation, coupled with negative assays for VP1 antigen in muscle from CFS patients, suggested the presence of a defective infection in which EV RNA persisted without normal EV nucleic acid replication and without expression of the full complement of EV proteins (Cunningham et al. 1990; Douche-Aourik et al. 2003). One case report described the detection EV RNA by PCR in the hypothalamus, brainstem, heart, and muscle of an individual with CFS who committed suicide; control tissues were negative (McGarry et al. 1994).

4.2 Evidence from Europe Not Implicating EVs

A study from the University of Glasgow, one of the centers that had previously published studies supporting a role for EV infections in CFS, found EV by PCR in 26% of 121 muscle biopsies from CFS subjects and in 20% of biopsies from 101 controls with non-inflammatory muscle disease. The authors suggested that EVs may localize in damaged muscle and concluded that a pathogenic role of persistent EV infection in CFS was unlikely, refuting their earlier work (Gow et al. 1994). A study from another center conflicted with this Glasgow study and other studies of muscle tissue from CFS patients, finding no evidence of EV by PCR in muscle biopsies from 34 CFS patients, thus also arguing against a role of EVs in CFS (McArdle et al. 1996).

4.3 New Evidence Implicating EVs: The American Experience

Recently described work of American investigators has revived speculation that EVs may be a cause of CFS. These investigators reported finding persistent elevations in neutralizing antibodies to EVs in >50% of CFS patients, lasting ≥ 48 months, compared with controls. (This contrasts with earlier European studies in which levels of neutralizing antibodies did *not* differ between CFS patients and healthy controls, despite more frequent detection of EV RNA in CFS patients.) They also described detection of EV by PCR in peripheral blood mononuclear cells in approximately 35% of CFS patients, compared with a rate of approximately 4–8% in control subjects; they did not find significant yields in plasma, however. (This contrasts with European studies that identified excess detection of EV RNA in serum but not in peripheral blood leukocytes of CFS patients). The RNA detected by PCR was resistant to RNase digestion, leading the authors to suggest that double-stranded EV RNA was detected and hypothesize that persisting EV double-stranded RNA and viral proteins induced a chronic inflammatory state in CFS (Chia and Chia 2003; Chia 2005).

These investigators also described their experience with therapeutic trials targeting EV infection in CFS patients. Fourteen patients with elevated antibody titers to Coxsackie B viruses were treated with one or more interferons (alpha, beta, or gamma), with or without the oral antiviral ribavirin. Decreases in symptoms and in levels of antibody and EV RNA in peripheral blood mononuclear cells were reported in eight of these patients, with relapses occurring after discontinuation of therapy. Another four CFS patients with high levels of EV antibody and/or EV RNA in peripheral blood mononuclear cells were treated with the antiviral pleconaril. Three treated for 1 week had no clinical improvement or change in antibody titers, whereas one patient treated for 1 month was reported to have symptomatic improvement and a decrease in EV antibody titers, with relapse following discontinuation of treatment and no response to re-treatment (Chia 2005). It should be noted that the primary data described by these investigators were published in meeting abstracts but not in peer-reviewed journal articles and the therapeutic trials were done without controls.

4.4 Evidence Linking EV Infection and CFS: Possible Explanations

The role of EVs in CFS, if any, is uncertain. EVs may play a causal role in a subset of CFS patients. Alternatively, EVs may be present in some patients, but consistent with the absence of specific histologic abnormalities, may not have a causal role. Immune or muscle disturbances described in CFS may be permissive of EV infection or activate a persistent EV infection. It is possible that studies demonstrating EV RNA or antigens in specimens from CFS patients may have

detected cross-reactive moieties rather than actual viral target. Finally, in light of numerous conflicts among the reported findings, there is a real possibility of methodologic errors in some of the studies.

5 Conclusions

EV 71 has emerged as a very real pathogen, capable of causing widespread epidemics and severe neurologic disease with life-threatening cardiopulmonary complications in young children. Prospective surveillance is required to monitor the extent of its circulation and pathogenicity. As global efforts to defeat the polioviruses reach their goal, it is reasonable to anticipate that increased attention will need to be devoted to the development of antiviral therapies and vaccines that target EV 71.

Recent findings suggesting that EVs may cause an expanded spectrum of congenital infections are intriguing and biologically plausible. However, currently this association can be best described as speculative. Consistency of experimental findings and confirmation in independent laboratories are required to establish that EVs are a greater threat to the fetus and newborn than has been previously recognized.

Likewise, the suggestion that EVs may contribute to the etiology of some cases of CFS is interesting, but “really speculative.” As for all chronic diseases to which EVs have been linked, definitive proof is needed that amplicon being detected by PCR in these conditions truly represents EV RNA. Further work must also be done to define whether EVs persist in humans, whether they can reactivate, and whether persistent or reactivating EVs cause disease. Additionally, for studies associating EVs with CFS, consistency of experimental findings, with sufficient control data, and confirmation in independent laboratories need to be demonstrated before an etiologic link can be accepted.

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Science and Society: The HIV Epidemic and South African Political Responses

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1 Introduction

The incapacity of the South African political system to respond effectively to the HIV epidemic in the years between 1994 and 2004 is just as astonishing as the sheer magnitude of the public health crisis in democratic South Africa. The lack of an effective reaction challenges Amartya Sen's argument that a political democracy cannot afford to ignore a major disaster within its own borders as well as raising significant questions about the public place of scientific argument in a developing country.

South Africa's catastrophic delay in tackling HIV/AIDS contrasts with much more successful programs in countries like Uganda or Brazil that, on paper, seem to have far poorer political and cultural resources with which to manage the epidemic. An observer on the eve of South Africa's first free election in 1994 – noting the similarity of the scale of the epidemic in these three different countries – might well have predicted that South Africa would be most capable of reducing the spread of the disease over the course of the next decade. What went wrong?

The story is complex and asks for comparative research which has not yet been completed. In this essay we chart the interaction between the rise of the epidemic in South Africa and the faltering and finally downright evasive and denialist reactions of the democratic government. We suggest a variety of factors which determined a South African outcome in which an estimated almost 5 million citizens out of a total of 42 million are infected in 2006.

The nation's racial polarization, the disparity between the political visions of the country's leadership and the embarrassing realities of HIV transmission, the centralization of political power in one legitimate revolutionary party – the African National Congress (ANC) – and the personality of the men and women at the top of the government are all parts of the larger story in which the greatest threat to the country's health was ignored, denied, and distorted in the face of both internal and external opposition. It is one of the few cases in which a modern and enlightened democracy has challenged the scientific enterprise at a cost to its own people which now runs into the millions.

The beginning, at least, of South Africa's story is not unique on the continent. Africa as a whole was slow to respond to the threat and scale of the epidemic. There was, initially, a forceful rejection on the part of governments as well as intellectuals of the proposition that HIV originated in Africa (a theory subsequently confirmed by evidence of the molecular evolution of the virus.) Writers like Susan Sontag predicted that the medical chaos created by the epidemic would be accompanied by a parallel cultural chaos and their prediction was borne out as much in Africa as in Western Europe and the United States.

This early phase of denial was related, in part, to African political and cultural sensitivities and to an African sense of dignity which had to be painfully cultivated in societies built in the aftermath of colonialism. Africans believed that the charge of African origins was racism revived and their governments were inhibited by urgent and visible priorities – such as tuberculosis, malaria, malnutrition, and famine – whereas HIV was almost invisible for many years (Jones 2001c). And this is to say nothing of political instability, armed conflict, and the drain on African health services caused by structural adjustment policies imposed by the World Bank and International Monetary Fund (Heald 2003).

There were exceptions. In Senegal, a robust response from the leadership and the people, probably accounts for the very low levels which prevail in Dakar to this day. In Uganda, where the response was delayed initially, after which the response was vigorous, the epidemic was controlled and the prevalence reduced.

Some credit has been given to the organizational capacity of the main political movements in these countries, the NRA (National Resistance Army) in Senegal and NRM (National Resistance Movement) in Uganda. De Waal concludes from these examples that policies are most effective when implemented through energetic institutions, and with the full backing of the state (de Waal 2003). The lag period between the first reported case of AIDS and initiation of a national AIDS control programme was about a year in Sudan, 2 years in Kenya, 3 years in Burundi, 4 years in Rwanda and Uganda (Mutuluza 2006).

During the early stages of the epidemic, programmes were mainly designed to prevent infection. Prevention interventions were probably 28 times more cost effective than antiretroviral (ARV) treatment. When ARVs became accessible (mainly due to a precipitous drop in prices and the availability of affordable generics) a vigorous debate ensued as to whether the emphasis on prevention was still useful. In sub-Saharan Africa, only one in six of the 4.7 million who require ARVs now receive it. However those receiving treatment increased more than eightfold between 2004 and 2005, from 100,000 to 810,000, and more than doubled in 2005. Coverage increased from 2% in 2003 to 17% by 2005 (WHO and UNAIDS 2006). We have reached a point where there is a developing consensus that the choices are not either prevention or treatment but both simultaneously. (Coovadia and Hadingham 2005; Ivers et al. 2005).

Meanwhile South Africa has 0.6% of the world's population but carries more than 10% of the global burden of HIV/AIDS. It is the worst affected region in the world with close onto 5–6 million HIV infected individuals of the worldwide total of about 40 million. HIV has affected almost every aspect of this uncertain nation, infects all groups, but Blacks are disproportionately involved.

2 South Africa: The AIDS Advisory Committee

There had never been reason to doubt the commitment of the internal as well as external anti-apartheid movement to fighting the epidemic, a commitment expressed clearly in the Maputo Declaration of April 1990 and continued into the first few years of the return of the political exiles. An AIDS Advisory Committee was appointed by the new government in the mid-1990s. One of the authors of this paper (H.M. Coovadia) was Chairperson, and it was widely representative of civil society. The credibility of this body was never in doubt. Indeed many of its members had been part of the struggle against apartheid. The Committee undertook detailed analyses of issues to inform and correct government policies but in vain. Health Minister Zuma and her Director-General did not respond to its findings.

The decision on the use of Zidovudine (AZT) for prevention of Mother-to-Child-Transmission (pMTCT) of HIV is a case in point. Government offered a variety of reasons for its refusal to implement a pMTCT programme based on AZT. Minister Zuma cast doubt on the results of the trial which had provided evidence for the efficacy of AZT. She also spoke of a lack of Primary Health Services and budget constraints. "If you have limited resources, you may decide to put your resources into preventing mothers getting infected in the first place" (Taitz 1999). This argument is in itself unexceptionable but for the fact that we should prevent infection to the child as well as to the mother.

The South African government's attack on evidence based proposals to reduce transmission of HIV to infants was unexpectedly far-reaching. President Mbeki accused the pharmaceutical industry of charging exorbitant prices for the drug, whilst Minister Manto Shabalala warned "The fact is some of the mice (tested with AZT) have contracted cancer. It attacks the bone marrow. It is very toxic" (Nicodemus 1999). In a remarkably brutal speech, a government spokesperson – Dr Ian Roberts – made the chilling comment that "... there was nothing to suggest that in impoverished rural areas, saving the life of a child would affect mortality statistics later on" (Jones 2001b).

After turning a deaf ear to a number of recommendations the government summarily disbanded the Committee. In April 1999, the Department of Health announced plans to make AIDS a notifiable disease (Marais 2000b). The Advisory Committee was opposed to this decision on a number of grounds, not least that members of the public who had been identified as HIV positive had been discriminated against and even murdered. However minister Zuma argued that "we cannot be dictated by human rights or AIDS activists. We need to do what is right. We want to know who is dying of AIDS" (Taitz 1999).

This barrage of counterproductive and dismissive responses from key individuals in the administration was an early indication of the assumption that only the government (the ANC really) reflected society's interests. Other groups, scientific or lay, friend or foe, were dismissed out of hand. In desperation, a number of colleagues (including Archbishop Njongkukulu Ndungane and Judge Edwin Cameron) with impeccable anti-apartheid histories, and one of the authors (H.M. Coovadia), wrote a private letter to President Mbeki urging him to reconsider the

scientific advice he was being given. He sent back a rather long and rambling response quoting numerous accounts published in the scientific literature reporting on the side effects of AZT. It is impossible to convey the despair we felt at such a simplistic evaluation of the use of a drug.

The years thereafter, which saw a withering of unity and conflicting views on implementation between these players, were characterized by a succession of appalling decisions made by the President and his Health Minister. A brief account follows of the key mistakes of the government.

3 Sarafina

On World AIDS Day, 1st December 1995, H.M. Coovadia was invited to the opening of *Sarafina II*, an “AIDS” musical financed by Minister Zuma and produced by Mbongeni Ngema, a well-known Black artist. (Marais 2000c). In the audience were scientists, health professionals, funders, international agencies and the local literati, excited to celebrate one of the first public media events on AIDS. It was amateurish, superficial, and worthless as an instrument to inform, educate, or to encourage safe sex. Predictably, the show failed miserably and vanished into the realm of lost causes.

This venture by the Minister became a national scandal for three reasons. Firstly, she had bypassed the democratic process of inviting interested and capable organizations from bidding for the play. Secondly, she had spent a large sum (Rands 14.27 million) from the Ministry’s budget without properly assessing the playwright’s suitability for the project, and with no sense of the play’s aesthetic and advocacy value, nor its scientific accuracy. Thirdly, she did not consult colleagues, friends or experts. Instead she brusquely announced, “AIDS does not consult- it infects people!” (News Report 1996).

4 The Virodene Saga

A major blunder associated with the ANC’s first years in power is the Virodene debacle (Marais 2000d). Senior government figures trumpeted a wonder drug for AIDS. Three University of Pretoria medical and scientific individuals with no credible prior research record suddenly announced a successful treatment for AIDS. The wonder drug turned out to be an industrial solvent (dimethylformamide)! The three had avoided ethical procedures at their institution and did not have approval by the regulatory body (The Medicine Control Council (MCC)).

Peter Folb, an eminent scientist from the University of Cape Town, head of the MCC and who, in addition, had supported progressive causes during the apartheid years, refused to approve of Virodene (News Report 1997). Folb was replaced as head of the MCC. This was typical of the government’s intolerance of dissent from its policies.

5 The Presidential AIDS Advisory Panel (Mbeki Panel, 2001)

The government's pseudo-scientific crisis deepened rapidly. Indeed it even began to doubt the existence of an AIDS epidemic in South Africa. It appointed a new panel to pursue these conclusions.

The panel met on two occasions: 6–7th May, 2000 in Pretoria, and 3–4th July, 2000 in Johannesburg; 32 panelists met in Pretoria, 30 in Johannesburg. An additional 15 (mostly South Africans) were invited by the Secretariat. The panelists were split between those who subscribed to the current facts and theories established by scientific methods, and the “dissidents” who did not subscribe to these.

The Panel was a spectacular failure, costing between R2.5 million to R4 million (News Report 2002a). Yet none of the major controversies which led to the formation of the Panel were resolved. No resolution of the key differences between the scientists and the dissidents was achieved. Nothing which was not already known, and which was of practical value in halting the epidemic and reversing its spread, emerged from these discussions. In fact, “discussions” is too generous a term, for very soon after the start, the participants broke into their two constituent groups (dissident vs. conventional science) and pursued their deliberations in parallel. Coming together in a plenary session was merely an occasion to restate familiar positions and remain entrenched on two sides of a very wide gulf of disagreement.

A set of recommendations finally emerged: on further research, on testing for HIV, on surveillance, on mortality data, treatment and prevention, and on socio-economic factors. It is noteworthy that the participants could not agree on even a single set of recommendations or experiments. Many of these recommendations seek to re-open issues already well established by experiment and observation over the past 20 years.

No amount of scientific evidence could shift the fixed positions of the dissidents. The latter presented little or no evidence of any kind and their chief technique was either simple denial of well established and proven facts, or ex-cathedra assertions of dubious authenticity. Indeed, after analyzing the Report, the government could have claimed with justification that there was equal support for dissident and conventional scientific views. They could have discontinued programmes on information, education, counseling, management of sexually transmitted diseases and testing of blood for HIV, and halted nascent efforts at reducing mother-to-child-transmission of HIV. Policies based on reducing risky sexual behavior and promoting safer sex could have been scrapped. The “dissidents” recommended the cessation of all HIV testing, the abandonment of HIV surveillance based on these tests, and avoidance of ARVs for HIV/AIDS.

It was the clear dismissal of the dissident views and the unstated belief among important decision-makers that the entire exercise could not be taken seriously, which prevented a crisis exploding into a genocidal disaster. So even the government did not take dissident views seriously.

This dalliance with the “dissidents” was a monumental miscalculation on the part of Mbeki which dented his image irrevocably. The country wasted significant

resources in money and time, and it squandered an opportunity for taking early steps to halt the spread of the infection. What will be remembered is the failure of a newly democratic government, whose many leaders were educated, intelligent and courageous, to appreciate the scientific process and the urgency of translating research into practice in the throes of an unprecedented national crisis. The event was constructed as an extension of the methodologies of politics such as negotiation and compromise, into the realms of scientific debate. It was a false and superficial understanding of the experimental, observational and theoretical paths followed by scientists.

A hugely under-rated consequence of the circumstances leading to and flowing from the “Dissident” affair was the effect on the country’s democratic fabric. An astonishing feature of the years during which dissident issues on HIV were in the foreground of the national landscape, was the silence within the higher reaches and activist ranks of the ANC. From Cabinet to branch levels, there were very few voices raised in protest at what was manifestly an unacceptable set of propositions emanating from the Presidency. Our view is that brave and influential persons were quiet and repressed, not because they were confused or in agreement, but were unwilling to criticize the leadership and their comrades.

When the leading political party in the South Africa is perceived to challenge the basic tenets and findings of science, then civic alienation is deepened and trust in government is imperiled. This is because science is one of the great accomplishments of our civilization. Loyal citizens are wont to ask “... if they can be so wrong in something as basic as this, how can we be sure they are right in any of their other policies?”

As a result of the pseudo-debate MTCT programmes were delayed and, without doubt, many thousands of infants who could have been protected, were infected by HIV. This is an ineradicable stain on our society. The effects may be especially damaging among youth and adolescents who are the driving force of the epidemic and vulnerable to ostensibly radical views. In a recent survey of a representative sample of South Africans carried out by the Helen Suzman Foundation, 39% of respondents were confused on government policy on HIV/AIDS, while 25% disagreed (News Report 2002b).

6 The Durban Declaration

On a wintry morning on the 27th June, 2000, Slim Karim (Chair of the Scientific Programme Committee of the AIDS Conference) and one of the authors (H.M. Coovadia) met the Minister of Health, the Director-General and the Chief Director (AIDS Directorate), in government offices in Pretoria. It was one of a handful of occasions when they had a relatively civil discussion. They were concerned that the proposed Durban Declaration to be publicly announced during the Conference proceedings would be an attack on President Mbeki. Attempts to allay their fears failed.

The year preceding the XIII International AIDS Conference in Durban, South Africa, had been marked by an escalation of the government's rhetoric which challenged critical issues on HIV/AIDS. In October 1999 President Thabo Mbeki had disputed the use of AZT for the prevention of MTCT of HIV; yet AZT had been shown conclusively to reduce MTCT by almost two-thirds. President Mbeki pointed to legal cases pending in the UK and US against AZT: "There also exists a large volume of literature alleging that... the toxicity of this drug is such that it is in fact a danger to health." (Marais 2000a) He was backed up by his Minister of Health Dr Nkosazana Zuma.

During that entire summer and autumn South Africa witnessed an unprecedented attack by the state on the scientific understanding of one of the worst pandemics to plague humankind. The huge irony of such ill-informed doubts expressed by the leaders of the first democratically elected government, was that South Africa was by that time the country most affected by HIV/AIDS in the world.

Many consider the Durban meeting one of the most successful and significant AIDS Conferences ever held. The conference was the first to be held in a developing country, intended to draw global attention to the predominance of AIDS in Africa. The event focused the world's attention to where the epidemic was and led to the first steps challenging the high cost of ARVs produced by the large research-based pharmaceuticals in the western countries. Yet the integrity of the AIDS Conference in Durban was threatened by the government's attacks.

In the summer of 2000, a few months before this AIDS Conference was to be held in Durban, Mbeki stunned the world by an amazing communication to his peers on the issue of AIDS. He wrote to Kofi Annan, Secretary General of the United Nations, President W. Clinton of the United States, and other Heads of States, that the "assumption of a link between HIV and AIDS by mainstream doctors, media and others amounted to "intellectual intimidation and terrorism" and that the alleged link required further investigation" (Hooper 2001). He described the criticisms of his views and the widely-held view that he stay out of the debate altogether, as censorship.

Equally revealing are the President's comments at the opening session of the 13th International AIDS Conference in Durban on 9th July 2000. He bemoaned the attitude of some people who considered that questions he and the government had raised on HIV/AIDS "as asking to grave criminal and genocidal misconduct" (Mbeki 2000a) He emphasized that international authorities (WHO) had identified "extreme poverty (as) the world's biggest killer and greatest cause of ill health and suffering across the globe." He recounted the debilitating nutritional deficiencies and numerous serious infections in Africa which led to a "collapse of the immune systems among millions of our people, such that their bodies have no natural defense against attack by many viruses and bacteria." It seemed to him "that we could not blame everything on a single virus." And so he wondered whether "Safe sex, condoms and anti-retroviral drugs [are] a sufficient response to the health catastrophe we face!"

It may be argued that this is denial on a grand scale, enlarging many times over the psychological barricades to acceptance constructed by the individual infected by HIV. In such a construct friends can be seen as opponents and critics as enemies.

Helen Schneider expresses the view that this resolves into a question of power – who decides policy on HIV/AIDS? Is it the President or civil society? (Schneider 2001)

More than 5,000 scientists from around the world, including 12 Nobel Laureates, as well directors of leading research institutes, and presidents of academies and medical societies, were moved by these events to sign the Durban Declaration. It affirmed “the evidence that AIDS is caused by HIV-1 or HIV-2 is clear-cut, exhaustive and unambiguous, meeting the highest standards of science. The data fulfill exactly the same criteria as for other viral diseases, such as polio, measles, and smallpox.” To “tackle the disease, everyone must first understand that HIV is the enemy. Research, not myths, will lead to the development of more effective and cheaper treatments, and ... a vaccine.” It concluded on a note of optimism: “Science will one day triumph over AIDS, just as it did over smallpox. Curbing the spread of HIV will be the first step. Until then, reason, solidarity, political will and courage must be our partners.”

7 The Enigma of President Mbeki

It is difficult to understand the reasons for the President’s unorthodox views. Our own conclusions are that he was conditioned by a number of different factors, chief of which are four major phenomena: post-colonial personal reactions to previous prejudices; political pragmatism; the urgent and onerous duties of building simultaneously a non-racial society and a country; and an unanticipated, deadly, and explosive epidemic of HIV/AIDS.

We see one significant impulse behind the President’s views as his affirmation of anti-colonial perspectives which developed in the latter half of the twentieth century. Before then European pictures of African populations were dehumanizing in the extreme. Europeans viewed Africans as a people not perfectly human, living by the most basic of instincts – an unrestrained sexuality, raging passions, cruelty, and lack of civilized inhibitions. They were supposed to be unhygienic, and diseases, especially sexually transmitted infections and mysterious degrading disorders, were imagined to be rampant amongst Africans.

Thabo Mbeki was nurtured by the vision of an alternative liberating set of beliefs, and a strategy of a rational attack on atrocious colonial prejudices. Yet HIV seems to be a horrifying return of these very same prejudices, spread as it is by promiscuity and enveloped in stigma, shame and discrimination. More than 70% of all those infected are Africans, and the risk factors are linked to cultural practices.

President Mbeki occupies a pre-eminent position in the array of African leaders; he often speaks for the continent (as in the African Renaissance, African Union, and NEPAD). Tim Trengrove Jones has provided an excellent account of these issues (Jones 2001a). Mbeki has a grand vision of his role; educated and refined, schooled in political and economic essentials, literate in the arts and sciences, and an intellectual with capabilities to deal effectively across the borders of multiple disciplines and subjects. He is at once an African Galileo, an Adam Smith, and a Simon Bolivar; in short a Renaissance man! He wants to restore the ancient glories

of African societies, repair the shabby image of the continent created by colonialism, and project Africa to the high ground of global perceptions. He wishes to “reassert the fundamental concept that we are our own liberators from oppression, from under-development and poverty, from the perpetuation of an experience from slavery, to colonization, to apartheid, to dependence on alms.” (Mbeki 1997). He pores scorn on the distortions that Africa is “home to an unending spiral of anarchy and chaos” (Mbeki 2000b). These beliefs of marginalization and dismissal of African cultures shape his response to HIV/AIDS.

Therefore Mbeki has to defend Africa from this new racism and neo-colonialism embedded in the discourse on the AIDS epidemic. He has to refute once again the suggestion of “degenerate Africans.” He challenges the scientific paradigm, is challenged, and finds refuge in denial. Indeed it may be argued that this is denial on a vastly inflated scale, a presidential magnification of the psychological barriers of denial formed by many an infected individual.

In support of our inclination to the “anti-colonial” view we quote two pieces, one from the President, the other from an ANC publication probably authored by ANC senior member Peter Mokaba.

Peter Mokaba:

“Yes we are sex-crazy”

“Yes we are diseased”

“Yes we spread the deadly HI Virus through our uncontrolled heterosexual sex”

“Yes among us rape is endemic in our culture”

“Yes what we need and cannot afford, because we are poor, are condoms and anti-retroviral drugs” (Hlongwane).

President Mbeki:

And thus does it happen that others who consider themselves to be our leaders take to the streets carrying their placards, to demand that, because we are germ carriers and human beings of a lower order that cannot subject their passion to reason, we must perforce adopt strange opinions to save a depraved and diseased people from perishing from a self-inflicted disease ... convinced that we are but natural-born promiscuous carriers of germs They proclaim that our continent is doomed to an inevitable mortal end because of our devotion to the sin of trust. (Forest and Streek 2001)

A prominent weekly *Mail and Guardian* (2001) suggests that this implies that those who advance a viral explanation of AIDS believe black people are unclean, uncivilised and sexually promiscuous.

8 Pragmatism

Another argument runs as follows: Mbeki has made a careful assessment of the costs of responding effectively to HIV/AIDS, come to the conclusion that the country simply cannot afford it, and retreated into a defensive rejection of the existence of the disease. We find this unconvincing: the MTCT programme which is feasible, affordable, cost effective and relevant, has generated the most heat in the country; the association with the “Dissidents,” and the intensity and range of arguments made by the Presidency against accepted notions on HIV/AIDS, do not accord with a limi-

tation imposed by budgetary constraints, and finally, South Africans can easily appreciate a government case based on unaffordability.

A deeply insulting view to the President is that he is completely hard-nosed and cynical. He knows the epidemic is catastrophic for South Africa and there is little to offer for treatment, care and prevention. So let those infected die, avoid identifying the cause, and hope for an African Renaissance emerging from the ashes of our devastated society (shades of the fourteenth Century Black Death in Europe)! We mention this only to illustrate the perverse ideas which may be thrown up in an atmosphere of uncertainty and confusion.

9 Preoccupation with Governance

Some of the misconceived AIDS policies have been attributed to the dislocations of transition. A new government has a million priorities and few resources. Qualified personnel are difficult to find and policy errors proliferate. The failure to act on HIV/AIDS may have been due to preoccupation with the immense pressures to rebuild the country after independence; there were internal terrorist attacks, a civil war with Inkatha in Kwazulu/Natal, a recalcitrant bureaucracy, unstable institutions, and complex political negotiations with the apartheid regime. There was a right-wing campaign to link AIDS to the arrival of political exiles from other African countries, and a corresponding reluctance to face the AIDS crisis among exiles who may have been vulnerable to HIV. The battle with large pharmaceuticals on the price of drugs has great resonance amongst most of those in the liberation struggle; but can also be seen as evidence of standing up to the marauding capitalists from the west, and their destructive ideologies.

10 The Politics of Negotiation

The Mbeki Panel was an attempt to project the prescriptions of political negotiation into the methods of science. For much of his career Mbeki had been involved in negotiations; this was striking in the CODESA negotiations between the liberation forces and the apartheid regime when power was being transferred to the majority and ‘consensus’ became the operative word. Indeed a “sufficient consensus” was established as the arbiter of decision making. Since then South Africans have become accustomed to the idea of agreement, reconciliation and harmony (The Truth and Reconciliation Commission; the “Rainbow Nation”; and the “Miracle” of the South African “Revolution”). The success of these processes convinced Mbeki of the value of “negotiation.”

In a tragic error he transferred this conviction to the world of science, where decisions on what is the “truth” in nature and humankind are not made by “consensus” but by observation and/or intellectual creativity, experiment and/or logical and rational discourse, probability, and theory. The repeatability of experiments and confirmation of propositions are also critical for establishing these

“truths.” It is not by “consensus,” except where conflicting or incomplete results require agreement for practical purposes.

11 AIDS and the Global Context

The global context of these rejections of accepted facts is a widely, but not universally, popular strain of postmodern thought which repudiates rational methods of arriving at the truth about reality. Vocal groups within the lay community are often anti-science: against controlled experiments and trials, determination of risk factors in complex situations, and quantitative analyses in getting at the truth. These groups display, in our view, elements of unjustified paranoia about a scientific community which is whole-heartedly engaged in studying and attempting to reverse the epidemic. They stress the relative and unstable nature of scientific knowledge, emphasize the supposed collaboration of the scientific enterprise with political and cultural power, without taking seriously the speed and seriousness with which many governments and scientific establishments have tried to tackle this public health disaster. The result is that people seek alternative therapies – which often are illusionary and outright quackery.

The unusual features of the collision between state and society in South Africa, almost unique in any modern democracy, encouraged us to look through the pages of history to identify any similar rifts. The oppression and unrestrained attacks by fascist states, including the apartheid regime, on science are well known and not directly applicable to this discussion; these will not be detailed here. Lysenko ruined Russian agriculture through his state supported rejection of orthodox genetics (Dombrowski 2001). Two Nobellists (in Physics) during Hitler’s rule, Phillip Lenard and Johannes Stark, attacked Einstein’s theories and attempted to establish an Aryan science (Beyerchen). In ancient Greece those individuals whose speculations and ideas appeared to challenge the prevailing beliefs in cosmology, religion and the divinities, were subjected to severe criticism, punishment, and even death. In 411 BC Protagoras was accused of being an impostor because of his cosmological observations, and brought to trial for “impiety” and banished (Gribbin 2002). Anaxagoras was charged with reducing the glory and divine status of the gods by suggesting that “the sun, the moon, and all the stars are red hot stones ...” and threatened with expulsion from Athens (Gribbin 2002). Socrates was tried and condemned to death in 399 BC for “meddling in the affairs of the heavens” (Gribbin 2002). He had explained the nature of rain and thunder on the basis of cloud behavior, and had thereby ignored and insulted Zeus. The case of Galileo is well known. He advocated Copernican theories on the motion and position of the planets and sun; these displaced Man from the centre of the universe and affronted current theological beliefs. Despite warnings on his beliefs, he published a defense of his theories – “Dialogue concerning the two chief world systems” – and was tried in 1632 for heresy by the inquisition in Rome. He recanted and was sentenced to life imprisonment, commuted to house arrest in Tuscany (Olson 1982). Edmond Halley was a distinguished scientist and midwife to publication of Isaac Newton’s “Principia” in 1687. He failed to get the Savilian Chair

of Astronomy in Oxford, as he was brought into conflict with the church over his attempts to estimate the age of the earth. Creation had already been dated to 4004 BC by Archbishop Ussher in 1620 (Gribbin 2002).

In addition there are several interesting characteristics of the individual, and of context, which may have resulted in the relentless deterioration in national AIDS policies in South Africa. In this Post Modern age there is a rejection of the philosophy of the Enlightenment: a negation of the exaltation of science, of universality of moral principles, of the sanctity of individual volition, and of the prospects of social progress of humankind. In contrast Post Modernism holds that all knowledge is local not universal, and the product of social class, circumscribed by its own interests, prejudices, and historical conditions (Gross and Levitt 1994). Amartya Sen believes in the “reach of reason” which persuades individuals in the solution of problems, and quotes Jonathan Glover, Oxford philosopher, who looks beyond these issues and ascribes the behavior of some powerful and intelligent individuals, to “strongly held personal beliefs,” and to “instincts” (Amartya 2005).

12 Conclusion

As the smoke and dust of the events we have described settle the realization takes hold in that science and rationality have a fragile standing in our societies and are under constant attack. It is critical that medical researchers and health professionals express their concerns, speak up, and act. Karl Popper, the great philosopher of science, warned scientists of the dangers of considering science as only an instrument of command over nature. We should equally well remind those in power that attempting to command science and its practitioners is self-defeating. As scientists we are charged by the very freedom we require, to pursue an unhindered search for the means to alleviate human suffering and to eliminate its preventable causes.

President Mbeki has framed the denialist case as a story like that of Galileo Galilei's – as an instance of a brave group of denialists challenging the established wisdom of the day – but in the view of the authors they more closely resemble the legendary King Canute. The Wikipedia, the internet encyclopedia, tell us that “King Canute's people thought he was a god, so he had his throne taken down to the ocean and told his people that ‘if I can hold back the tide, I must be a god’; however, the tide came in. His people decided, then, that he was not a god.”

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3

Pathogenesis of Group A Streptococcal Infections and Their Sequelae

Madeleine W. Cunningham

1 Introduction

Streptococcus pyogenes or group A streptococci are Gram positive extracellular bacterial pathogens which colonize the throat or skin and are responsible for a number of suppurative infections and non-suppurative sequelae (Cunningham 2000). As pathogens they evade host defense mechanisms and exhibit a group of virulence determinants. Group A streptococci are a common cause of bacterial pharyngitis, scarlet fever or impetigo. The concept of distinct throat and skin strains arose from decades of epidemiological studies, where it became evident that there were serotypes of group A streptococci with a strong tendency to cause throat infection, and similarly, there were other serotypes often associated with skin infections (Bisno 1995b). The group A streptococcus is most well recognized for streptococcal toxic shock syndrome and necrotizing fasciitis which involves destruction of the skin and soft tissues in severe cases (Stevens 2000).

The two major post-infectious streptococcal sequelae are acute rheumatic fever and acute glomerulonephritis (Bisno 1995a). In acute rheumatic fever, which affects children globally, rheumatic heart disease is the most serious autoimmune sequela of group A streptococcal infection, while arthritis is the most common. Rheumatic carditis, migratory polyarthritis, Sydenham's chorea, a neurologic disorder involving abnormal movements, erythema marginatum, a circinate skin rash, and subcutaneous nodules are all considered to be major manifestations of acute rheumatic fever based on the Jones criteria (Jones 1944; Dajani et al. 1989).

The Lancefield classification scheme of serologic typing identified beta-hemolytic *Streptococcus pyogenes* as group A streptococci based on their surface group A carbohydrate containing *N*-acetyl-glucosamine linked to a rhamnose polymer backbone (McCarty 1956). Group A streptococci were further separated serologically based on their surface M protein serotype. Currently, over 100 M protein serotypes have been identified using a molecular approach to sequence the *emm* (M protein) gene (Beall et al. 1996). Vaccines containing the anti-phagocytic streptococcal M protein as well as other surface components are under investigation for prevention of streptococcal infections and their sequelae (Fischetti 1991; Bessen and Fischetti 1997; Dale 1999). Group A streptococcal M protein serotypes which

cause impetigo and skin infections are in general different M protein serotypes from those that cause pharyngitis (Bessen et al. 1995, 1996). Skin serotypes are associated with production of acute post-streptococcal glomerulonephritis which occurs seasonally during summer months and in temperate climates (Bisno 1995). The infection is limited to the epidermis usually on the face or extremities and is highly contagious.

2 Virulence Determinants and the Host Response

Host-pathogen interactions are important in the balance between the streptococcal virulence factors versus the host response to prevent colonization or disease manifestations. Adherence of group A streptococci to pharyngeal or dermal epithelial cells leads to colonization of the host. Without strong adherence mechanisms, group A streptococci could not attach to host tissues and would be removed by salivary fluid-flow mechanisms and exfoliation of epithelium. In colonization of the skin, a site of previous damage is important in breaking through the dermal barrier. Specific adhesion allows for competition between normal flora and group A streptococci for tissue sites where normal flora reside. Streptococcal adhesion requires multiple group A streptococcal adhesins reported by several investigators and described in excellent reviews (Hasty et al. 1992; Hasty and Courtney 1996; Cunningham 2000; Courtney et al. 2002; Bisno et al. 2003). Adherence involves a two step process whereby weak interaction with the mucosa initiates adhesion and is followed by stronger adherence to tissue specific, high avidity ligands. The streptococcus has the advantage of multiple adhesins which enhance the overall virulence (Hasty et al. 1992; Cunningham 2000). Adhesins may be important for colonization in a specific tissue and infiltration of the skin into deeper tissues may depend on specialized adhesion mechanisms (Okada et al. 1994). In addition, inflammation may be a result of adhesion since cytokine production and inflammatory responses are linked to adherence of streptococci to epithelia (Okada et al. 1994, 1995; Wang et al. 1997).

Major virulence factors of group A streptococci include the surface exposed M protein and hyaluronic acid capsule (Fischetti 1991; Wessels et al. 1991, 1994). Antiphagocytic mechanisms involve the binding of factor H which inhibits the activation of the complement pathway (Horstman et al. 1988; Kotarsky et al. 2001; Giannakis et al. 2002; Jarva et al. 2003). Factor H is a regulatory component of the complement pathway which inhibits the deposition of soluble C3b. Factor H binds to the C repeat region of the M proteins, and deletion of the C1 and C2 repeat regions reduces Factor H binding (Fischetti 1991).

The group A streptococci are covered with an outer hyaluronic acid capsule, while the group A carbohydrate antigen and the type-specific M protein are attached to the bacterial cell wall and membrane (Pancholi and Fischetti 1988). Both the M protein and the capsule are considered to be virulence factors conferring antiphagocytic properties upon the streptococcal cell (Wessels et al. 1994; Dale et al. 1996). The M protein extends from the cell surface as an alpha-helical coiled-

coil dimer which appears as fibrils on the surface of group A streptococci (Fischetti 1989). Absence of M protein allows rapid phagocytosis of the streptococcus. The anti-phagocytic property of M protein is due to its binding to complement regulatory protein factor H (Horstman et al. 1988) and fibrinogen (Whitnack and Beachey 1982; Wang et al. 1995). The binding of fibrinogen also leads to the acquisition and activation of plasminogen which is then converted by streptokinase to active plasmin. Human kininogen has also been reported to bind to M protein with the subsequent release of bradykinin, a vasoactive peptide released in plasma (Herwald et al. 2003). In addition, M protein has been shown to form complexes with fibrinogen which induce vascular leakage that could contribute to the overall toxic streptococcal shock syndrome and necrotizing fasciitis (Herwald et al. 2004).

The C5a peptidase is a proteolytic enzyme (endopeptidase) found on the surface of group A streptococci and is considered a virulence factor (Cleary et al. 1992). It is a highly specific 130kDa serine peptidase that is anchored to the streptococcal cell wall. The peptidase cleaves the complement derived chemotaxin C5a at its PMN binding site and inhibits the recruitment of phagocytic cells to the site of infection. The C5a peptidase is believed to be important in virulence of group A streptococci. Mutations in the *scpA* gene increase clearance of streptococci from subdermal sites of infection and from the nasopharyngeal mucosa of intranasally infected mice (Ji et al. 1996, 1997). Intranasal immunization of mice produced a vigorous serum and secretory antibody response that enhanced clearance of the bacteria from the oral mucosa of mice (Ji et al. 1997). Less than 15% of children under 10 years old exhibit antibody against C5a peptidase while most adults have a strong immune response to C5a peptidase (Shet et al. 2003).

Type specific and opsonizing anti-M protein antibody is essential for host immune clearance of group A streptococci by polymorphonuclear leukocytes or neutrophils. Non-opsonic antibodies are produced against other epitopes in the M protein molecule and these antibodies do not protect against infection with homologous type group A streptococci. Once the host is exposed to the type specific epitopes, a primary response occurs and long term immunity to the infecting serotype is acquired. Although opsonic antibodies are important in clearance of group A streptococci from tissues through the Fc mediated mechanism of clearance by the neutrophil, mucosal immunity at the surface of epithelium in the pharynx is important in protection against pharyngeal colonization by group A streptococci (3–6). Adults have significantly fewer group A streptococcal infections than children due to acquired immunity during childhood. Protection seen in adults may be due to mechanisms preventing colonization in the pharynx. Studies have investigated the role of IgA at mucosal surfaces in protection against group A streptococcal infection. In mice, passively administered M protein specific IgA provided protection against mucosal infection and also delayed disseminated infection and death (Bessen and Fischetti 1988a, b, c). IgA blocks adherence of bacteria to mucosal surfaces and plays a key role in host protection at mucous membranes. IgA could also cause the streptococci to become trapped in mucous and cleared by host fluid flow mechanisms. In summary, protective immunity constitutes two major mechanisms. Group A streptococci entering the pharynx can be blocked from attachment to mucosal surfaces by

IgA, and those organisms penetrating into host blood and tissues are effectively eliminated by opsonization with type specific antibody and complement with subsequent phagocytosis and killing. M protein vaccine strategies have taken advantage of the epitopes conferring these two modes of protection from infection (Bessen and Fischetti 1990; Dale 1999).

A large number of superantigens play a role in toxic streptococcal syndrome acting as superantigens which recognize certain major histocompatibility complex class II molecules and a limited number of V β regions of the T lymphocyte receptor to activate large numbers of T cells non-specifically with liberation of inflammatory cytokines and interleukins, tumor necrosis factor and gamma interferon (Kotb 1995; Kotb et al. 2002). The pyrogenic exotoxins are potentially responsible for at least some of the manifestations of toxic streptococcal syndrome. Several superantigens of group A streptococci are likely to be involved in the pathogenesis of toxic shock, invasion of soft tissues and skin, and necrotizing fasciitis. The streptococcal superantigens include exotoxins A, B, and C, exotoxin F (mitogenic factor) and SSA (streptococcal superantigen), Spe G, Spe H, Spe J and SMEZ and SMEZ-2 (Chatellier et al. 2000; Proft et al. 2000, 2003; Gerlach et al. 2001; Kazmi et al. 2001; Muller-Alouf et al. 2001; Unnikrishnan et al. 2002).

3 Acute Rheumatic Fever

Rheumatic fever is a delayed sequel to group A streptococcal pharyngitis. The disease manifestations include an inflammation of the joints (arthritis), heart (carditis), central nervous system (chorea), skin (erythema marginatum) and/or subcutaneous nodules (Stollerman 1988, 1997). These five major clinical manifestations were established by the Jones criteria and revised by the American Heart Association (Jones 1944; Dajani et al. 1989; Dajani 1992). Although carditis is the most serious major manifestation, migratory, polyarticular arthritis is the most common. Arthritis does not produce permanent damage, while injury to the heart valves can be serious and require replacement of the damaged valve (s). The carditis presents as mitral and/or aortic regurgitation with a heart murmur upon auscultation and/or evidence by Doppler echocardiography (Veasy 2001). Other manifestations observed in rheumatic fever but less frequently are subcutaneous nodules, Sydenham's chorea, and erythema marginatum (Dajani 1992). Sydenham's chorea is a neurological disorder causing involuntary movements, muscle weakness, and emotional disturbances (Swedo et al. 1993; Swedo 1994). After puberty, chorea is seen only in females (Stollerman 1975). Chorea can be the only major manifestation present in rheumatic fever, but in the presence of an elevated anti-streptolysin O or anti-DNAse B titer, diagnosis of rheumatic fever can be ascertained. Anti-brain antibodies reactive with neurons of basal ganglia and other brain tissues have been reported in the sera of patients with Sydenham's chorea (Husby et al. 1976). Experimental therapy by plasma exchange has been reported to be rapidly curative in patients with chorea (Garvey and Swedo 1997).

Erythema marginatum is a distinct red circinate rash which is characteristic of rheumatic fever. However, it is rarely seen in the disease. Subcutaneous nodules occur over the surfaces of joints and spine, and they are not unique to rheumatic fever since they are seen in cases of rheumatoid arthritis and systemic lupus erythematosus (Stollerman 1975). The minor characteristics of rheumatic fever include fever, elevated erythrocyte sedimentation rate, elevated C-reactive protein, leukocytosis, prolonged P-R interval on ECG and arthralgia. Supporting evidence of a recent streptococcal infection, either by serology or by culture, is required to make the diagnosis of acute rheumatic fever (Bisno 1993, 1995).

The disease is autoimmune in nature and most likely is a result in part from the production of autoreactive antibodies and T cells shown to be crossreactive with components of the group A streptococcus and host tissues. The medical importance of rheumatic fever is due to serious cardiac involvement with myocarditis or valvulitis leading to death or valve replacement (Stollerman 1988; Veasy 1995). Massell has published a most extraordinary historical account of rheumatic fever from the earliest records of the disease to the most current research investigations (Massell 1997). Acute rheumatic fever is a leading cause of heart disease in children world-wide (Kaplan 2004), and since 1985 a resurgence of ARF has been observed in the United States (Veasy et al. 1987, 1994, 2004). The pathogenesis of acute rheumatic fever is complex and may depend on a number of streptococcal and host factors, particularly autoimmune and inflammatory responses in host tissues (Kaplan and Dallenbach 1961; Kaplan et al. 1964; Zabriskie 1967, 1985; Zabriskie et al. 1970). Immune crossreactivity or molecular mimicry between group A streptococcal M protein, *N*-acetyl-glucosamine, the dominant group A carbohydrate epitope, and cardiac myosin may in part lead to tissue destruction in acute rheumatic fever (Galvin et al. 2000).

Host susceptibility is also an important factor in the development of ARF. In a highly defined group with mitral valve disease such as mitral regurgitation and mitral stenosis, the frequency of DRB1*0701 and DQA1*0201 alleles and the DRB1*0701-DQA1*0201 and DRB1*13-DQA1*0501-3-DQB1*0301 haplotypes were found more often in the rheumatic heart disease patients than in ethnic controls (Guedez et al. 1999). The data suggested that DRB1*0701, DR6, and DQB1*0201 confer susceptibility to rheumatic fever. Although there is a high prevalence of streptococcal pharyngitis in populations, only a small percentage of individuals develop acute rheumatic fever. The studies of families suggest that the disease is familial, but that the genetic factor, characterized as autosomal recessive, has limited penetrance (Zabriskie 1985), and it is believed that there is genetic susceptibility to rheumatic fever (Gibofsky et al. 1998).

However, twins do not usually both develop rheumatic fever, suggesting that environmental factors play a role in susceptibility to disease (Taranta et al. 1959). Repeated exposure to streptococcal infections plays a central role in development of the rheumatic fever. Genes and environmental factors, other than a group A streptococcal infection, which may play a role in the disease, remain virtually unknown.

Acute rheumatic fever is an important model to study autoimmune disease following a bacterial infection, and is an excellent example of molecular mimicry

between host and pathogen (Cunningham 2000). Sera of patients with acute rheumatic fever contain heart-reactive or myosin-reactive antibodies, frequently in high titers (Cunningham et al. 1988, 1989; Zabriskie et al. 1970). The initiating event following streptococcal infection is the production of the cross-reactive autoantibodies against the heart and valve tissues. Studies suggest that immune responses against group A streptococci and the autoantigen cardiac myosin may play a significant role in pathogenesis of acute rheumatic fever (Krisher and Cunningham 1985; Galvin et al. 2000). Antibodies and complement were observed deposited in the myocardium and valves of acute rheumatic fever patients (Kaplan et al. 1964). Studies of anti-streptococcal/anti-heart monoclonal antibodies (mAbs) from rheumatic carditis have revealed that cardiac myosin, and *N*-acetylglucosamine, the immunodominant epitope of the group A carbohydrate antigen, are the crossreactive antigens involved in antibody deposition on the valve (Galvin et al. 2000). Previous work by Dudding and Ayoub demonstrated the persistence of increased levels of anti-group A carbohydrate antibody in rheumatic valvular heart disease and their association with a poor prognosis (Dudding and Ayoub 1968).

Although progress has been made in our understanding of rheumatic fever and its pathogenesis as an autoimmune disease, there is still much to be elucidated about the disease process. Disease susceptibility factors, including the major histocompatibility antigens and potential tissue specific antigens, are under investigation as potential risk factors in the disease (Guedez et al. 1999). Autoantibodies which develop during streptococcal infection and in rheumatic fever are being investigated for their potential role in the disease (Galvin et al. 2000; Kirvan et al. 2003), while it is evident that T lymphocytes play a key role in the pathogenesis of rheumatic carditis (Guilherme et al. 1995; Fae et al. 2006). Pathogenic epitopes of streptococcal and host antigens which cause autoimmune disease in animal models have been defined (Huber and Cunningham 1996; Cunningham et al. 1997; Quinn et al. 2001).

3.1 Immune Mechanisms in Rheumatic Carditis

3.1.1 Antibody Reacts with Valve Endothelium

In acute rheumatic carditis, anti-streptococcal antibodies may attack the valvular endothelium which becomes inflamed and upregulates expression of adhesion molecules on valvular surface endothelium or endocardium. The adhesive endothelium/endocardium attracts lymphocytes which extravasate into the valve (Galvin et al. 2000; Roberts et al. 2001). mAb 3B6 derived from rheumatic carditis recognized both cardiac myosin in the myocardium and laminin at the valve surface and within the basement membrane of the valve. Laminin is a large 900 kDa molecule composed of three chains, A, B1 or B2, which contain alpha-helical coiled-coil domains which are highly homologous with streptococcal M proteins

and cardiac myosins. The laminin sequence HTQNT, shared between cardiac myosin and laminin, was shown to inhibit the reactivity of the anti-streptococcal/anti-myosin antibody with the valve endothelium and basement membrane (Galvin et al. 2000). The mechanism for antibody deposition on the valve would indicate that anti-streptococcal antibodies recognize the valve surface endothelium and/or basement membrane where antibodies may target the valve surface and further enhance the upregulation of inflammatory signals such as vascular cell adhesion-1 (VCAM-1) by the valve endothelium (Roberts et al. 2001).

3.1.2 T Cell Infiltration Through Valve Endothelium

Studies of the valve surface endothelium in humans with acute rheumatic fever suggested that it first becomes inflamed by some mechanism, such as by antibody and complement binding. T cell infiltration occurs through an activated valvular surface endothelium. It is believed that the surface endothelium of the valve is the initial site of entry of lymphocytes in rheumatic heart disease (Roberts et al. 2001). Since the valve is considered to be an avascular tissue, the most logical explanation or hypothesis is that the valve is inflamed by antibody deposition and subsequent lymphocytic infiltration. The evidence in support of this hypothesis comes from studies of rheumatic valves from young children (Roberts et al. 2001). Immunochemical staining of rheumatic valves demonstrated the attachment and infiltration of valve surface endothelium with CD4+ or CD8+ T cells. T lymphocytes in rheumatic heart lesions react with streptococcal M protein and cardiac myosin sequences (Fae et al. 2006). Human T cell clones confirmed crossreactivity between cardiac myosin sequences and streptococcal M proteins (Ellis et al. 2005).

The data support a two-hit hypothesis considering that mimicry between alpha-helical cardiac myosins and group A carbohydrate and the alpha-helical streptococcal M protein are important in producing rheumatic heart disease. Therefore, there may be a role for both M protein and group A carbohydrate in the development of rheumatic heart disease. The anti-group A carbohydrate antibody may initiate the disease by reacting with the valvular endothelium, and the T cells specific for M protein and cardiac myosin shared epitopes infiltrate the valve and lead to a TH1 response by the T cells and scarring of the valve. Once the endothelium is activated, T cells infiltrate and inflammatory Th1 cytokines (Guilherme et al. 2004) would be produced in the valve and scarring occurs. The scarred tissue eventually becomes neovascularized with vessels developing in the previously avascular valve tissue. The neovascularization within the scar allows disease to progress within the valve. Scarring and neovascularization in the valve tissues leads to murmurs and endstage disease including irreversible deformation of the valve and chordae tendinae and malfunction of the heart. In summary, evidence from rheumatic heart disease supports the hypothesis that the valve endothelium becomes activated allowing M protein specific T cells to enter the valve and produce disease.

3.1.3 Immune Mechanisms in Sydenham's Chorea

The understanding of Sydenham's chorea and its pathogenesis developed from the discovery that three human mAbs from Sydenham's chorea patients reacted with the surface of neuronal cells and demonstrated antibody crossreactivity with the group A carbohydrate epitope *N*-acetyl-beta-D-glucosamine and lysoganglioside (Kirvan et al. 2003). However, one of the mAbs, 24, was found to induce elevated calcium calmodulin dependent (CaM) protein kinase II levels in a neuroblastoma cell line and to possess the highest avidity for ganglioside (Kirvan et al. 2003).

Subsequent study of acute and convalescent Sydenham's chorea sera led to the discovery that antibodies in the acute sera also had similar reactivity to mAb 24. Acute chorea sera produced elevated CaM kinase II levels in neuroblastoma cells while the convalescent sera did not (Kirvan et al. 2003). Therefore, the antibody-mediated neuronal cell signaling produced by antibodies in chorea was associated with disease and disappeared when the symptoms resolved. Further study indicated that mAb 24 induced tyrosine hydroxylase activity after intrathecal transfer of purified mAb 24 into Lewis rats and sera and mAb 24 induced tritiated dopamine release in vitro. The evidence supports the hypothesis that in Sydenham's chorea antibodies are produced which cross the blood-brain barrier and trigger antibody mediated cell signaling and dopamine release in the caudate putamen region of the brain which would lead to the movement disorder.

4 Post-Streptococcal Glomerulonephritis

Group A streptococci which invade the skin and cause impetigo are different M protein serotypes from those that cause pharyngitis (Bessen et al. 1996, 1998, 2000). In addition, some of the skin strains are associated with production of acute post-streptococcal glomerulonephritis. The skin infections and nephritis are seasonal, usually occurring during the summer months and in temperate climates. The infection is limited to the epidermis usually on the face or extremities and is highly contagious (Bisno 1995a, b). Streptococcal strains which cause pyoderma are less likely to cause rheumatic fever. Staphylococci may be mixed with streptococci in impetigo, thus the treatment of choice is not penicillin for penicillinase-producing staphylococci (Bisno 1995). Group A streptococcal strains may enter the skin through abrasions and other types of lesions to penetrate the epidermis and produce erysipelas or cellulitis. Acute post-streptococcal glomerulonephritis occurs primarily in children and young adults with males affected twice as often as females, and individuals over 40 can also be subject to development of the disease (Bisno 1995a). The epidemiology of acute post-streptococcal glomerulonephritis is related to its presence in southern and temperate climates where pyoderma associated glomerulonephritis demonstrated peak occurrence in the summer while rheumatic fever peaked in the autumn and winter months of the year (Bisno 1995a). In northern climates, acute glomerulonephritis has been associated with throat infection (Silva 1998). However, frequently the same organism infecting the skin in impetigo will also infect the

throat. Risk factors such as crowding, poor hygiene, and poverty are also associated with acute glomerulonephritis outbreaks. Long term prophylaxis with penicillin in patients following a post-streptococcal acute glomerulonephritis attack is not recommended (Silva 1998). Unlike rheumatic fever, the outbreaks of acute glomerulonephritis have continued to decline and may be due to changes in the streptococci or the host (Silva 1998). Regions of the world which still exhibit a high incidence of post-streptococcal acute glomerulonephritis include Africa, the Caribbean, South America, New Zealand and Kuwait.

Nephritogenicity of group A streptococci appears to be related to specific M protein serotypes of *S. pyogenes* which cause acute glomerulonephritis, and certain strains within the serotype are nephritogenic (Bisno 1995a, b). Thus, not all strains of the same M protein serotype are nephritogenic. Both pharyngeal or skin infection can lead to glomerulonephritis. However, predominant M protein serotypes associated with pyoderma or skin infections and glomerulonephritis are M types 2, 49, 42, 56, 57 and 60, while M types 1, 4, 12 and 25 are associated with throat infections and glomerulonephritis (Bisno 1995a, b). It is well-documented that of M type 12 strains not all are nephritogenic (Bisno 1995a).

Characteristics of post-streptococcal acute glomerulonephritis include edema, hypertension, hematuria, urinary sediment abnormalities and decreased serum complement levels with little fever (Bisno 1995a). A latent period of 1–4 weeks (average of 10 days) between the streptococcal infection and development of acute glomerulonephritis is observed, and anti-streptococcal antibody titers for anti-DNAase B or anti-hyaluronidase are elevated. In glomerulonephritis, the latent period from infection to symptoms may be 3–6 weeks and the anti-streptolysin O titers are generally low. After throat infection, the latent period may be 1–2 weeks, and the anti-streptolysin O titers may be higher. Clinical manifestations include discolored or coffee-colored urine due to hematuria, edema of the face and extremities which has a sudden onset, and circulatory congestion due to renal impairment (Bisno 1995a). Recurrent attacks of glomerulonephritis do not cause more severe disease, and in general there is no permanent damage to the kidney in children following the disease attack. A previous review describes pathological and clinical outcomes of postinfectious glomerulonephritis (Silva 1998).

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4

Snakes, Jellyfish and Spiders

Bart J. Currie

1 Introduction

Our knowledge of animal and human physiological processes owes much to over a century of study of natural toxins. The neurotoxins of kraits (bungarotoxins from *Bungarus* spp.) and cobras (*Naja* spp.) have helped define the acetylcholine receptor and neuromuscular transmission. Axonal trafficking and sodium channels have been studied using tetrodotoxin from the blue-ringed octopus (*Hapalochlaena* spp.) and puffer fish (fugu). Unravelling the complexities of the human coagulation pathways (intrinsic and extrinsic) has involved studies using haemotoxins from various snakes, including Russell's vipers (*Daboia russelii*), the saw-scaled vipers (*Echis* spp.) and the Australian taipans (*Oxyuranus* spp.). Ancrod is a snake venom enzyme from the Malayan pit viper (*Calloselasma rhodostoma*) which has been successfully used to treat thrombotic stroke.

The kallikrein – kinin (bradykinin) system was discovered and elucidated by the Brazilian scientists Mauricio Rocha e Silva and Sergio Ferreira, who were looking at the properties of the South American pit vipers, including *Bothrops jararaca*. In 1949, Rocha e Silva discovered that bradykinin, a hypotensive peptide, is produced when *B. jararaca* venom is injected into the blood circulation of mammals (Rocha e Silva et al. 1949). Ferreira subsequently worked on this venom with the Nobel Laureate John Vane, resulting in the discovery and subsequent manufacture of the multi-billion dollar angiotensin converting enzyme class of drugs – “That would not have happened without the “blue-sky” research on the snake venom which started in Brazil and then went on in my laboratories in London. There were so many extraordinary coincidences that were needed in order for that process to fructify, including Ferreira’s choice to visit my laboratory rather than Oxford.” http://www.medschool.lsuhsu.edu/neuroscience/bluesky_research.asp (accessed October 2006).

Snake venom peptides continue to be used in contemporary research. For instance while the small polypeptide neurotoxic bungarotoxins have been used for decades to study the nicotinic acetylcholine receptor, more recently these peptides and their receptors have been used to study trafficking and function of other receptors, such as AMPA and GABA receptors (Sekine-Aizawa and Huganir 2004;

McCann et al. 2006). In addition, there remains enormous potential for novel therapeutics in yet to be discovered natural toxins from other animals such as cone snails, scorpions and poisonous frogs.

2 Toxins and Clinical Relevance

While laboratory scientists discover and evaluate individual toxins from venomous creatures, clinicians are primarily interested in the clinical syndromes resulting from envenoming by an individual species. These clinical syndromes are the result of combined actions of the multiple toxins present in the envenoming animal's venom. Sometimes venoms contain toxins which are potent in vitro, but which rarely or never cause clinical problems. An example is the Australian "brown snake paradox." The Australian brown snakes (*Pseudonaja* spp.) contain complex presynaptic neurotoxins such as textilotoxin, yet neurotoxicity is extremely rare after bites from brown snakes. It is thought likely that slow or inefficient toxin-receptor binding may account for this, coupled with early antivenom use in the majority of envenomed cases where rapid onset coagulopathy dominates the clinical picture (Currie 2004). Furthermore, the specific toxins responsible for some envenoming syndromes remain to be characterised. Such is the case with the early collapse and loss of consciousness seen after bites from some species of snakes.

3 Snakebite

The epidemiology of snakebite is poorly documented, but the most recent estimate is that there are 5 million snakebites globally each year, with 2.5 million envenomed and up to 125,000 deaths (Chippaux 1998). Around a quarter of deaths are in children, suggesting possibly up to 30,000 deaths annually (D. Warrell, personal communication). As with most envenoming scenarios, children have a higher mortality because of their smaller body mass being exposed to a (relatively) fixed venom dose. The vast majority of severe envenomings are from snake species from the family Elapidae (cobras, kraits, mambas, Australasian elapids and sea snakes) or the family Viperidae (true vipers, lance-headed pit vipers and rattlesnake pit vipers) (Gutierrez et al. 2006). The species most responsible for snakebite fatalities are saw-scaled (true) vipers (*Echis* spp.) in northern Africa, cobras (*Naja* spp.) and kraits (*Bungarus* spp.) in Asia and lance-headed pit vipers (*Bothrops asper* and *B. atrox*) in South and Central America (Gutierrez et al. 2006). Paradoxically, two of the most feared snakes, with reputations for great speed, agility, biting prowess and venom potency, have been associated with far fewer well documented fatalities; the black mamba (*Dendroaspis polylepis*) of eastern and southern Africa and the Australian taipan (*Oxyuranus scutellatus*). In Europe the diminutive but widely distributed European adder (*Vipera berus*) still causes occasional deaths, although

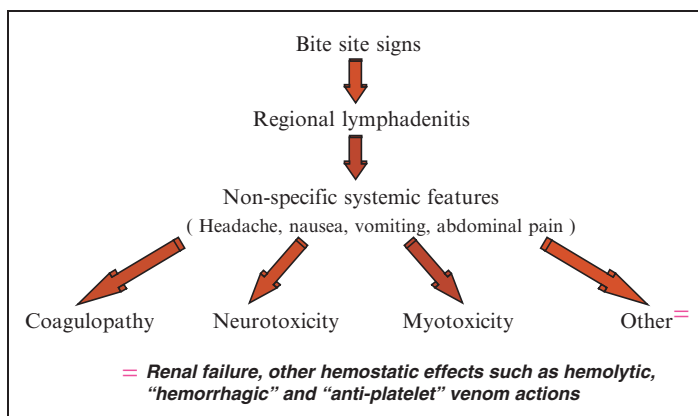


Fig. 1 Template for clinical features of snakebite

it is several decades since the last death from *V. berus* envenoming in the United Kingdom (Warrell 2005) (D. Warrell, personal communication).

There is an enormous diversity in snakebite clinical syndromes globally, reflecting the diversity of snake species and their venoms (Cheng and Currie 2004; Gutierrez et al. 2006). While a summary clinical flow chart can be usefully applied to the features of any snakebite (Fig. 1), the clinical specifics for each region's snakes need to be clearly elucidated for health staff working in any region where they may need to treat victims of snakebite (for a summary see Currie (2004) Table 1).

In some fatal Australasian elapid bites, bite site signs such as fang marks can be minimal or even not visible, while local and extending necrosis at the bite site can be the major and a potentially fatal clinical feature of many Viperidae bites. Regional lymphadenitis is a specific and moderately sensitive indication of envenoming, provided there is a clear difference in pain and/or tenderness to palpation on comparing the inguinal or axillary nodes on the side of the bite with the non-bitten limb's draining nodes. The four cardinal "non-specific" systemic features of envenoming are headache, nausea, vomiting and abdominal pain. These are moderately sensitive and specific for the presence of circulating venom. The importance of regional lymphadenitis and the non-specific systemic features is that they alert the clinician to probable envenoming and mandate careful assessment of the specific features of systemic envenoming that are potentially life-threatening – neurotoxicity, coagulopathy/vasculopathy and myotoxicity.

In addition to severe local tissue damage, death can result from (i) progressive neuromuscular paralysis (neurotoxins – cobras, kraits, mambas and some Australasian elapids); (ii) bleeding internally (eg intracranial haemorrhage) or from bite site or wounds (haemotoxins and/or haemorrhagins – most Viperidae and some Australasian elapids); (iii) rhabdomyolysis resulting in secondary renal failure (myotoxins – some pit vipers, Russell's viper (*Daboia russelii*) and some

Australasian elapids) and (iv) renal failure from other poorly elucidated mechanisms (some pit vipers, Russell's viper (*D. russelii*) and the Australian brown snakes (*Pseudonaja* spp.).

Access to safe and effective antivenoms is the key to decreasing deaths from snakebite. In recent years there has been a global crisis in snake antivenom availability, with some manufacturers ceasing production for commercial reasons (Cheng and Currie 2004; Gutierrez et al. 2006). A comprehensive summary of available snake antivenoms has recently been published, together with a summary of their uses (Lalloo and Theakston 2003). There is considerable variability in the quality and efficacy of antivenoms, with a high rate of antivenom reactions with some. Historically horses have been used for production of snake antivenoms, but more recently some sheep Fab-based antivenoms have been successfully produced and even camel and hens have been considered for antivenom production because of specific favourable properties of their antibodies (Gutierrez et al. 2006). There has been an impressive move towards good clinical trials of new antivenoms and international partnerships to address the shortfall in antivenom availability and the need for development of regionally appropriate antivenoms (Laing et al. 2003, 2004; Pardal et al. 2004; Smalligan et al. 2004; Gutierrez et al. 2005). The proposed global partnership to improve the production, deployment and accessibility of snake antivenoms is at a critical stage and requires ongoing support in the face of many other competing priorities in those locations where snakebite deaths are occurring for lack of antivenom (Gutierrez et al. 2006).

4 Jellyfish Stings

In 1913 Charles Richet was awarded the Nobel Prize for medicine for his recognition of anaphylaxis. His studies began with investigating the sting of the Portuguese man-of-war (*Physalia physalis*) while on a scientific cruise on the yacht of Prince Albert of Monaco. Subsequently he demonstrated sensitisation of dogs injected with sea anemone extract, with subsequent fatal anaphylaxis on re-exposure. A number of hypersensitivity reactions to jellyfish stings have been described (Burnett et al. 1987), including possible anaphylaxis. Initially under recognised but found in around half of those stung by box jellyfish is a delayed hypersensitivity reaction, with intensely itchy papular urticaria occurring on days 7–14 after the sting (O'Reilly et al. 2001). These reactions respond well to topical steroid cream and are attributed to retained jellyfish stinging cell (nematocyst) products in the dermis.

Although much has been made about “reactions” to jellyfish contact, the dramatic demise of those with severe box jellyfish stings is entirely due to envenoming. Envenoming from the major box jellyfish, *Chironex fleckeri* is arguably the fastest natural envenoming process seen. Death, if it occurs, is within minutes and this is why *C. fleckeri* has been called “the world's most venomous animal” (Endean 1988). The potency of the venom has evolved to enable virtually instantaneous death of the jellyfish prey, to avoid damage to the delicate tentacles

from any struggling of the entangled shrimp and small fish. However the only prospective study of *C. fleckeri* envenoming has shown that severe envenoming and death are very uncommon in human encounters (Currie and Jacups 2005). Most stings do not have enough tentacle contact to be life-threatening, although even minor stings are extremely painful and can result in blisters and scarring. However the last 13 deaths in Australia from *C. fleckeri* envenoming have all been in children, reflecting the greater risk of a smaller body mass exposed to the billions of nematocysts on jellyfish tentacles (Currie and Jacups 2005).

While the fatal cases of *C. fleckeri* envenoming are thought to result from cardiotoxicity with arrhythmias, the “lethal factor” in *C. fleckeri* venom remains to be isolated and characterised. It is thought that the mechanism of action involves abnormalities in ionic transport across nerve and muscle membranes. This limited understanding of such a potent natural toxin contrasts with the vast knowledge of snake toxins. Furthermore, while there is an ovine antivenom for use in *C. fleckeri* envenoming, there remain uncertainty about its efficacy (Endean and Sizemore 1988; Currie 2003; Currie and Jacups 2005). Given the rapidity of the fatalities, the opportunities to trial *C. fleckeri* antivenom as a life-saving therapy are extremely limited.

There are a number of other related multi-tentacled box jellyfish which have also caused fatalities, most notably in the Philippines and Japan (1996). A small number of deaths have also been attributed to the Portuguese man-of-war (*Physalia physalis*) (1996). Recently there has been one probable and one confirmed death in far north Queensland, Australia from intracranial haemorrhage resulting from the Irukandji syndrome, the first being a 58 year old tourist from the United Kingdom visiting the Great Barrier Reef (Fenner and Hadok 2002; Huynh et al. 2003). This enigmatic syndrome follows stings from a number of small four-tentacled box jellyfish species, most of which are yet to be characterised (Little et al. 2006). It results in delayed onset (10–40 min after jellyfish contact) “systemic pain,” profuse sweating, piloerection and hypertension. Subsequent cardiogenic pulmonary oedema can occur at 12–24 h. The Irukandji syndrome has been attributed to an endogenous catecholamine storm precipitated by unknown toxins in the jellyfish (Corkeron et al. 2004; Winkel et al. 2005).

Box jellyfish evolved more than 300 million years ago, but they display some remarkable properties. *C. fleckeri* has 24 eyes, including some with a primitive cornea, lens and retina (Kavanau 2006). Visual cues are processed by a neural pathway without a brain and recent observations suggest that after being metabolically active *C. fleckeri* spend periods of time “asleep” on the sea floor (Seymour et al. 2004). The envenoming mechanism involves a stylet-like folded collagen spring in the nematocyst everting to fire a harpoon-like thread, which pierces the external barrier of the prey or the human victim’s dermis and delivers the venom. This process takes only 3 ms – 10 times faster than a vehicle air bag. This is considered evolutionarily to be the earliest known use of collagen and the force generated has been estimated at 40,000 G force or over 7 GPa, which is in the range of technical bullets (Nuchter et al. 2006). *C. fleckeri* are also efficient and agile swimmers and can move at 1.5 knots in a purposeful direction.

5 Spider Bites

Deaths from spider bites are extremely uncommon. From clinical experience and animal lethal dose studies, the most venomous spiders to humans and other primates worldwide are the male Australian funnel-web spiders. Funnel-web spiders comprise around 40 species of the genera *Atrax* and *Hadronyche* and they are found primarily on the eastern seaboard of Australia, with Sydney a most notable habitat. The envenoming syndrome of funnel-web spider bite is often rapid in onset, beginning in the most comprehensive study from 8 to 175 min after the bite (median 28 min) (Isbister et al. 2005). Clinical features reflect the toxins, which modulate sodium channel function to prolong action potentials, resulting in spontaneous, repetitive nerve firing and transmitter release. Muscle fasciculations are a particular finding, plus autonomic features such as diaphoresis, increased salivation, piloerection, lacrimation and pupillary changes. Hypertension or hypotension and arrhythmias can occur and subsequent death from pulmonary oedema has been documented.

There have been only 13 confirmed deaths from funnel-web spider envenoming. There has been excellent clinical response to the rabbit-derived IgG funnel-web spider antivenom which was first used in 1981, with no deaths since its introduction (Isbister et al. 2005). There are four widow spider (*Latrodectus* spp.) antivenoms available, including the American black widow (*L. mactans*) antivenom and the Australian red-back spider (*L. hasselti*) antivenom. Antivenoms are also available for recluse spiders (*Loxosceles* spp.) These are the spiders definitively associated with necrotic arachnidism, but there is little evidence to support the effectiveness of antivenom in preventing or decreasing the local necrosis (Isbister et al. 2003).

As with snakebite and jellyfish stings, prospective studies of spider bites are allowing firmer definitions of envenoming syndromes and responses to therapies such as antivenoms. A critical aspect of this improved evidence base is the restriction of analysis to cases where a spider was seen to bite the victim and the spider was retrieved and formally identified by an expert (Isbister and Gray 2002; Isbister and White 2004). The most important recent advance from this approach has been the debunking of the “myth of necrotic arachnidism.” For several decades there has been an increasing attribution of ulcerating skin lesions to necrotic arachnidism. This has occurred in Europe as well the USA and Australia. The propagation of this global myth has been through not just the public media, but also through medical journals.

Necrotic arachnidism was originally attributed to bites from the recluse spiders (*Loxosceles* spp.) in South America and the USA and called loxoscelism. Subsequently it was determined that bites from at least one species of recluse spider did indeed cause distinct necrotic skin ulceration. This is the brown recluse spider (*L. reclusa*) of central and southern states of the USA and the necrosis is thought to be caused by the enzyme sphingomyelinase D. However even in the USA the vast majority of lesions attributed to necrotic arachnidism have alternative diagnoses (Swanson and Vetter 2005). Indeed doctors and patients not uncommonly attribute a lesion to

necrotic arachnidism in locations where there are no recluse spiders and even when a bite from a spider was not witnessed or considered likely.

Sphingomyelinase D has not been found in the other species of spiders accused of being responsible for necrotic arachnidism and recent careful studies have systematically exonerated them. These include wolf spiders (family Lycosidae), the Australian white-tailed spiders (*Lampona cylindrata* and *L. murina*) and black house spiders (*Badumna* spp.), the hobo spider (*Tegenaria agrestis*) and yellow sac spiders (*Cheiracanthium* spp.) (Vetter et al. 2006). In Australia the attribution of necrotic arachnidism to white-tailed spider bites rapidly increased after it was first raised as a possibility in 1982. Many doctors began making the diagnosis of white-tailed spider bite on the presence of a skin ulcer (Isbister 2004). It wasn't until a prospective study of 130 confirmed bites by white-tailed spiders showed no cases of necrotic ulcers (Isbister and Gray 2003) that the myth began to unravel in the same journal that published much of the unsubstantiated early claims (White 2003).

So if it isn't a spider bite, what does cause that necrotic skin ulcer? Table 1 lists conditions that could be potentially misdiagnosed as necrotic arachnidism. Two notable recent situations have been the initial misdiagnosis of one of the 2001 USA anthrax bioterrorism cases as *Loxosceles* spider bite (despite brown recluse spiders not being endemic on the East Coast USA) (Swanson and Vetter 2005) and the surprisingly common misdiagnosis of community-acquired methicillin-resistant *Staphylococcus aureus* skin sepsis as a spider bite (Dominguez 2004).

Table 1 Conditions potentially misdiagnosed as necrotic arachnidism

Infective ulcers

Bacterial

- Staphylococcal (including MRSA) and streptococcal
- Mycobacterium ulcerans*, other atypical mycobacteria, *M. tuberculosis*
- Environmental bacteria; cutaneous nocardia, *Chromobacterium violaceum*
- Ecthyma gangrenosum; *Pseudomonas* spp.
- Cutaneous anthrax, tularaemia, melioidosis, rickettsial infection, Lyme disease

Fungal

- Sporotrichosis, chromoblastomycosis, cryptococcosis

Viral

- Herpes simplex, herpes zoster

Parasitic

- Leishmaniasis
 - Tropical ulcer
 - Pyoderma gangrenosum
 - Vasculitic ulcer
 - Polyarteritis nodosa, leukocytoclastic vasculitis, Wegener's granulomatosis
 - Malignant skin ulcer
 - Chemical cellulitis with ulcer
 - Insect bites, plant contact dermatitis
 - Diabetic and pressure ulcers
-

6 Conclusions

Prospective studies of bites and stings from case series where the envenoming animal is formally identified are laying the foundation of a more comprehensive evidence-based knowledge of the diverse range of envenoming syndromes from the natural world. With randomised comparative studies of new therapies, best practice is being formulated. However production, deployment and accessibility of good quality snake antivenoms remains the most pressing need in global clinical toxinology.

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Current Status of Group A Streptococcal Vaccine Development

James B. Dale

1 Introduction

Group A streptococci (GAS) are ubiquitous human pathogens that cause a wide spectrum of clinical syndromes. The acute infections range from uncomplicated pharyngitis, cellulitis, and pyoderma to necrotizing fasciitis, sepsis, pneumonia, and streptococcal toxic shock syndrome. The non-suppurative sequelae that may follow GAS infections are acute rheumatic fever (ARF) and post-streptococcal glomerulonephritis (PSGN). The burden of disease caused by GAS in economically developed and developing countries is significant. The search for a safe and effective vaccine to prevent GAS infections and their complications has been ongoing for more than 80 years. This chapter details the worldwide burden of disease, the obstacles encountered in vaccine development, current vaccine strategies and an update on the clinical development of M protein-based vaccines.

2 Burden of Disease Caused by GAS Infections

There is a distinct dichotomy in the burden of GAS infections and their sequelae between economically developed and poor countries of the world. In the US, Western Europe and other developed countries the majority of these infections present as uncomplicated pharyngitis or pyoderma. The recent worldwide resurgence of invasive infections has impacted most populations of the world (Carapetis et al. 2005). In the U.S. the Centers for Disease Control estimate that there are approximately 10,000 serious, invasive infections each year resulting in as many as 1,700 deaths. The direct and indirect cost of uncomplicated pharyngitis in the US alone is estimated to be over \$2 billion per year (Bisno et al. 2005). Thus, a vaccine aimed at the most common forms of GAS infections would certainly be cost effective and preventing even a fraction of the invasive infections could have a significant impact. These data are important for the eventual commercial development of GAS vaccines, since revenues from the sale of vaccines in economically advantaged countries might offset the costs of manufacturing and distributing the vaccines to poor countries.

Without a doubt, the largest worldwide burden of disease caused by GAS infections is ARF and rheumatic heart disease (RHD). Prior to World War II, ARF was relatively common in all parts of the world. In the late 1930s, there began a remarkable and steady decline in the number of ARF cases in the U.S., Western Europe, and other industrialized nations (Stollerman 1997). The decline was variably attributed to the introduction of penicillin, improved living conditions, less crowding, and better access to medical care (Stollerman 1975). All of these theories were supported, at least in part, by the observation in the 1960s and 1970s that in the U.S., ARF had become a disease of the inner city poor (Land and Bisno 1983). Apart from an impressive resurgence of ARF in the late 1980s in Utah (Veasey et al. 1987), Ohio, and a few other U.S. locations (Stollerman 2001), the incidence of the disease has remained at low levels in the economically developed countries of the world. This is clearly not the case in economically deprived countries where rheumatic fever and rheumatic heart disease remain rampant.

The W.H.O. estimates that 400,000 people die each year from complications of RHD (WHO 1992). In addition, approximately 12 million people currently suffer from RHD, and 1 million are in need of open-heart surgery and valve replacement. Only a small fraction of these life-saving procedures are actually performed because of their high costs. The prevalence of RHD among school-age children ranges from 0.7/1,000 to 14/1,000 with an average of 4/1,000 (Steer et al., 2002). The W.H.O., in its Global Burden of Disease Report 2001, calculates the disability-adjusted life years (DALYs) attributed to RHD as 6,112,465, which represents a significant worldwide reduction in productive life span due to premature heart disease. Primary and secondary prevention strategies based on antibiotic administration have only been marginally successful in developing countries. Primary prevention with antibiotics requires ready access to medical care and is best suited for economically developed countries (Bisno 2001). In addition, it has also been recognized that as many as 50% of all cases of ARF may be preceded by asymptomatic infections, which would preclude their prevention by this strategy altogether (Veasey et al. 1987). Secondary (long term) prophylaxis is labor intensive and requires a public health infrastructure for implementation and documentation of compliance, which is often quite low (WHO 1992). Vaccine prevention of even a fraction of the total number of cases of ARF could have a major impact on the health of millions of children and young adults, as well as reducing the economic burden of this devastating disease.

3 Current Strategies for Vaccine Development

Current strategies for vaccine development are based on our understanding of the molecular pathogenesis of group A streptococcal infections and the virulence determinants that contribute to pathogenesis. Group A streptococci are human-specific pathogens that are maintained in the population and disseminated among individuals through symptomatic infections of the mucous membranes and skin.

The organisms are spread via droplets or by hand-to-mouth contact. Attachment to mucosal surfaces is thought to be an important first step in the pathogenesis of pharyngeal infections. A number of ligands that mediate adherence to specific host cells have been defined including lipoteichoic acid, several fibronectin binding proteins, and M proteins (Hasty et al. 1992). Firm attachment allows the organisms to colonize the mucosal surface where extracellular toxins and possibly specific invasins promote tissue damage and penetration into deeper tissues, respectively.

Once in contact with blood or exudate, the organisms must resist opsonization and phagocytosis in order to establish infection. Streptococcal surface M protein has been considered the major determinant of resistance to phagocytosis (Lancefield 1962). Some M proteins bind plasma fibrinogen, which coats the bacterial surface and blocks activation of the alternate complement pathway as well as nonspecific deposition of C3b (Whitnack and Beachey 1982). M protein and fibrinogen also bind factor H, a potent regulator of the complement cascade, which prevents the generation of C3b (Fischetti et al. 1995). Some M proteins and M-like proteins bind immunoglobulins through nonimmune mechanisms (Lindahl and Stenberg 1990), which may mask the surface of the organism and prevent nonspecific immune recognition. Another important virulence factor is the hyaluronate capsule, which for some serotypes appears to be the major determinant of resistance to phagocytosis (Dale et al. 1996; Moses et al. 1997). Group A streptococci also express C5a-peptidase that specifically inactivates the potent chemoattractant and reduces the influx of neutrophils (PMNs) into the area of infection (Ji et al. 1996). Approximately half of all GAS express serum opacity factor (SOF), which is a virulence determinant and in some cases also serves as a protective antigen (Courtney et al. 2003). Extracellular products of group A streptococci that have an important impact on the host include the streptococcal pyrogenic exotoxins (SPEs), most of which have both toxic and superantigenic properties and likely play an important role in the pathogenesis of severe infections and streptococcal toxic shock syndrome (Kotb 1995). SPE B is also a precursor for cysteine protease, which appears to be an important determinant of virulence (Kapur et al. 1994). Streptolysin O and streptolysin S are extracellular hemolysins with broad host cell specificity and likely are important mediators of infection and inflammation.

Many of the virulence determinants mentioned above are shared among most or all serotypes of group A streptococci and are being considered as vaccine candidates. The C-repeat region of M protein (Fig. 1) contains common sequences that evoke protective antibodies in animals (Bessen and Fischetti 1988; Bronze et al. 1992; Bisno 2001). In studies by Fischetti and his coworkers, the C-repeat peptides evoked cross-protective immune responses in mice after intranasal administration (Bessen and Fischetti 1988). The common, protective C-repeat peptides have been expressed on the surface of *Streptococcus gordonii*, which is designed to serve as a live carrier for the mucosal vaccine (Bolken et al. 2002). A series of reports by Good and colleagues demonstrated the presence of opsonic antibodies in animals after parenteral immunization with synthetic peptides copying a portion of the C-repeat sequence (Pruksakorn et al. 1994; Brandt et al. 1997, 2000). This approach has recently been extended to include the C-repeat peptide

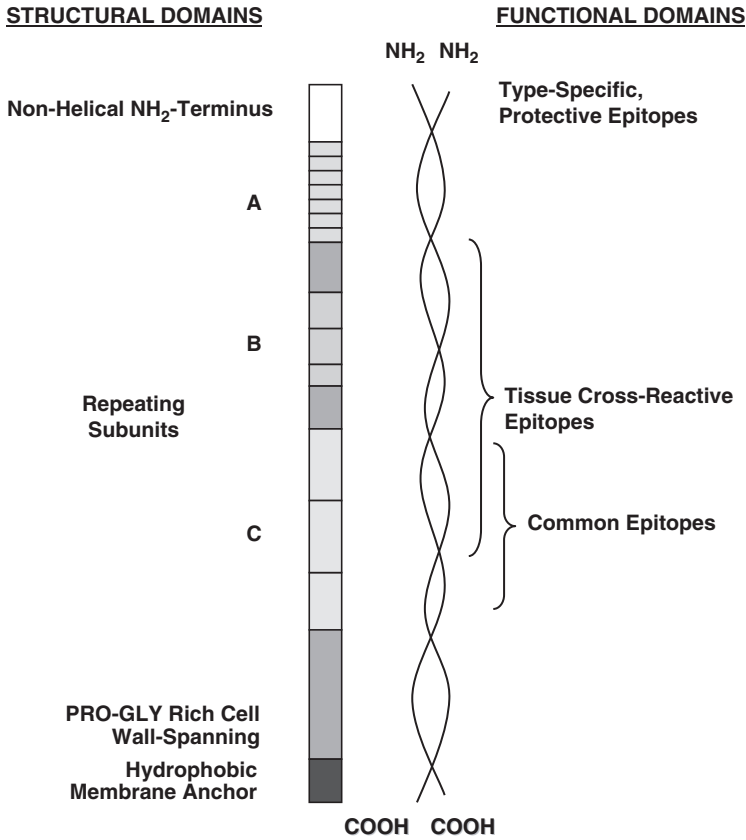


Fig. 1 Schematic representation of the structural and functional domains of streptococcal M protein

coupled to type-specific M protein epitopes from serotypes that are epidemiologically associated with ARF in Australia (Brandt et al. 2000; Olive et al. 2006). Extracellular products of group A streptococci have also demonstrated protective efficacy in animal models. C5a-peptidase evoked antibodies that neutralized the enzyme, which enhanced phagocytic killing of the organisms (Ji et al. 1996) and protected animals from challenge infections (Jones et al. 1986). SPE-B, which is the precursor of cysteine protease also showed some protective efficacy in animals (Kapur et al. 1994). Additional common antigens that are being considered as potential vaccine candidates include the group carbohydrate (Zabriskie et al. 1997; Sabharwal et al. 2006), several fibronectin binding proteins (Courtney et al. 1994; Kawabata et al. 2001), streptococcal pyrogenic exotoxins (Roggiani et al. 2000), streptolysin S (Dale et al. 2002), a new streptococcal protective antigen, Spa (Dale et al. 1999), and serum opacity factor (Courtney et al. 2003).

4 Rationale for M Protein-Based GAS Vaccines

Although there are sufficient data in animals to suggest that the virulence determinants described above may contribute to protective immune responses, the observation remains that most individuals acquire more than one streptococcal infection in a lifetime. Group A streptococci have evolved a complex array of type-specific, protective M protein epitopes that largely accounts for the immunologic diversity of the species (Fischetti 1989; Cunningham 2000). We believe the best evidence developed over the longest period of time supports the use of multivalent, type-specific, M protein-based vaccines designed to elicit bactericidal antibodies against epidemiologically significant serotypes of group A streptococci. The M proteins of group A streptococci have been functionally defined as surface proteins that (i) confer resistance to phagocytosis, and (ii) contain protective (opsonic) epitopes. They exhibit some common structural features, including high alpha-helical potential throughout the majority of the protein and conserved C-terminal sequences (Fischetti 1989). The M proteins extend as coiled-coil dimers (Phillips et al. 1981) from the cell wall with the N-terminus oriented outward and the C-terminus buried in the cytoplasm (Fig. 1). In general, the N-terminal 20–35 amino acids of the mature proteins are non-helical and are distinct from one serotype to another. Some M proteins contain internal tandem A and B repeats in the N-terminal half, and all contain common C repeats in the C-terminal half.

Perhaps the most significant challenge in the development of M protein-based streptococcal vaccines has been the finding that some M proteins are immunologically cross-reactive with human tissues (Cunningham 2000). Although there is no direct evidence linking M protein tissue cross-reactive antibodies with the pathogenesis of ARF, until the pathogenesis is understood, we are obligated to make every effort to exclude tissue cross-reactive epitopes from vaccine preparations. A series of studies from our laboratory (Dale et al. 1983; Beachey and Seyer 1986; Dale and Beachey 1986; Dale et al. 1993) and others (Jones et al. 1985; Jones and Fischetti 1988) has shown that protective epitopes may be separated from tissue cross-reactive epitopes. The N-terminal, hypervariable regions of M proteins contain epitopes that evoke antibodies with the greatest bactericidal activity (Dale et al. 1983; Beachey and Seyer 1986; Dale and Beachey 1986; Jones and Fischetti 1988) and are least likely to evoke tissue cross-reactive antibodies. Most of the cross-reactive epitopes identified have been located in the middle of the M proteins (Fig. 1) and can be separated from the N-terminal, protective epitopes. Together, these results now serve as the basis for our current strategy for M protein-based vaccine design, which is to incorporate limited N-terminal peptide fragments of multiple M proteins into recombinant, multivalent vaccine constructs. We now have a considerable amount of data indicating the feasibility of this approach.

5 Preclinical Evaluation of a 26-Valent M Protein-Based Vaccine

Current epidemiologic data indicate that the majority of group A streptococcal infections in the U.S. are caused by relatively few serotypes. Surveillance of invasive disease conducted by the U.S. Centers for Disease Control and Prevention has shown that during the years 1998–2000, 19 serotypes accounted for 84% of the total isolates (Schuchat et al. 2001). In ongoing studies to determine the serotype distribution of group A streptococci recovered from pediatric cases of pharyngitis in the U.S., it was shown that 16 different serotypes accounted for 97% of all cases of pharyngitis (Shulman et al. 2004). These data indicate that a multivalent vaccine containing M protein fragments from a limited number of serotypes could potentially have a significant impact on the overall incidences of streptococcal infections within a population. Therefore, a 26-valent vaccine was designed to include N-terminal M peptides from epidemiologically important serotypes of group A streptococci. These include the serotypes commonly responsible for serious infections, uncomplicated pharyngitis in children, and the serotypes that are currently or historically associated with acute rheumatic fever. Based on this information, serotypes included in the 26-valent vaccine (Fig. 2) account for 78% of all invasive infections, 80% of all cases of uncomplicated pharyngitis, and theoretically 100% of all “rheumatogenic” serotypes.

The 26-valent vaccine consists of four component fusion proteins (Fig. 2) that were mixed in equimolar ratios and formulated with alum to contain 400 µg of

Hexa A.1						
M24	M5	M6	M19	M29	M14	M24

Septa B.2							
M1.0	M12	Spa	M28	M3	M1.2	M18	M1.0

Septa C.2							
M2	M43	M13	M22	M11	M59	M33	M2

Septa D.1							
M89	M101	M77	M114	M75	M76	M92	M89

Fig. 2 Composition of the 26-valent M protein-based GAS vaccine. 5' *emm* gene fragments encoding the hypervariable type-specific epitopes of multiple M proteins were incorporated into four different hybrid genes. Recombinant proteins were purified and mixed to constitute the final vaccine product

protein/dose (Hu et al. 2002). Three rabbits that received three I.M. doses of the vaccine at 0, 4, and 16 weeks developed broadly opsonic antibodies that were not cross-reactive with human tissues. Antibody titers were determined by ELISA using serum obtained at 18 weeks against each of the purified recombinant dimeric peptide components of the vaccine. All preimmune titers were less than 200. Of the 81 immune serum titers determined (27 antigens \times 3 rabbits), 69 titers (85%) increased by fourfold or greater. The vaccine elicited fourfold or greater increases in antibody levels against 25 of the 26 serotypes represented in the vaccine. To determine the functional activity of the M protein antibodies evoked by the 26-valent vaccine, in vitro opsonization and bactericidal tests were performed using each of the 26 serotypes of group A streptococci. Opsonization assays were designed to determine the percentage of neutrophils that engulfed or were associated with streptococci after rotation in nonimmune human blood that contained either preimmune or immune rabbit serum. The preimmune sera from all three rabbits resulted in $\leq 10\%$ opsonization of each of the 26 serotypes tested, indicating that the donor blood used for these assays did not contain antibodies against the test organism and that each organism was fully resistant to opsonization in nonimmune blood. Using 30% opsonization in the presence of immune serum as a positive threshold result, 18 of the 26 serotypes (69%) were opsonized by at least one of three immune rabbit sera.

Bactericidal assays were also performed as an additional measure of the potential protective efficacy of the 26-valent vaccine. In these assays, each of the 26 serotypes of group A streptococci was rotated in nonimmune blood for 3 h in the presence of either preimmune or immune rabbit sera. In all experiments, the test mixture containing preimmune serum resulted in growth of the organisms to eight generations or more, again indicating that the human blood did not contain opsonic antibodies against the test strains and that each organism was fully resistant to bactericidal killing in nonimmune blood. Using 50% reduction in growth (percent killing) after the 3 h rotation in blood containing immune serum compared to the preimmune serum as a positive threshold, bactericidal activity was observed against 22 of the 26 serotypes tested. When the results of the opsonization and bactericidal assays were combined, 24 of the 26 serotypes (92%) tested were opsonized by the immune sera in one or both assays. These results show that a highly complex 26-valent M protein-based vaccine was immunogenic in rabbits and evoked broadly opsonic antibodies against the vast majority of vaccine serotypes. Further experiments indicated that the vaccine did not evoke potentially harmful tissue cross-reactive antibodies.

6 Clinical Experience with the 26-Valent GAS Vaccine

The 26-valent vaccine described above has now completed phase I and II clinical trials to determine its safety and immunogenicity in adult volunteers. These studies were under the direction of Scott Haperin, MD and Shelly McNeil, MD at the IWK

Grace Health Center, Dalhousie University, Halifax, Canada. In the phase I component of this study, 30 adult volunteers received 400 µg of the 26-valent vaccine formulated with alum administered I.M. at 0, 4, and 16 weeks (McNeil et al. 2005). Clinical and laboratory follow-up included assays for tissue cross-reactive antibodies, type-specific antibodies against the component peptides of the vaccine, and functional assays to detect bactericidal antibodies. The vaccine was found to be safe and well-tolerated. Local reactogenicity was comparable to other alum-based vaccines in adults. None of the subjects had laboratory or clinical evidence of rheumatic fever or nephritis. None of the subjects developed tissue cross-reactive antibodies. 26 of 27 of the vaccine peptides evoked a >4-fold increase in the geometric mean antibody titer over baseline. Significant bactericidal activity was observed after immunization for all vaccine serotypes of GAS. A phase II study that included 70 adult volunteers has recently been completed which showed similar results.

7 Development of Vaccines Designed to Prevent Rheumatic Fever

The 26-valent vaccine described above was designed based on epidemiologic data from North America. Based on currently available data, it is predicted that this vaccine would be most effective in economically developed countries. Other reports suggest that the epidemiology of GAS infections in developing countries where ARF is common may be quite different (Pruksakorn et al. 2000; Ho et al. 2003; Dey et al. 2005). For this reason, we have begun prospective studies to determine the molecular epidemiology of streptococcal pharyngitis in Nicaragua and Mali. A similar protocol is being conducted in Fiji under the direction of Michael Good and Jonathan Carapetis from Australia. Both of these projects are funded by the US National Institutes of Health (National Institute of Allergy and Infectious Diseases) and are designed as cooperative agreements between the investigators and the NIH. The overall goal is to determine the emm-types of GAS that are prevalent causes of infection so that new vaccines can be developed based on the epidemiology of infections in areas where ARF and RHD are common. Ultimately, the goal is to determine if ARF is truly a vaccine-preventable disease.

8 Summary and Conclusions

We now have a much more detailed understanding of the molecular pathogenesis of GAS infections. These discoveries have led to the identification of several vaccine candidates which are in various stages of development. One of the leading candidate antigens is the surface M protein, which confers protection against infection in animal models. In addition, M antibodies in human serum correlate

with protection against infection with the homologous serotype of GAS. Molecular techniques have been used to genetically engineer highly complex multivalent M protein-based vaccines that appear to be free of potentially harmful tissue cross-reactive epitopes. A 26-valent vaccine has been shown to be well-tolerated and immunogenic in adult volunteers and is now being considered for pediatric trials, which is the primary target group for the vaccine. Ongoing efforts are now addressing the epidemiology of GAS infections in developing countries so that new vaccines can be designed to prevent the infections that may trigger ARF and RHD. Successful deployment of safe and effective vaccines to prevent GAS infections and their complications could potentially have a significant impact on the health of millions of people around the world.

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6

New Aspects on Diagnosis and Transmission of Hepatitis B in Pediatric Patients and Pregnant Women

Robert A. de Man, Annemiek van der Eijck, and Irene Veldhuijzen

1 Horizontal Transmission of Hepatitis B and the Paediatric Patient

Epidemiologic studies of hepatitis B virus (HBV) show varying levels of endemicity world-wide. The prevalence of chronic HBV in developed nations of Western Europe and North America is about 0.1–0.5% and HBV infection in these societies is usually acquired in adult life through parental or sexual contact. In contrast, in less developed countries as sub-Saharan Africa, Asia and the Far East, HBV is highly endemic and the majority of infections occur in infancy and childhood through horizontal transmission (ie that occurring without apparent parental, sexual or perinatal exposure).

A seroepidemiological survey of HBV infections in a rural district in Ghana showed an increase in seroprevalence of HBV with age. The prevalence of at least one marker of HBV infection was about 33% at the age of 1, 60% at the age of 6 and 83% at the age of 12. Specific risky behaviours significantly associated with horizontal transmission were sharing of bath towels, sharing of chewing gum or partially eaten candies, sharing of dental cleaning materials and biting of fingernails in conjunction with scratching the back of others.

A study investigating the distribution of responses to HBV infection in the rural community of Tip, Senegal showed also that the proportion of infected persons increased rapidly with age, indicating a predominance of horizontal transmission.

It should be noted that here is a continuing risk for horizontal HBV transmission among children born in a developed country, but whose parents are refugees from a high endemic area with HBV. A baseline seroprevalence survey of HBV in US-born Southeast Asian children showed that children born to HBsAg-negative women and living with carriers were 5.4 times more likely to have evidence of HBV infection than were children who did not live with carriers. In the same survey, approximately one third of children with serologic evidence of HBV infection lived in households without carriers and were probably infected by other family members or children from their neighbourhood. In these communities the prevalence of chronic HBV infection approaches that of the country of origin.

2 Infectivity of Body Fluids

The development of molecular diagnostic assays has revolutionised the ability to detect viruses both qualitatively and quantitatively. More information regarding the precise amounts in the different body fluids provides insight in the potential infectivity of these fluids. HBV DNA, which indicates potentially infectivity, has been shown to be present in other body fluids than serum and semen, such as saliva and urine.

The U.S. Public Health Service has published guidelines for the management of occupational exposure to HBV, HCV and HIV and recommendations for post exposure prophylaxis. Blood, body fluids containing visible blood, semen and vaginal secretions are considered potentially infectious. Also considered potentially infectious are: cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid and amniotic fluid. Further the report states that faeces, nasal secretions, vomitus, tears, sputum, saliva and urine are not considered as potentially infectious, unless they contain blood. In addition it is stated that the risk for transmission of HBV, HCV and HIV infection from these fluids and materials is extremely low.

Recently a study was published which describes the results of paired quantitative HBV DNA measurements in serum, saliva and urine in 150 chronic HBV patients. In the study of van der Eijk et al. a non-linear correlation was found between the level of HBV DNA in serum and the level of HBV DNA in saliva or urine (see Fig. 1a and 1b). HBV DNA levels above 10^5 copies/mL were found in 15% of saliva and 1% of urine samples. None of the samples showed a higher HBV DNA level in saliva or urine compared with the paired serum sample. The results showed in both HBeAg-positive as in HBeAg-negative patients HBV DNA levels up to 10^7 copies/mL in clear saliva samples (Fig. 2a, 2b and 2c). The significant amounts of HBV DNA in saliva and urine in chronic HBV patients with high viremia in serum could have implications for the understanding of hepatitis B epidemiology, as the origin of infections especially in horizontal transmission remains unknown in up to 20% of cases. In experimental studies using gibbons, saliva from HBsAg-positive donors was administrated subcutaneously and orally. The animals inoculated subcutaneously developed HBsAg followed by anti-HBs whereas none of the gibbons exposed orally developed evidence of HBV infection. These experiments show that the HBV DNA particles in saliva may still be infectious. A case of acute hepatitis B infection that developed after a human bite in a sheltered accommodation was further investigated and genotypic evidence of viral transmission with saliva being the vehicle is described by Hui et al. Heermann et al. stated that approximately 10% of HBV particles detected in serum are in fact infectious. The percentages for saliva and urine are unknown and therefore transmission studies in appropriate models to establish the transmission risk for each log level of viremia for the different body fluids should be performed before further translating these findings in public health advisory practice.

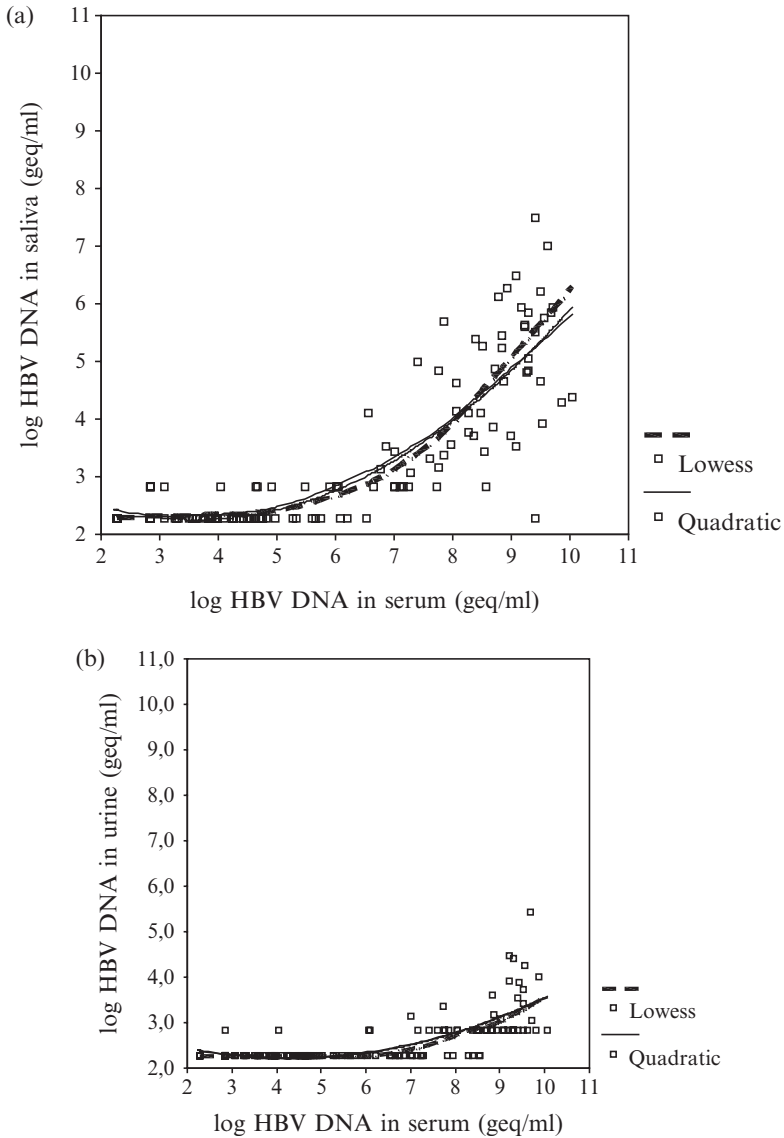
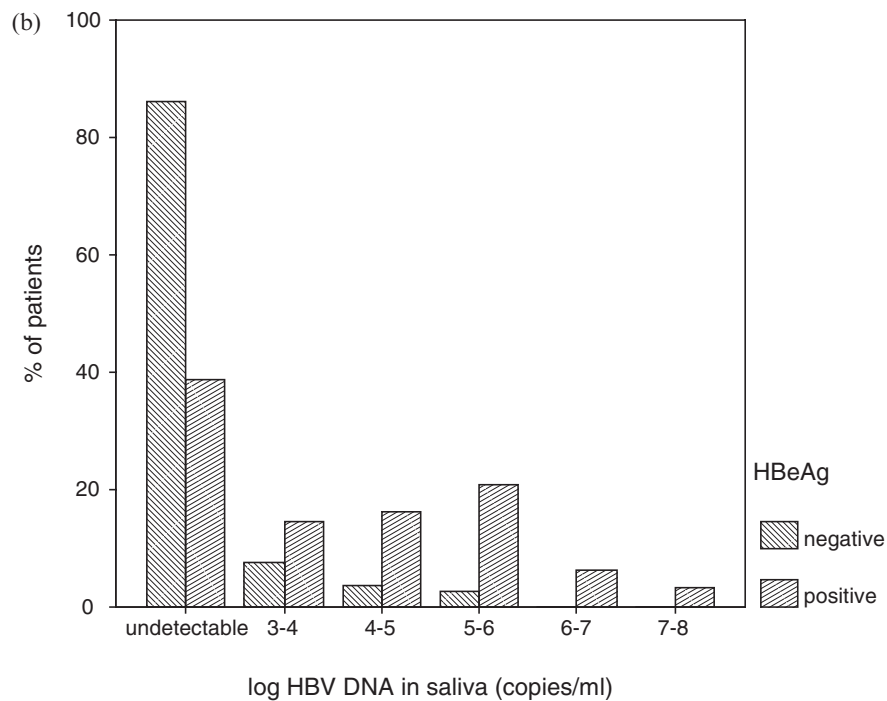
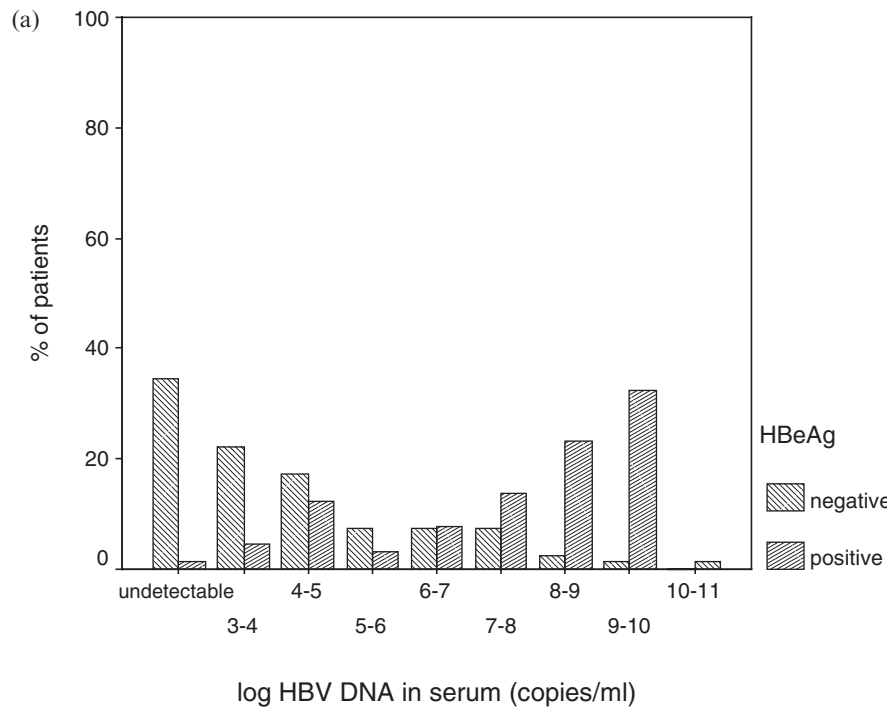


Fig. 1 Individuals with low viraemia (less than 10^5 copies/mL) had undetectable or very low levels of HBV DNA in saliva or urine. High virus levels (more than 10^5 copies/mL) in saliva were found in individuals with serum HBV DNA of 10^7 copies/mL or more. High virus levels in urine were only found in individuals with serum HBV DNA of 10^9 copies/mL or more. (a) Association between HBV DNA (copies/mL) in serum and saliva. The dotted line represents the Lowess fit; the black line represents the fitted curve of the quadratic model. (b) Association between HBV DNA (copies/mL) in serum and urine. The dotted line represents the Lowess fit; the black line represents the fitted curve of the quadratic model



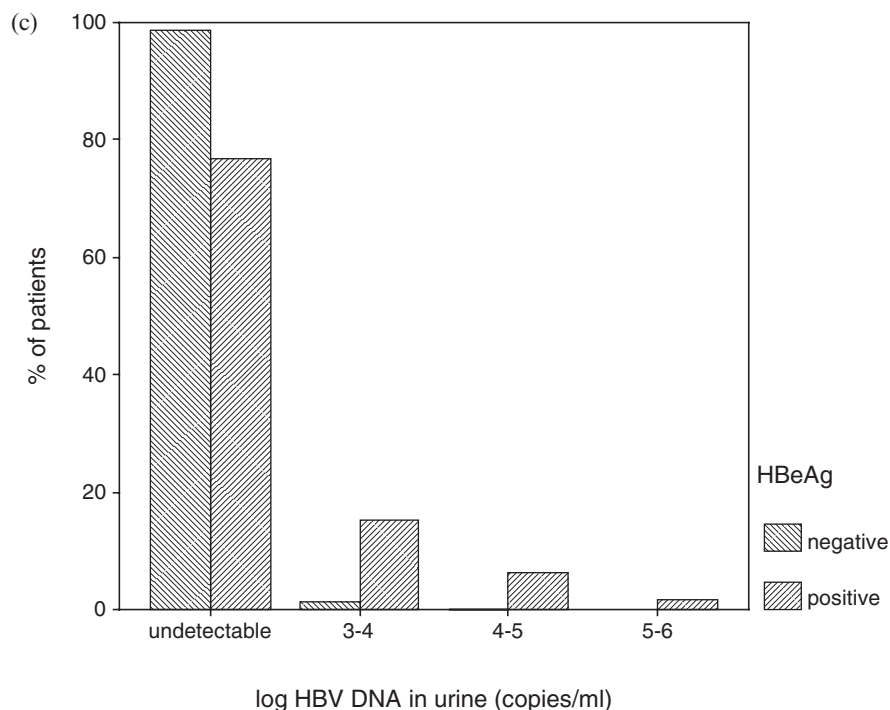


Fig. 2 (a) Quantitative log HBV DNA levels (copies/mL) in serum versus HBeAg status of the patient. (b) Quantitative log HBV DNA levels (copies/mL) in saliva versus HBeAg status of the patient. (c) Quantitative log HBV DNA levels (copies/mL) in urine versus HBeAg status of the patient

3 Implications for the Paediatric Patient

Horizontal infection of young children contributes considerably to the seroprevalence of antibody markers in older children and adults. Horizontal transmission among children may result from close bodily contact, leading to transfer of HBV infected body fluids. These body fluids could inoculate HBV into cutaneous scratches, abrasions, burns, other lesions or on mucosal surfaces. A HBV DNA level of 10^5 copies/mL is a level below which virus transmission via needlestick or mucosal scratch is highly unlikely. HBV DNA levels above this level are reached in saliva and urine in a selected group of patients. For saliva these levels are found in patients with a HBV DNA level in serum above 10^7 copies/mL, for urine these levels are namely found in patients with HBV DNA levels above 10^9 copies/mL in serum.

In a selected group of patients with high (HBV DNA levels above 10^7 copies/mL) virus levels in serum a potential role for saliva in transmitting HBV is therefore postulated.

Experiments in gibbons showed no evidence of oral transmission of HBV. Separate dishes and eating utensils for infected persons are thus not required. With appropriate immunoprophylaxis, including hepatitis B immunoglobulin and hepatitis B vaccine, breast-feeding of infants of chronic HBV carriers poses no additional risk for the transmission of HBV.

However, transmission of HBV following a human bite or a spit in the eye has previously been described and in these cases appropriate measurements such as hepatitis B immunoglobulin and hepatitis B vaccination should be taken. Viable HBV can persist on environmental surfaces and household objects and exposure to these surfaces and objects eg, razors, toothbrushes and other sharp objects can lead to transmission of HBV.

4 Chronic Hepatitis B in Pregnancy

The hepatitis B virus (HBV) can be transmitted from mother to child during delivery. The risk of transmission is highly dependent on the HBeAg status of the mother. In many countries pregnant women are screened for chronic HBV in order to identify children of carrier mothers and protect them from getting infected by timely vaccination against HBV. The efficacy of immunisation of these infants is abundantly proven (Lee et al. 2006). Despite immunisation a small proportion of infants get infected with HBV. The probability of such breakthrough infections is related to the level of HBV DNA in the mothers blood (Ip et al. 1989; del Canho et al. 1997). The level of viral DNA is a marker for viral replication and can be measured in serum with a PCR test. If a pregnant woman is positive for HBeAg as well as HBsAg a high level of HBV DNA is likely (Seo et al. 2005). A study in the UK showed that HBeAg negative pregnant women can also have detectable HBV DNA levels, although they were below 10^5 copies/mL (Holtby and McCarron 2004). In an evaluation of the Dutch immunisation programme of children of carrier mothers, del Canho et al. found a protective effect of vaccination in 92% of children (del Canho et al. 1997). The only factor of influence was the level of virus DNA in the mothers serum, vaccine efficacy was 100% in children of mothers with a viral load <150 pg/mL and 68% with a viral load ≥ 150 pg/mL. All children that tested positive for HBsAg at the age of 12 months were born from HBeAg positive mothers. A study to evaluate the British immunisation programme found HBV infection in 4.9% of the total group of 543 children. This was 7% (24/365) in children of HBeAg positive and anti-HBe negative mothers (Sloan et al. 2005).

The probability of breakthrough infection can be reduced by giving pregnant women with high viral loads antiviral treatment in the last trimester of the pregnancy. In a small Dutch study by van Zonneveld et al. out of eight highly viraemic pregnant women (viral load >150 pg/mL) treated one child was HBsAg positive (12.5%) compared to 7 of 24 children (28%) in a control group (van Zonneveld et al. 2003). Due to the small study group this difference was not statistically significant. In a case report another breakthrough infection has been reported where transmission to

the child occurred despite the reduction of viral DNA in the mothers serum to undetectable levels (Kazim et al. 2002). In a randomised controlled study in China where pregnant women with a viral load $>1,000$ MEq/mL were treated with lamivudine or a placebo in the lamivudine group 10 out of 56 (18%) children were HBsAg positive at age 12 months compared to 23 of 59 (39%) in the placebo group (Xu et al. 2004). The higher proportions of breakthrough infections in the Chinese study compared to the Dutch study might be related to the HBV genotype. In China genotype C is predominant and genotype C is known to be associated with more active viral replication (Guettouche and Hnatyszyn 2005).

Besides the positive protective effect on the child, treatment of pregnant women with high viral loads is favourable for the pregnant womens own health (van Zonneveld et al. 2004; Lau et al. 2005).

However pregnant women often don't get referred to specialist care due to a lack of knowledge on treatment options during pregnancy in primary care (midwives, GP's). Pregnant women are probably also more focussed on their pregnancy than on their hepatitis B virus infection.

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7

Pertussis Immunisation in Adolescents and Adults

Ulrich Heininger

1 Introduction

Pertussis, or “whooping cough,” is an acute bacterial infection of the respiratory tract which is caused by *Bordetella pertussis* and, less frequently, by *B. parapertussis* (Cherry and Heininger 2004) and that occurs worldwide. It is a widely held belief that pertussis is an exclusive childhood disease while in reality it affects all age groups. However, it is most frequently recognised in children. Wide-spread immunisation in children has controlled the disease successfully since its introduction in the early 1940s for several decades. Yet, the incidence of reported pertussis has increased continuously in many countries over the last approximate 30 years in all age groups, but especially among young infants, adolescents, and adults (Mortimer 1990; Cromer et al. 1993; Mink et al. 1994; Aoyama et al. 1995; Baron et al. 1998; Cherry 1999; Güris et al. 1999; De Serres et al. 2000; de Melker et al. 2000; Yih et al. 2000; Senzilet et al. 2001; Tanaka et al. 2003; Gzyl et al. 2004). Moreover, pertussis has been re-discovered as a frequent cause of prolonged cough in adolescents and adults in the recent past but the diagnosis still is often missed unless specific diagnostic tests are applied (Mink et al. 1992; Deville et al. 1995; Postels-Multani et al. 1995; Schmitt-Grohé et al. 1995; Nennig et al. 1996; Birkebaek et al. 1999; Gilberg et al. 2002; Lee et al. 2004). Pertussis can be effectively prevented by immunisation with whole-cell and, more recently, with acellular component vaccines. The introduction of combined reduced antigen diphtheria-tetanus-acellular pertussis component vaccines offers the option to expand pertussis immunisation efforts beyond childhood into adolescence and adulthood. This will be necessary to better control the increase of pertussis and its associated outbreaks.

2 Epidemiology

2.1 Incidence of Pertussis Disease Compared to *B. pertussis* Infections in Adolescents and Adults

Before immunisation, the annual incidence of reported pertussis in the United States was approximately 150 per 100,000 population and the case fatality rate was 4% compared with 230 per 100,000 population and 1.4% in England and Wales, respectively (Cherry 1984). On top of endemic disease, epidemic peaks occurred in cycles approximately every 2–5 years. With the introduction of universal pertussis immunisation in children, incidence and mortality of pertussis dropped significantly overall but cyclic peaks continued to occur (Fine and Clarkson 1982; Cherry 1984). However, over the last two decades, a modest but constant increase of pertussis disease incidence has been observed in the United States and many other countries (Mortimer 1990; Cromer et al. 1993; Mink et al. 1994; Aoyama et al. 1995; Baron et al. 1998; Cherry 1999; Güris et al. 1999; De Serres et al. 2000; de Melker et al. 2000; Yih et al. 2000; Senzilet et al. 2001; Tanaka et al. 2003; Gzyl et al. 2004). In the United States, this increase was most prominent in infants (with highest rates among all age groups), but was also significant in adolescents and adults, the age groups with the sharpest relative increase of almost tenfold (Güris et al. 1999).

At least five possible causes may be responsible (Cherry 2003):

1. Waning vaccine-induced immunity as a consequence of a decreased chance for natural boosting in the vaccination era and/or use of somewhat less efficacious acellular pertussis component vaccines (compared to most previously used whole cell vaccines) in the recent past.
2. Increased awareness of pertussis, stimulated by press reports and scientific literature on the “resurgence of pertussis,” promotional activities that accompanied acellular pertussis vaccine efficacy trials, and the consecutive licensure of new vaccines over the last two decades.
3. Increasing availability of improved diagnostic tools such as PCR techniques and improved serological assays and single-serum analyses.
4. Genetic changes of circulating strains of *B. pertussis* which may be less susceptible to vaccine-induced immunity.
5. Lessened potency of pertussis vaccines currently in use.

Of note, a rise of fatalities due to *B. pertussis* infection has been observed in infants in the United States during the last decade: while 1.67 deaths per million infants per year were reported in the 1980s, the rate increased to an average of 2.40 in the 1990s. The increase almost exclusively affected infants <4 months of age, which is in support of a true change in epidemiology (Vitek et al. 2003).

The epidemiology of *B. pertussis* infections should be discerned from that of pertussis as a disease (Cherry 2005). Based on longitudinal serologic studies (irrespective of clinical disease) with use of pertussis toxin (PT) as the only test antigen which is specific for *B. pertussis*, it is estimated that the incidence of

B. pertussis infections in adolescents and adults varies between 1,000 and 8,000 per 100,000 (Cromer et al. 1993; Deville et al. 1995; Wright et al. 1995; Hodder et al. 2000; Ward et al. 2005). Similarly, active surveillance studies – in which *B. pertussis* infections were diagnosed primarily by serology in defined populations of adolescents and adults with prolonged cough illnesses – resulted in estimated incidences between 69 and 1,500 per 100,000 in the United States (Mink et al. 1992; Nennig et al. 1996; Hodder et al. 2000; Strebel et al. 2001; Ward et al. 2005) and 133 per 100,000 in one study from Germany (Schmitt-Grohé et al. 1995). These estimates are up to a 1,000-fold higher compared to those of reported disease in national surveillance in the United States, which have recently been determined to be 3.4 and 0.5 per 100,000 in adolescents and adults, respectively (Güris et al. 1999). This discrepancy can be explained by a high degree of under-consultation, under-recognition, under-diagnosis, and under-reporting of cough illnesses caused by *B. pertussis* infections in these age groups in the absence of study conditions.

Furthermore, studies which applied confirmatory laboratory tests for *B. pertussis* infections revealed that pertussis accounts for 1–17% of prolonged cough diseases in adolescents and adults. However, since most of these studies relied on significant serum antibody responses to PT and approximately 10% of adults infected with *B. pertussis* do not mount an antibody response to PT, the true percentage is probably higher (Birkebaek et al. 1999).

Reported deaths due to pertussis in infants have also been increasing recently and are probably still underestimated (CDC 2002; Crowcroft et al. 2002; Vitek et al. 2003; Heininger et al. 2004). In contrast, deaths due to pertussis appear to be extremely rare in adolescents and relatively rare in adults (Güris et al. 1999; Mertens et al. 1999; Gil et al. 2001; CDC 2004).

Along with the increase in incidence, outbreaks in schools, colleges and universities have been reported in increasing frequency during the last decades (Greenberg 2005; Mattoo and Cherry 2005).

2.2 *Adolescents and Adults as the Source for Pertussis in Infants*

Pertussis in adolescents and adults is known to be an important source of *B. pertussis* infection and disease in unimmunised or incompletely immunised children (Hennes 1921). This phenomenon was re-discovered in the recent past (Linnemann and Nasenbeny 1977; Nelson 1978; Mortimer 1990; Cherry 1999; Edwards 2005; Schellekens et al. 2005). As immunisation coverage in childhood increased constantly over the decades, child to child transmission of *B. pertussis* decreased whereas the relative importance of adolescents and especially adults (with waning immunity after natural infection or immunisation in childhood) as the source of infection in infants increased (Baron et al. 1998). Among adults, mothers (Hoppe 1996; Izurieta et al. 1996; Crowcroft et al. 2003) and health care workers (Kurt et al. 1972; Shefer et al. 1995; Gehanno et al. 1999; Nouvellon et al. 1999)

have been found to be significant transmitters of *B. pertussis* infections to young infants. However, even when pertussis was still epidemic due to low vaccine uptake, e.g. in Germany in the early 1990s, adults with pertussis were a frequent source for infections in young children (Schmitt-Grohé et al. 1995).

Analysis of seasonality patterns of pertussis in the United States between 1980 and 1999 supports the major role of adults (more so than adolescents) as the source for *B. pertussis* infections in young infants. The proportion of reported pertussis cases by age and month of onset showed peaks in infants in July and August overlapping with those in adults (July to September). In contrast, peaks in adolescents (10–19 years of age) were observed in the period October to December (Tanaka et al. 2003).

3 Disease

Typical pertussis is a three phase illness (Cherry and Heininger 2004). The catarrhal phase lasts for about 1–2 weeks and is characterised by flu-like symptoms such as rhinorrhea, sneezing, and non-specific cough. The paroxysmal phase is characterised by an increase of frequency and severity of coughing spells with a typical whoop and frequently also posttussive vomiting of food or viscous mucus. Paroxysms may present several times per hour, sometimes intensified at night. Finally, after a few days to several weeks, symptoms gradually improve (convalescence). Fever is usually absent.

Severity of *B. pertussis* infection varies greatly and correlates with young age, low number of previous immunisations, no previous infection, and comorbidities (De Serres et al. 2000; Greenberg 2005). Adolescents and adults usually are partially immune due to previous immunisations and/or natural infection and this explains the frequent mild symptoms on re-infection (Deville et al. 1995; Schmitt-Grohé et al. 1995). In a study of our group performed in Germany, 64 laboratory-confirmed cases of pertussis in adults were identified. Of these, 70% had paroxysmal cough, 38% had whooping, 66% had posttussive phlegm, and 17% experienced posttussive vomiting (Schmitt-Grohé et al. 1995). In a further study from Germany, pneumonia, rib fracture, inguinal hernia, and severe weight loss were noted as complications of pertussis in adults (Postels-Multani et al. 1995). Moreover, encephalopathy and deaths due to pertussis have been reported in adults (Halperin and Marrie 1991; Güris et al. 1999; Mertens et al. 1999; Gil et al. 2001; CDC 2004). Recently, we provided evidence that *B. pertussis* infection may trigger exacerbations in adults with chronic obstructive pulmonary disease (Bonhoeffer et al. 2005).

In a large study of *reported* cases of pertussis in the United States, 76% of adolescents 10–19 years old presented with prolonged cough (≥ 4 weeks) and 82% with paroxysms (Farizo et al. 1992), and posttussive vomiting and whoop occurred in 53% and 47% of patients, respectively. It should be emphasised that less typical cases, the great majority of *B. pertussis* infections in adolescents and adults, are

likely to be missed in clinical practice. Yet, they still have a negative impact on daily life such as absenteeism at work or school and cause medical costs like those that occur in typical cases (Nennig et al. 1996; De Serres et al. 2000; Lee and Pichichero 2000).

Therefore, pertussis in adolescents and adults is a significant public health problem.

4 Diagnosis

In every prolonged cough (≥ 7 days) in an adolescent or adult *B. pertussis* infection should be considered a likely diagnosis. Most typical cases can be diagnosed on clinical grounds, whereas less characteristic cases require specific microbiological tests (Müller et al. 1997) and/or the presence of an epidemiological link. Although not always readily available, isolation of *B. pertussis* by culture from the nasopharynx of a coughing patient is the diagnostic gold standard. Nasopharyngeal secretions can be obtained by aspiration or use of calcium alginate or dacron swabs; specific media are necessary to recover *Bordetella* spp. in the laboratory (Cherry and Heininger 2004). Polymerase chain reaction (PCR) has significantly improved the diagnosis of pertussis and is increasingly available. Its sensitivity is markedly higher when compared to culture, especially in atypical cases and in patients who have been started on antibiotics (Heininger et al. 2000). Nowadays, real-time detection of the amplification products and Light Cycler PCR provide results within a few hours of specimen collection (Templeton et al. 2003).

Serologic tests are the most sensitive technique for the diagnosis of *B. pertussis* infections (Heininger et al. 2000). Enzyme immunoassays using purified *B. pertussis* antigens are most frequently being used. Although IgG antibody assays provide sufficient sensitivity, additional IgA assays are helpful (Hallander 1999). Unfortunately, serology tests are still not standardized and interpretation of results may be difficult in the presence of vaccine induced pre-existing antibodies (Wirsing von König et al. 2002).

Importantly, none of the diagnostic tests is 100% sensitive and therefore negative results do not rule out a diagnosis of pertussis in cough illnesses.

5 Treatment

As has been shown in children, antibiotic treatment will rapidly terminate contagiousness in an individual with *B. pertussis* infection (Hoppe and the Erythromycin Study Group 1992). Erythromycin has been used most frequently in the past and the recommended dose for adolescents and adults is 2 g/day given every 6–8 h for 14 days (Cherry and Heininger 2004). Based on in vitro studies and limited clinical data, newer macrolide antibiotics with improved gastrointestinal

tolerability such as clarithromycin and azithromycin are valid alternatives and gradually substitute for erythromycin (Hoppe and Eichhorn 1989; Martinez et al. 2001).

Importantly, antibiotic treatment should be started during the catarrhal stage of disease to be most effective (Cherry and Heininger 2004), which in the absence of an epidemiological link is rather unrealistic. Yet, treatment is recommended also in progressed disease mainly for the purpose of terminating contagiousness

6 Prevention by Immunisation

6.1 Successful Childhood Programmes

Efficacy of pertussis immunisation with acellular pertussis component vaccines (APV) in children has been clearly demonstrated (summarised by Cherry and Heininger 2004; Mattoo and Cherry 2005). These studies were performed in the late 1980s and early 1990s and several countries have introduced differently composed vaccines from various manufacturers since. All APV available contain pertussis toxoid (PT) and one or more other purified antigens, namely filamentous haemagglutinin (FHA), pertactin, and fimbriae. Other components such as diphtheria and tetanus toxoids, inactivated polio vaccines, *Haemophilus influenzae* type B conjugate, and hepatitis B are frequently included, too.

All countries recommend a primary series of pertussis immunisation comprising two or three doses in the first year of life. However, timing and number of further reinforcing booster doses are highly variable in different countries (Table 1). Conflicting opinions about the necessity of booster immunisations, uncertainties about the duration of protection after completion of the primary immunisation series, and concerns about additional costs mainly account for this heterogeneity.

Some limited data obtained with one specific APV by our group suggests that protection will last at least 6 years after the fourth dose in the second year of life (Lugauer et al. 2002).

6.2 Expanding Programmes Beyond Childhood

Currently, only Austria, Australia, Canada, France, Germany, and the United States recommend pertussis immunisation in adolescents as a fifth or sixth dose (Table 1). Introduction of further booster doses in adults so far has only been achieved in Austria and the United States for the general population and in Germany for so called-high risk groups such as parents and other care-givers of young children or health care workers. However, unfortunately, implementation of these recommendations has not yet been enforced.

Table 1 Recommended pertussis immunization schedules in Europe and selected other Countries^a

Country	Number of doses	Type of vaccine ^b	Schedule (recommended minimal age)	Comments
Australia	5	APV	2–4–6 months/ 4 years/15 years	
Austria	≥5	APV	2–3–4 months/ 12 months/ 13 years ^c	
Belgium	5	APV	2–3–4 months/ 15 months/5 years	
Canada	6	APV	2–4–6 months/ 18 months/ 4 years/14 years	Not yet implemented in all provinces
Denmark	4	APV	3–5–12 months/ 5 years	
Estonia	4	WPV	3–4–6 months/2 years	
Finland	5	WPV/APV	3–4–5 months/ 20 months/6 years	APV for doses 2–4 if adverse events experienced after WPV; dose 5: APV
France	5	WPV/APV	2–3–4 months/15 months/11 years	WPV for doses 1–3, APV for doses 4 and 5
Germany	6	APV	2–3–4 months/ 11 months/ 5 years/9 years	Single further dose in adults at high risk ^d
Greece	5	WPV/APV	2–4–6 months/ 18 months/4 years	WPV or APV for all doses
Hungary	5	WPV	3–4–5 months/ 3 years/6 years	
Iceland	4	APV	3–5–12 months/ 5 years	
Ireland	4	APV	2–4–6 months/4 years	
Italy	4	APV	3–5–11 months/ 5 years	
Latvia	4	WPV/APV	3–4–6 months/ 18 months	APV for dose 4
Lithuania	4	WPV/APV	2–4–6 months/ 18 months	APV for dose 4
Luxemburg	3	APV	4–6–12 months	
Malta	3	WPV	2–3–4 months	
The Netherlands	5	WPV/APV	2–3–4 months/ 11 months/4 years	APV for dose 5
Norway	4	APV	3–5–12 months/ 7 years	
Portugal	5	WPV/APV	2–4–6 months/ 18 months/5 years	APV if WPV contraindicated
Romania	5	WPV	2–4–6 months/ 12 months/3 years	

(continued)

Table 1 (continued)

Country	Number of doses	Type of vaccine ^b	Schedule (recommended minimal age)	Comments
Slovakia	5	WPV	3–5–9 months/2 years/5 years	
Slovenia	4	APV	3–4–5 months/18 months	
Spain	5	APV	2–4–6 months/15 months/4 years	
Sweden	3	APV	3–5–12 months	
Switzerland	5	APV	2–4–6 months/15 months/4 years	
United Kingdom/N. Ireland, Scotland	5	APV	2–3–4 months/3 years/5 years	
USA	6	APV	2–4–6 months/15 months/4 years/11 years	

^aUpdated and modified from Heininger 2001, and WHO (http://www.nt.who.int/immunization_monitoring/en/globalsummary/countryprofileselect.cfm)

^bAPV, acellular pertussis component combination vaccine; WPV, whole cell pertussis component combination vaccine

^cFollowed by regular booster doses in adults every 10 years (every 5 years from age 65 onwards)

^dHealth care workers, women before pregnancy, parents and other close contacts to newborns

The major role of adolescents and adults in transmission of *B. pertussis* to young, unprotected infants and the burden of disease for themselves should give pertussis immunisation programs in these age groups high public priority. However, remaining hurdles such as misconceptions about the epidemiology of *B. pertussis* infections, cost-effectiveness issues, implementation difficulties, and neglect of the scientific evidence of pertussis disease in adolescents and adults need to be overcome. The work of the Global Pertussis Initiative which recently proposed various strategies for the control of pertussis including adolescents and certain groups of adults, may be of help in this regard (Forsyth et al. 2004).

Over the last two decades, a number of APV have been developed and studied in specific formulations for use in adolescents and adults (Tables 2 and 3). Most of these vaccines contain reduced amounts of pertussis antigens and/or diphtheria or tetanus toxoids.

Two major lessons could be learnt from clinical studies with these new vaccines:

1. Pronounced antibody responses to antigens contained in the respective vaccines were induced after a single dose. Reduced amounts of antigen (compared with those in childhood formulations of the same vaccines) were usually sufficiently

Table 2 Clinical studies with acellular pertussis component vaccines in adolescents

Authors (year of publication)	Vaccine ^a (manufacturer)		Study subjects	Immunogenicity of pertussis antigens	Reactogenicity
	Antigens	Doses			
Begue et al. (1998)	DTPa-IPV (Glaxo-SmithKline, Belgium)		10–13 years of age	Significant ab value rises (≥2-fold in initially seropositives or above cut-offs in initially seronegatives) in 66% (PT), and 100% (FHA and pertactin)	Local (up to 85% for pain) and systemic (up to 40% for headache in DT and 27% in DTPa recipients) reactions were generally mild and occurred at similar rates in both groups
	PT (25 µg), FHA (25 µg), Pertactin (8 µg); ≥40 IU tetanus toxoid, ≥30 IU diphtheria toxoid plus polio viruses 1–3 (40, 8 and 32 D antigen units)		<i>n</i> = 59 (safety) <i>n</i> = 53 (immunogenicity)	All subjects had pre-immunisation anti-PT ab above cut-off	Follow-up ended on day 3 post-immunisation
	One dose		Controls: DT-IPV (<i>n</i> = 53, safety; <i>n</i> = 49, immunogenicity)		
Minh et al. (1999)	DTPa or pa (GlaxoSmithKline, Belgium)		10–13 years of age	Significant ab value rises (≥2-fold in initially seropositives or above cut-offs in initially seronegatives) in 92–96% (PT), 97–98% (FHA) and 99–100% (pertactin) with no significant differences between groups	Local (up to 83% for pain in Td and 79% in dTPa recipients) and systemic (up to 50% for fatigue in Td and 56% in dTPa recipients) reactions were generally mild and occurred at similar rates in all groups
	PT (8 µg), FHA (8 µg), Pertactin (2.5 µg); plus 5 Lf tetanus toxoid and 2.5 Lf diphtheria toxoid for dTPa		dTPa <i>n</i> = 448 (safety) <i>n</i> = 447 (immunogenicity)	Humoral immune response correlated strongly with cell-mediated immune response	Great majority of local reactions occurred within 48 h of immunisation whereas most fever reactions occurred between days 3 and 15 post-immunisation (median day 5)

Halperin et al. (2000)	<ul style="list-style-type: none">One dose	Td + pa (1 month later) <i>n</i> = 60/59 (safety) <i>n</i> = 57 (immunogenicity)	12–19 years of age	Significant ab value rises (≥4-fold) in 86–90% (PT), 78–89% (FHA), 93–97% (pertactin), and 89–95% (Fimbriae 2 and 3) with no significant differences between groups	Pain at injection site in up to 88% (Tdap-IPV) and 93% (Td-IPV) and other local reactions in up to 22% of vaccinees with no significant increase for addition of aP component; systemic reactions (up to 41% for headache) occurred at similar rates in all groups
	<ul style="list-style-type: none">PT (2.5 µg), FHA (5 µg), Pertactin (3 µg), Fimbriae 2 and 3 (5 µg); plus 5 Lf tetanus toxoid, 2 Lf diphtheria toxoid, polio viruses 1–3 (40, 8 and 32 D antigen units) for Tdap-IPV	Tdap-IPV or aP (Connaught, Canada)	Tdap-IPV, <i>n</i> = 350	Anti-PT, anti-FHA, and anti-Fim GMT higher after aP compared to Tdap-IPV	
	<ul style="list-style-type: none">One dose	Tdap-IPV, followed by aP 1 month later, <i>n</i> = 116	11–17 years of age	Significant ab value rises (≥2- to 4-fold) in 92% (PT), 86% (FHA), 95% (pertactin), and 95% (Fimbriae 2 and 3) of vaccinees	
Pichichero et al. (2005)	<ul style="list-style-type: none">PT (2.5 µg), FHA (5 µg), Pertactin (3 µg), Fimbriae 2 and 3 (5 µg); 5 Lf tetanus toxoid, 2 Lf diphtheria toxoid	Tdap (Sanofi Pasteur, Canada)	11–17 years of age	Significant ab value rises (≥2- to 4-fold) in 92% (PT), 86% (FHA), 95% (pertactin), and 95% (Fimbriae 2 and 3) of vaccinees	

Table 2 (continued)

Authors (year of publication)	Vaccine ^a (manufacturer)		Study subjects	Immunogenicity of pertussis antigens	Reactogenicity
	• Antigens	• Doses			
Vergara et al. (2005)	• One dose		Controls: Td (<i>n</i> = 792, safety; <i>n</i> = 516, immunogenicity)		
	DTPa±IPV or DTPa-IPV	(GlaxoSmithKline, Belgium)	10–14 years of age	Significant ab value rises (≥2-fold in initially seropositives or above cut-offs in initially seronegatives) against all pertussis antigens in ≥97% irrespective of vaccine	Local (up to 95% for pain) and systemic (up to 62% for headache) reactions were generally mild and occurred at similar rates in all groups with a tendency for less frequent occurrence after dTpa component vaccines
	• dTpa PT (8 µg), FHA (8 µg), Pertactin (2.5 µg); ≥20 IU tetanus toxoid, ≥2 IU diphtheria toxoid		dTpa <i>n</i> = 219 (safety) <i>n</i> = 217 (immunogenicity)	Higher GMT after DTPa-IPV compared to dTpa component vaccines	Great majority of reactions occurred within 48 h of immunisation
	• dTpa-IPV as above plus polio viruses 1–3 (40, 8 and 32 D antigen units)		dTpa-IPV <i>n</i> = 436 (safety) <i>n</i> = 429 (immunogenicity)		
	• DTPa-IPV PT (25 µg), FHA (25 µg), Pertactin (8 µg); ≥40 IU tetanus toxoid, ≥30 IU diphtheria toxoid plus polio antigens as above		DTPa-IPV <i>n</i> = 110 (safety) <i>n</i> = 217 (immunogenicity)		
	• One dose				

<p>Theeten et al. (2005)</p>	<p>DTPa (GlaxoSmithKline, Belgium)</p>	<p>10–18 years of age</p>	<p>Significant ab value rises (≥ 2- to 4-fold) in approximately 83–90% (PT), 96% (FHA), and 98% (pertactin)</p>	<p>Local (up to 91% for pain) and systemic (up to 48% for fatigue) reactions were generally mild and occurred at similar rates in all groups</p>
	<p>PT (8 μg), FHA (8 μg), Pertactin (2.5 μg); 5 Lf tetanus toxoid, 2.5 Lf diphtheria toxoid; 3 different amounts of aluminum content (0.13–0.5 mg)</p>	<p>10–18 years of age</p>	<p>No significant differences depending on aluminum content for % with ab rises but higher anti-PT GMT for 0.5 mg aluminum group</p>	<p>Tendency for more severe reactions (up to 8% with grade 3 fatigue) with 0.5 mg aluminum</p>
	<p>One dose</p>			<p>Great majority of reactions occurred within 48 h of immunisation</p>
<p>Southern et al. (2005)</p>	<p>Tdap \pm IPV (Sanofi Pasteur, Canada)</p>	<p>13–17 years of age</p>	<p>ab value rises against PT (8.2-fold vs. 3.5 and 4.5) and FHA (13.7-fold vs. 7.1 and 5.5) were higher for dTpa compared to Tdap \pm IPV; anti-pertactin ab value rises were similar between groups (19.4–38.3; no percentages given)</p>	<p>Local (up to 78% for pain) and systemic (up to 32% for headache) reactions were generally mild and occurred at similar rates in both groups</p>
	<p>PT (2.5 μg), FHA (5 μg), Pertactin (3 μg), Fimbriae 2 and 3 (5 μg); 5 Lf tetanus toxoid, 2 Lf diphtheria toxoid; polio viruses 1–3 (40, 8 and 32 D antigen units)</p>	<p>Tdap, $n = 74/74$ (immunogenicity/reactogenicity)</p>		<p>Great majority of reactions occurred within 72 h of immunisation</p>

(continued)

Table 2 (continued)

Authors (year of publication)	Vaccine ^a (manufacturer)	Study subjects	Immunogenicity of pertussis antigens	Reactogenicity
	<ul style="list-style-type: none">• Antigen• Doses• dTpa (GlaxoSmithKline, Belgium)• PT (8 µg), FHA (8 µg), Pertactin (2.5 µg); 5 Lf tetanus toxoid, 2.5 Lf diphtheria toxoid• One dose	<p>Tdap-IPV, n = 75/76</p> <p>dTpa, n = 68/68</p> <p>Controls: Td, n = 71/72</p> <p>11–18 years of age</p> <p>Tdap, n = 123/127 (immunogenicity/reactogenicity)</p> <p>No controls</p>		
Knuf et al. (2006)	<p>DTpa or pa (GlaxoSmithKline, Belgium)</p> <p>PT (8 µg), FHA (8 µg), Pertactin (2.5 µg); plus 5 Lf tetanus toxoid and 2.5 Lf diphtheria toxoid for dTpa</p> <p>One dose</p>		<p>Significant ab value rises (≥2-fold and ≥5 EU/mL) in 90% (PT), 100% (FHA), and 98% (pertactin)</p>	<p>Local and/or systemic reactions occurred in 9% of vaccinees; they were not serious and only 4% thought to be vaccine related</p>

^aUpdated from: Heininger U and Cherry JD. Pertussis immunisation in adolescents and adults – *Bordetella pertussis* epidemiology should guide vaccination recommendations. *Expert Opin Biol Ther* 2006 Jul;6(7):685–697 (reproduced with permission)

Table 3 Clinical studies with acellular pertussis component vaccines in adults

Pertussis vaccine ^a (manufacturer)		Study subjects		Immunogenicity of pertussis antigens	Reactogenicity
Authors (year of publication)	Antigens Doses				
Granström et al. (1987)	aP (Japanese National Institute of Health):	<i>n</i> = 47, with lack of or low anti-pertussis Ab		Significant ab value rises in 76% (FHA-IgG) and 68% (PT-IgG) of vaccinees	Any reaction in 85% of vaccinees versus 40% in placebo group, mostly mild
	• PT (7.5 µg), FHA (7.5 µg) • One dose	Controls: placebo (<i>n</i> = 20),			Delayed local reactions (days 6–8) in 47% of vaccinees
Rutter et al. (1988)	aP (Center of Applied Microbiology and Research, UK):	<i>n</i> = 35, with low anti-pertussis ab: PVA (<i>n</i> = 15), PVB (<i>n</i> = 20)		ab value rises (no percentages given) against FHA and PT independent of adjuvants, against fimbriae: PVA > PVB	Local reactions in 29% of vaccinees, mild or moderate in severity
	• PT (10 µg), FHA (10 µg), Fimbrien (10 µg) with aluminumhydroxide (PVA) or aluminum-phosphate (PVB) • One dose	Controls: Placebo (<i>n</i> = 19), with high anti-pertussis ab		No correlation between local reactions and ab values	Delayed local reactions (after day 6) in 8% of vaccinees
Ruuskanen et al. (1991)	aP (Smith Kline Beecham, Belgium):	<i>n</i> = 45, with primary immunisation series in childhood		Significant ab value rises (FHA-IgG and PT-IgG) in 100% of vaccinees	No fever, no systemic reactions Local reactions in 71% of vaccinees, mild or moderate in severity
	• PT (25 µg), FHA (25 µg) • One dose				Delayed local reactions (peak on day 8) in 16% of vaccinees

(continued)

Table 3 (continued)

Pertussis vaccine ^a (manufacturer)				
Authors (year of publication)	Antigens Doses	Study subjects	Immunogenicity of pertussis antigens	Reactogenicity
Edwards et al. (1993)	TdapP (Lederle/Takeda, USA):	<i>n</i> = 118, with primary immunisation series in childhood: TdapP 1/1 <i>n</i> = 30, TdapP 1/2 <i>n</i> = 30, TdapP 1/4 <i>n</i> = 28	Significant ab value rises in 100% against all vaccine antigens (independent of dosage)	Local reactions in 53% (TdapP 1/1), 53% (TdapP 1/2), 43% (TdapP 1/4) and 77% (Td) of vaccinees
	• PT (3 µg), FHA (36 µg), Pertactin 1.6 µg, Fimbriae 0.7 µg; 5 Lf tetanus toxoid; 2 Lf diphtheria toxoid	Controls: Td (<i>n</i> = 30)		No delayed local reactions
	• One dose (1/1, 1/2 or 1/4 concentration)			
Stehr et al. (1995)	DtaP (Lederle/Takeda, USA):	<i>n</i> = 630	Not done	Local reactions in 34% of vaccinees (19% mild, 15% moderate) No delayed local reactions
	• PT (3 µg), FHA (36 µg), Pertactin 1.6 µg, Fimbriae 0.7 µg; 5 Lf tetanus toxoid, 9 Lf diphtheria toxoid			
	• 1/2 dose			
Stehr et al. (1995)	aP (Lederle/Takeda, USA)	<i>n</i> = 185, without history of pertussis, with (<i>n</i> = 95) or without (<i>n</i> = 90) pertussis immunisations in childhood	Significant ab value rises independent of number of previous pertussis vaccine doses (no percentages given)	Pain at injection site in 62% of vaccinees and other local reactions in 10–20%

Lin and Chiang (1997)	<ul style="list-style-type: none">• PT (3 µg), FHA (36 µg), Pertactin (1.6 µg), Fimbriae 0.7 µg)		No correlation between rate and severity of local reactions and ab value rises	Delayed local reactions (days 3–7) in 17% of vaccinees
	<ul style="list-style-type: none">• One dose Tdap (Chemo-Sero-Therapeutic Research Institute, Japan)	<i>n</i> = 118, with primary immunisation series in childhood; Tdap 1/1 <i>n</i> = 26, Tdap ½ <i>n</i> = 27 Controls: Td (<i>n</i> = 27)	Positive correlation between post-immunisation ab values against PT and FHA and antigen content	Local reactions in 54% (Tdap 1/1), 48% (Tdap 1/2) and 59% (Td) of vaccinees
	<ul style="list-style-type: none">• PT (1 µg), FHA (4 µg) – 1/1 and ½dose; 5 Lf tetanus toxoid, 2 Lf diphtheria toxoid			Local and systemic reactions independent of pertussis antigen content
	<ul style="list-style-type: none">• One dose aP (Chiron Vaccines, Italy)	<i>n</i> = 300	Significant ab value rises (≥4-fold) in 91–100% of vaccinees	Pain at injection site in 45% (placebo: 19%) of vaccinees; erythema in 10% (placebo: 7%) Delayed local reactions (days 3–13) in 20% of vaccinees
Keitel et al. (1999)	<ul style="list-style-type: none">• PT (5 µg), FHA (5 µg), Pertactin (5 µg).	Controls: Placebo (<i>n</i> = 100)		
	<ul style="list-style-type: none">• One dose aP, various manufacturers, 5 different aP vaccines	<i>n</i> = 450	Significant ab value rises in correlation with vaccine antigen content	Pain at injection site in up to 90% of vaccinees and other local reactions in up to 21% Delayed local reactions (days 4–7) in up to 28% of vaccinees
Van der Wielen et al. (2000)	<ul style="list-style-type: none">• 1–4 pertussis antigens	Controls: Placebo (<i>n</i> = 31)		
	<ul style="list-style-type: none">• One dose Dtpa (GlaxoSmithKline, Belgium)	<i>n</i> = 295	Significant ab value rises (≥4-fold) in 94–99% of vaccinees	Pain at injection site in up to 89% of vaccinees and other local reactions in up to 35%, independent of vaccine used

(continued)

Table 3 (continued)

Pertussis vaccine ^a (manufacturer)		Study subjects	Immunogenicity of pertussis antigens	Reactogenicity
Authors (year of publication)	Antigens Doses			
Halperin et al. (2000)	PT (8 µg), FHA (8 µg), Pertactin (2.5 µg); 5 Lf tetanus toxoid, 2.5 Lf diphtheria toxoid	Tdap ± IPV, n = 490, with a second dose of aP in n = 120	Significant ab value rises (≥4-fold) in 80% (PT), 82–88% (FHA), 89–96% (pertactin), and 76–88% (Fimbriae 2 and 3) with no significant differences between groups	Delayed local reactions (day 3 and later) in 2% of vaccinees
	One dose			
Turnbull et al. (2001)	Tdap ± IPV or aP (Connaught, Canada)	aP, n = 126 controls: Td (n = 126) n = 548	Only anti-PT GMT higher after two doses (206 EU/mL) compared to one dose (120 EU/mL)	Pain at injection site in up to 88% and other local reactions in up to 27% of vaccinees with no significant increase for addition of aP component; systemic reactions (up to 41% for headache) occurred at similar rates in all groups Rates of local and systemic reactions did not increase with a second dose of Ap
	PT (2.5 µg), FHA (5 µg), Pertactin (3 µg), Fimbriae 2 and 3 (5 µg); plus 5 Lf tetanus toxoid, 2 Lf diphtheria toxoid, polio viruses 1–3 (40, 8 and 32 D antigen units) for Tdap ± IPV			
	One or two dose Dipa (GlaxoSmithKline, Belgium)		Significant ab value rises (≥4-fold) in 97–100% of both aP and Tdap vaccines	Lower rates of local reactions with pertussis component vaccines when compared to Td
	PT (8 µg), FHA (8 µg), Pertactin (2.5 µg); 5 Lf tetanus toxoid, 2.5 Lf diphtheria toxoid			
	One dose			Delayed local reactions (day 3 and later) in up to 4% of vaccinees

Schmitt et al. (2001)	aP (Connaught, USA)	n = 207	Significant ab value rises (≥4-fold) in 98% (PT) and 100% (FHA) of vaccines	Erythema (>1 cm) in 2%, induration (>1 cm) in 8% (>3 cm: 6%) of vaccines
	<ul style="list-style-type: none"> PT (23.4 µg), FHA (23.4 µg) One dose 			No delayed local reactions beyond day 5 post immunisation
Christie et al. (2001)	aP (Massachusetts Public Health Biologic Laboratories, USA)	n = 102	Significant ab value rises (≥2-fold) in 85% (PT) and 92% (FHA) of vaccines	Local (up to 82%) and systemic (up to 24%) reactions were generally mild and occurred at similar rates in both groups
	<ul style="list-style-type: none"> PT (25 µg), FHA (3 µg) One dose 	Controls: Meningo-coccal vaccine (n = 97)		
Bartels et al. (2001)	DtaP (Wyeth Lederle, USA):	n = 100	Significant ab value rises against PT (7.6-fold), FHA (22.5-fold), and fimbriae (5.6-fold); no percentages given	Pain at injection site in 89% of vaccinees and other local reactions in 34–37%
	<ul style="list-style-type: none"> PT (3 µg), FHA (36 µg), Pertactin (1.6 µg), Fimbriae (0.7 µg); 5 Lf tetanus toxoid, 9 Lf diphtheria toxoid One dose 	Controls: Td (n = 42), d (n = 38)	No correlation between rate and severity of local reactions and ab value rises	No delayed local reactions
Pichichero et al. (2005)	Tdap (Sanofi Pasteur, Canada)	n = 1,752 (safety) n = 743 (immunogenicity)	Significant ab value rises (≥2- to 4-fold) in 84% (PT), 83% (FHA), 94% (pertactin), and 86% (Fimbriae 2 and 3) of vaccines	Local (up to 64%) and systemic (up to 23%) reactions were generally mild and occurred at similar rates in both groups

(continued)

Table 3 (continued)

Pertussis vaccine ^a (manufacturer)		Study subjects	Immunogenicity of pertussis antigens	Reactogenicity
Authors (year of publication)	Antigens Doses			
Ward et al. (2005)	<ul style="list-style-type: none">PT (2.5 µg), FHA (5 µg), Pertactin (3 µg), Fimbriae 2 and 3 (5 µg); 5 Lf tetanus toxoid, 2 Lf diphtheria toxoid	Controls: Td (n = 573, safety; n = 510, immunogenicity)		
	<ul style="list-style-type: none">One dose			
	aP (GlaxoSmithKline, Belgium)	n = 1,391 (Pa)	Efficacy trial; vaccine efficacy: 92% (95% confidence interval: 32–99)	No serious adverse events were considered to be vaccine-related
	PT (8 µg), FHA (8 µg), Pertactin (2.5 µg)	Controls (n = 1,390): Hepatitis A vaccine		

^aT = tetanus, D = diphtheria toxoid, d = reduced diphtheria toxoid, Lf = limits of flocculation, aP/Pa = acellular pertussis component, ap = reduced acellular pertussis component; PT = Pertussis toxin; FHA = Filamentous haemagglutinin; IPV = inactivated polio viruses; Ab: Antibody values against specific *B. pertussis* antigens

immunogenic. Antibody responses usually exceeded those after three doses with APV with similar antigens in infants, for whom efficacy has been demonstrated in efficacy trials. By analogy it can be assumed that APV will also be efficacious in adolescents and adults.

2. When compared to placebo or other vaccines without pertussis antigens, reactogenicity of APV generally was not increased. Earlier concerns regarding delayed local reactions (between days 4 and 14 after immunisation) have not been confirmed in more recent trials. Further, there was no evidence for occurrence of partial or whole limb swellings which have been observed in increasing numbers after four or five consecutive doses of APV and numerous other vaccines in children (Woo et al. 2003).

So far, only one study investigates the efficacy of pertussis immunisation in adults (Ward et al. 2005). In this study, 1,391 and 1,390 subjects, 15–65 years old, received a single dose of APV (produced by Glaxo SmithKline, Belgium) containing 8 µg PT, 8 µg FHA, and 2.5 µg pertactin in a 0.5 mL dose) or hepatitis A vaccine as control, respectively, followed by active surveillance for cough illnesses ≥ 5 days duration over a median of 22 months. Overall, 2,672 cough illnesses were reported with similar frequencies in both groups. However, laboratory confirmed pertussis was more frequent in controls ($n=9$) compared to pertussis vaccinees ($n=1$). This resulted in an estimated vaccine efficacy of 92% (adjusted for duration of cough; 95% confidence interval, 32–99%) which is slightly higher compared to point estimates of 71% (Greco et al. 1996) and 81% (Schmitt et al. 1996) efficacy by use of comparable case definitions after three doses of the same 3-component APV (with threefold higher antigen content) in infants.

Today, APV for use in adolescents and adults from two manufacturers are generally available (Table 4). Both vaccines contain tetanus toxoid, reduced diphtheria toxoid, and reduced acellular pertussis, adsorbed vaccines with or without added inactivated polioviruses and have been tested in several clinical studies (Minh et al. 1999; Halperin et al. 2000; Van der Wielen et al. 2000; Turnbull et al. 2001; Vergara et al. 2005; Theeten et al. 2005; Pichichero et al. 2005; Southern et al. 2005).

Based on data submitted to the US Food and Drug Administration (<http://www.fda.gov/ohrms/dockets/ac/05/briefing/2005-4097b1.htm>), immunogenicity and safety of the two vaccines can be summarised as follows:

6.2.1 Boostrix

Data on approximately 2,700 healthy adolescents, 10–18 years of age, with Boostrix given as a fifth dose showed seroprotective anti-diphtheria and anti-tetanus toxin antibodies (each ≥ 0.1 IU/mL) in 99.9 and 100% of vaccinees, respectively. Further, 98.9% (anti-PT), 99.7% (anti-pertactin) and 100% (anti-FHA) of vaccinees achieved seropositive antibody values (≥ 5 EU/mL) after immunisation. Seropositivity rates against PT, FHA, and pertactin varied between 84.5 and 95.4%. Also, tolerability was good. Any pain at the injection site (75% vs. 71%

Table 4 Characteristics of acellular pertussis component vaccines licensed for use in adolescents and/or adults

Characteristic	Vaccine	
Tradename		
Europe	Covaxis/Repevax	Boostrix/Boostrix-IPV
US/Canada	Adacel	Boostrix
Volume	0.5 mL	0.5 mL
Pertussis antigens		
PT	2.5 µg	8 µg
FHA	5 µg	2.5 µg
Pertactin	3 µg	2.5 µg
Fimbriae	5 µg	–
Diphtheria toxoid	≥2 I.U.	≥2 I.U.
Tetanus toxoid	≥20 I.U.	≥20 I.U.
IPV	40, 8 and 32 D antigen units	40, 8 and 32 D antigen units
Adjuvant	0.33 mg aluminumphosphate	0.5 mg aluminumhydroxide
Indications (Age)		
Europe ^a	≥4 years (Repevax) ≥10 years (Covaxis)	≥4 years
United States	11–64 years	11–18 years

PT = Pertussis toxin; FHA = Filamentous haemagglutinin; IPV = inactivated polio viruses 1, 2, and 3 (included in Repevax and Boostrix-IPV)

^aNo upper age restriction

in controls who received Td) was the most frequently observed adverse event but this interfered with normal activity in only 4.5% (compared to 3.7% after Td) of vaccinees. Fever (defined as $\geq 38^{\circ}\text{C}$) within 72 h of vaccination was rare (1.8% vs. 1.5%). Importantly, no serious adverse events occurred within 30 days after immunisation.

6.2.2 Covaxis (Adacel)

Safety and immunogenicity has been assessed in 5,841 and 3,316 individuals, respectively, 11–64 years of age. Amongst adolescents (age 11–17 years), Covaxis as a fifth dose induced anti-diphtheria and anti-tetanus toxin antibodies ≥ 0.1 IU/mL in 99.8 and 100% of vaccinees, respectively. In adults (age 18–64 years), respective figures were 94.1% and 100%. Seropositivity rates against PT, FHA, and pertactin were induced in 82.7–94.9% of vaccinees. Any pain at the injection site (adolescents: 78% vs. 71% in controls – who received diphtheria and tetanus vaccine only; adults: 66% vs. 63% in controls) was the most frequently reported adverse event. Fever ($\geq 38^{\circ}\text{C}$) within 72 h of immunisation was rare (2.9% vs. 1.5% in adolescents and 0.8% vs. 0.4% in adults). Finally, a total of 89 serious adverse events throughout the entire 6 month study period were reported. Most of them were hospitalisations, they occurred mainly in adults, and they were rated to be independent of immunisation. No deaths were reported.

Recommending bodies and health care providers internationally are now faced with the challenge to make best use of these products and to find innovative ways

to foster their implementation in adolescents and adults who generally have suboptimal immunisation coverage with recommended standard vaccines.

7 Summary

The burden of disease in affected adolescents and adults, their important contribution to the ongoing circulation of *B. pertussis* in the population, and the associated direct and indirect costs make prevention of pertussis by immunisation beyond childhood an attractive goal. The recent introduction of booster doses in adolescents in Austria, Australia, Canada, France, Germany, and the United States are an important step in the right direction. However, this most likely will not have a significant impact on reduction of disease in young infants and adults. It will be a challenge for the next few years to convince authorities, health care providers, and the general population about the necessity of regular pertussis vaccine booster doses in adults if better control of this potentially devastating disease is wanted. Although, as has recently been said (Cherry 2005), this “may be looked on by many as a pie-in-the-sky vision,” the availability of tetanus toxoid, reduced diphtheria toxoid, and reduced acellular pertussis, adsorbed (Tdap) vaccines with proven tolerability and immunogenicity make this goal achievable. Introduction and enforcement of regular pertussis boosters, e.g. every 10 years, will not only reduce the overall morbidity of pertussis in the population but most likely – by use of combination vaccines – will also improve the less than optimal coverage of diphtheria and tetanus immunisations in adults.

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Trachoma: Recent Developments

David Mabey

1 Introduction

Trachoma is a chronic kerato-conjunctivitis caused by the intracellular bacterium *Chlamydia trachomatis*. In the nineteenth century it was an important cause of blindness in Europe and North America, but it disappeared from more affluent parts of the world as living standards improved in the twentieth century. It is now a disease of poor rural communities, mainly in Africa and Asia, but it remains the leading infectious cause of blindness worldwide (Mabey et al. 2003).

The clinical signs of trachoma are best seen in the conjunctiva of the everted upper eyelid. Sub-conjunctival follicles are the characteristic sign of active disease (Fig. 1), which is usually seen in children in endemic communities. *C. trachomatis* can often be found in active cases, though follicles can persist for some months after infection has been cleared. In some cases, severe inflammation is seen in the subtarsal conjunctiva (Fig. 2). Such cases are particularly likely to progress, over many years and following repeated reinfection, to develop conjunctival scarring (Fig. 3). As the scars contract, the lid margin turns inwards, and the lashes rub against the cornea, a condition known as trichiasis (Fig. 4). This damages the cornea, eventually rendering it opaque (Fig. 5).

This review will discuss recent progress towards the elimination of blinding trachoma as a public health problem.

2 Epidemiology

In 1995, the World Health Organisation (WHO) estimated that trachoma was responsible for 15% of global blindness, with 5.9 million blind from the disease (Thylefors et al. 1995). In 1998 the World Health Assembly passed a resolution calling for the global elimination of blinding trachoma by the year 2020. A recent review by WHO estimated that, in the year 2002, trachoma was responsible for only 3.6% of global blindness, or 1.3 million blind (Resnikoff et al. 2004). Does this reflect significant progress towards the elimination of blinding trachoma, or merely uncertainty in the estimation of trachoma blindness? Probably both.

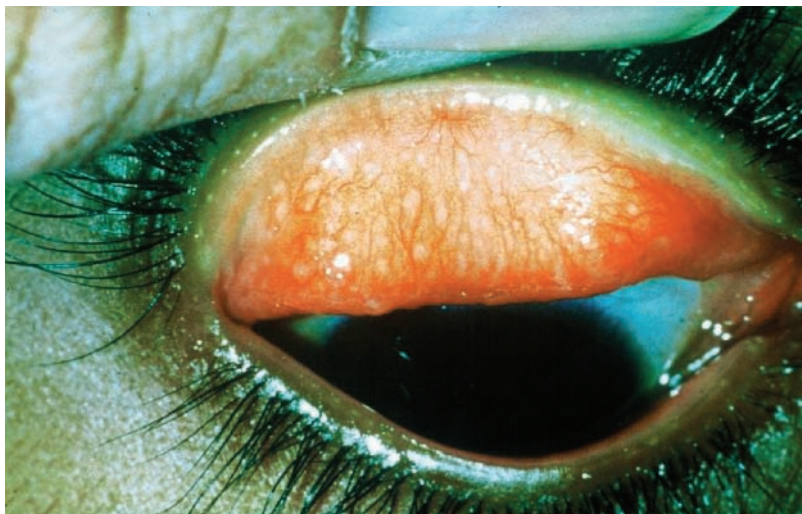


Fig. 1 Follicular trachoma



Fig. 2 Intense trachoma

Ranson and Evans drew attention to the uncertainty in estimating trachomatous blindness due to a lack of reliable data from many regions. Depending on which method they used to extrapolate global figures from available data, they estimated that either 0.64 or 2.9 million people were blind from trachoma, and either 1.5 or 6.7 million were visually impaired (Ranson and Evans 2003). Polack et al. (2005) recently mapped the distribution of active trachoma and of trachomatous trichiasis worldwide at the District level, using both published and unpublished data from



Fig. 3 Trachomatous scarring



Fig. 4 Trichiasis

139 population based surveys conducted since 1980. Surveys had been conducted in 33 of the 55 countries in which trachoma is believed to be endemic. These maps provide the most detailed available data on the current global distribution of trachoma, but also highlight major deficiencies in the data. In particular, in China and India, which potentially contribute the largest number of cases of trachoma, surveys had only been conducted in one or two districts. Encouragingly, national

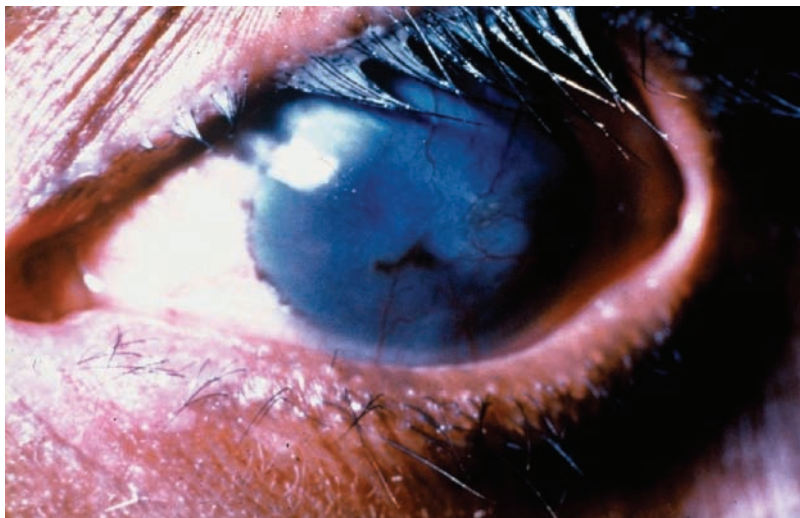


Fig. 5 Corneal opacity

trachoma prevalence surveys have now been conducted in several African countries (Dolin et al. 1998) (Schemann et al. 1998, 2003; Saal et al. 2003). However, the focal distribution of the disease makes it difficult to estimate prevalence at the national or regional level from surveys of a small number of communities.

Trachoma is certainly disappearing from some populations in which it has long been endemic. In Morocco, an impressive reduction in the prevalence of both active disease and trichiasis has followed a sustained and effective national control programme (Chami et al. 2004; Kuper et al. 2005). In other countries, eg Nepal and The Gambia, the prevalence of trachoma has declined for reasons which are less clear; possible explanations include improvements in education and living standards, or the increasing use, for other conditions, of antibiotics with anti-chlamydial activity (Dolin et al. 1998; Chidambaram et al. 2004; Jha et al. 2002). There is no doubt, however, that trachoma remains a serious public health problem in many countries, especially in marginalised communities and those afflicted by wars and famine. Recent surveys in Sudan and Ethiopia have found extremely high prevalences of both active trachoma and trichiasis, with 9% of adults aged over 15 years reported to have trichiasis in Southern Sudan (Cumberland et al. 2005; Ngondi et al. 2005).

3 Trachoma Assessment

The WHO currently recommends that the need for trachoma control activities in a community should depend on the prevalence of clinical signs of active trachoma in children aged 1–9 years, and the prevalence of trichiasis in adults. If the prevalence

of active trachoma in children exceeds 10%, that community should receive mass treatment with either a single oral dose of azithromycin, or 6 weeks of once daily 1% tetracycline ointment, annually for 3 years. Health education and other interventions to improve the environment, and hence reduce the transmission of ocular *C. trachomatis* infection should also be implemented (WHO 2002). For planning purposes, the key data required are (i) the number of communities requiring mass treatment, and (ii) the number of individuals per district who require trichiasis surgery.

4 Clinical Signs and *C. trachomatis* Infection

Since the advent of sensitive nucleic acid amplification tests (NAATs) for *C. trachomatis*, it has become increasingly clear that there is a mismatch between the presence of ocular *C. trachomatis* infection and the presence of clinical signs of active trachoma.

A number of recent studies have confirmed that, especially in communities where the prevalence of trachoma is falling, many subjects with clinical signs of active trachoma are not infected with *C. trachomatis*. These studies suggest that the characteristic conjunctival follicles, the presence of which in the sub tarsal conjunctiva defines active trachoma according to the WHO classification, may persist for months or even years after the infection has been eliminated (Thein et al. 2002; Bird et al. 2003; Burton et al. 2003; Miller et al. 2004; Mabey et al. 2003; Solomon et al. 2004a, b). This could lead to some communities receiving repeated rounds of mass antibiotic treatment long after transmission of ocular *C. trachomatis* has ceased. A simple, point-of-care test for *C. trachomatis* could save considerable resources by identifying communities in which infection has been eliminated, allowing control efforts to be focussed where they are most needed. Such a test, produced by Cambridge Diagnostics, has been evaluated in trachoma-endemic communities in Tanzania. It was found to be 84% sensitive and 99% specific compared to a PCR gold standard (Roche Amplicor; Michel et al. 2006).

The use of real-time PCR to quantitate ocular *C. trachomatis* infection, and of RNA amplification to detect metabolically active *C. trachomatis*, has cast some light on the relationship between infection and disease. By and large, residents of trachoma – endemic communities who have positive NAATs for *C. trachomatis* in the absence of active trachoma have low levels of infection (Burton et al. 2003; Solomon et al. 2003; West ES et al. 2005; West S et al. 2005). Chlamydial RNA cannot usually be detected in those without clinical signs (Burton et al. 2006). High chlamydial loads are found in young children, with the highest loads being found in those with intense inflammatory trachoma (TI according to the simplified WHO grading scheme) (Solomon et al. 2003; West ES et al. 2005; West S et al. 2005). Those with high chlamydial loads are less likely to be cured by antibiotic treatment, and more likely to infect their household contacts than those with low loads (West S et al. 2005).

5 Pathogenesis of Trachoma

Endemic trachoma is invariably due to the ocular serovars A, B, Ba and C. Caldwell et al. have determined the probable basis of the tropism of *C. trachomatis* strains. All ocular strains tested lacked a functional tryptophan synthase gene, whereas this gene was intact in all genital strains tested, including the rare serovar B genital isolates (Caldwell et al. 2003). Genital isolates were able to escape interferon (IFN)- γ mediated eradication in the presence of indole, which is produced by commensal bacteria present in the female genital tract, whereas ocular strains could not. IFN- γ is expressed in the conjunctival epithelium in active trachoma, and the level of expression is positively correlated with the expression of *C. trachomatis* 16SrRNA (Burton et al. 2004; Faal et al. 2005) it remains unclear how ocular *C. trachomatis* strains escape the neutralising activity of IFN- γ .

Recent studies using real time reverse transcriptase PCR have confirmed that the pro-inflammatory cytokines TNF- α and IL-1 β are expressed in the conjunctival epithelium of subjects with active trachoma. Increased expression of the fibrogenic molecule matrix metalloprotease-9 (MMP-9) and the anti-inflammatory cytokine IL-10 were also found. In subjects in whom *C. trachomatis* 16SrRNA expression could be detected, increased expression of IL-4, IL-12p40 and perforin were also found. 16SrRNA expression was more closely correlated with the presence of clinical signs of trachoma than was the presence of *C. trachomatis* DNA, suggesting that chlamydial metabolic activity may be a prerequisite for the development of clinical signs (Burton et al. 2006, 2004). Perforin expression at the site of infection suggests that CD8+ cytotoxic T cells are induced by ocular *C. trachomatis* infection. This was confirmed by the detection of circulating T cells capable of binding HLA class I tetramers presenting *C. trachomatis* MOMP-specific peptides in subjects with trachoma (Holland et al. 2006). These cells were more frequent in those with current ocular *C. trachomatis* infection, but their frequency was not related to clinical signs or to *C. trachomatis* bacterial load; nor did they predict the appearance or resolution of clinical signs.

The role of IL-10 in the pathogenesis of trachoma was clarified by two studies. In a case-control study of 651 Gambian subjects with scarring trachoma and age-, sex- and village-matched controls, an IL-10 haplotype was associated with increased risk of scarring (Natividad-Sancho et al. 2005). The second study used allele-specific quantification to study the relative expression of IL-10 transcripts in the conjunctival epithelium of subjects with active trachoma. Alleles containing a transcribed SNP tagging the risk haplotype was relatively more abundant in heterozygous subjects, suggesting that increased IL-10 production during acute infection confers increased risk of scarring sequelae (Natividad et al. 2006).

6 The SAFE Strategy

The WHO strategy for the elimination of blinding trachoma has the acronym SAFE: Surgery for turned eyelids (trichiasis), Antibiotics to treat ocular *C. trachomatis* infection, Face washing and Environmental improvement to reduce transmission of *C. trachomatis*.

7 Surgery

In Tanzania and The Gambia, long-term follow up studies showed that trichiasis recurs after surgery in up to 40% of cases. Ocular *C. trachomatis* infection was rarely found in recurrent cases, but severe inflammation, and other bacterial infections were common (Burton et al. 2005b; West ES et al. 2005a). In The Gambia, a randomised controlled trial of azithromycin given to patients and to children in their households at the time of surgery showed no benefit (Burton et al. 2005b) but in Ethiopia, where the prevalence of *C. trachomatis* infection is higher, azithromycin treatment reduced the incidence of recurrence by 30% (West et al. 2006). An Ethiopian study confirmed the findings of an earlier study in Tanzania, showing that the outcome of surgery performed by integrated eye care workers was similar to that of surgery performed by ophthalmologists (Alemayehu et al. 2004).

8 Antibiotic

Recently published studies suggest that community-based mass treatment with azithromycin may be more effective than previously believed. When high coverage is achieved, a single round of mass treatment has been shown to eliminate *C. trachomatis* infection from a meso-endemic community for up to 5 years (Solomon et al. 2004b; Harding-Esch et al. 2006). Infection is more difficult to eliminate from high prevalence communities (Melese et al. 2004; West S et al. 2005; Chidambaram et al. 2006) and may be rapidly re-introduced in highly mobile populations (Burton et al. 2005a). It has been suggested that there may be a threshold prevalence and intensity of infection, below which transmission will not be sustained and elimination is inevitable (Solomon et al. 2004; Chidambaram et al. 2005).

Azithromycin is well received by trachoma-endemic populations, who are often aware that it is effective against other diseases in addition to its effect on trachoma (Fry et al. 2002; Desmond et al. 2005). Its administration can be simplified by determining dosage on the basis of height rather than weight (Munoz et al. 2003), and recent studies have found no evidence that mass treatment leads to an increased prevalence of macrolide resistant *Streptococcus pneumoniae* or *C. trachomatis* (Fry et al. 2002; Batt et al. 2003; Gaynor et al. 2003; Solomon et al. 2005).

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9

Gonococcal Infections in Newborns and in Adolescents

Noni MacDonald, Tim Mailman, and Shalini Desai

1 Introduction

Gonococcal (GC) genital tract infection was first recognized by Hippocrates in the fifth century BC and later given its name – “gonorrhea” meaning “flow of semen” – by Galen in second century AD (Woods 2005). Despite the passage of time, much research, the advent of sensitive and specific diagnostic tests and effective antimicrobial therapy, gonorrhea remains one of the most prevalent bacterial sexually transmitted infections (STI), in both industrialized and developing countries. This chapter focuses on gonococcal infections in two groups – newborns who acquire infection from their mothers at the time of delivery, and adolescents: a group at the center of the ongoing worldwide STI epidemic.

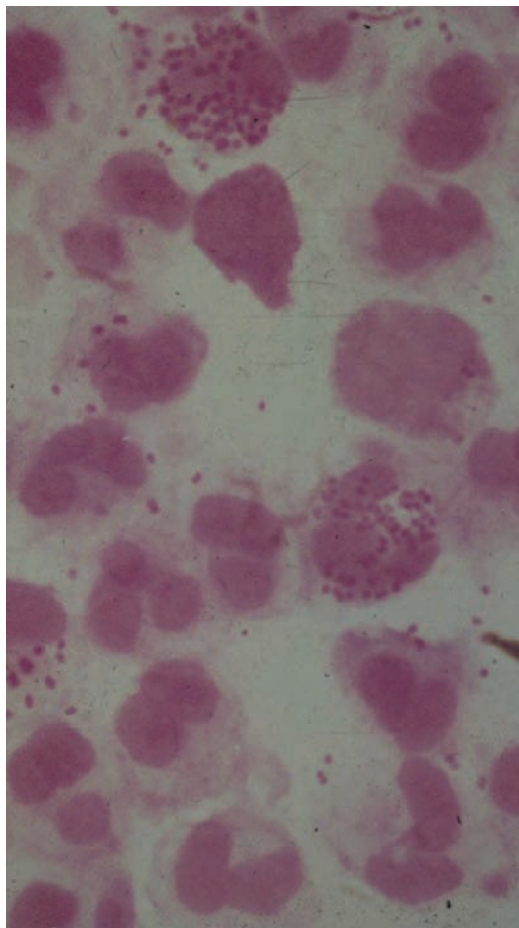
2 The Microbe

Neisseria gonorrhoeae is an aerobic, non-motile, Gram-negative coccus that characteristically grows in pairs (diplococcus) with flattened adjacent sides, yielding a “kidney bean” appearance (Fig. 1). First described by Neisser in 1879, the organism closely resembles *Neisseria meningitidis*, and several nonpathogenic *Neisseria* species. Members of the genus *Neisseria* are capnophilic, oxidase positive, and (with the exception of *N. elongata* subspecies) catalase positive. Most species grow optimally in humid environments at 35–37°C.

Traditionally, *N. gonorrhoeae* are differentiated from other *Neisseria* by isolation on enriched but selective (i.e. Thayer–Martin, Martin–Lewis, or New York City) agar and the abilities to produce acid from glucose but not maltose, sucrose or lactose, and to reduce nitrites. Suspect colonies may be screened with oxidase and superoxol tests (production of vigorous bubbling when exposed to 30% hydrogen peroxide) and should be confirmed on the basis of at least two distinct biochemical or antigenic features (i.e. pattern of acid production from carbohydrates plus a rapid antigenic test).

Molecular/nucleic acid-based detection of *N. gonorrhoeae* is becoming more widely available (Center for Disease Control and Prevention 2002; Gaydos 2005).

Fig. 1 Gram stain of urethral secretions showing gram negative intracellular diplococci and neutrophils. (Public Health Agency of Canada, 2002) (with permission Boehringer-Ingelheim (Canada) Ltd. From the Public Health Agency of Canada. Self-Learning Module. STD Slide Gallery 2002; Gram stain of urethral secretions. <http://www.phac-aspc.gc.ca/slm-maa/slides/other/pages/u206.html>)



These methods are highly sensitive and avoid inherent problems with culturing fastidious organisms. *N. gonorrhoeae*, in particular, is susceptible to temperature fluctuations or desiccation in transport; neither of which impact on the yield of nucleic acid amplification tests. Drawbacks of molecular techniques include false positives due to carryover contamination or horizontal genetic exchange among *Neisseria* species and false negatives due to sequence variations; both of which can have significant implications (see Diagnostic Testing).

Unlike other human *Neisseria* species, *N. gonorrhoeae* is always considered a pathogen, regardless of the site of isolation. It has multiple virulence features (Table 1) that enable it to evade host immunological defenses, bind to mucosal surfaces, and resist antimicrobials. Of particular note, strains causing disseminated gonococcal infection (DGI) are usually less susceptible to bactericidal activity of sera and do not express Opa proteins (Vogel and Frosch 1999; Woods 2005). Lack of Opa expression appears to facilitate evasion of host defense mechanisms: a critical step in the pathway to disseminated disease.

Table 1 *Neisseria gonorrhoeae*: key virulence factors (Vogel and Frosch 1999; Woods 2005)

Virulence factor	Role in pathogenesis
Surface structures	
Pili	Mediate attachment to mucosal surfaces Impede phagocytosis Regions of antigenic variation enhance immune evasion and make pilus-based vaccines a challenge
Lipooligosaccharides (LOS)	Evokes inflammatory response: complement activation, cytokine production, and neutrophil lysis
Outer membrane proteins	
PorI-PorA/PorB	Resistance to bactericidal effects of human serum Promote invasion of epithelial cells
Opa proteins	Augment pilus-mediated attachment to mucosal surfaces; facilitate invasion of epithelial cells; hypervariable regions contribute to immune evasion
IgA1 protease	Inactivates IgA1 at mucosal surfaces
Plasmids	Often carry antibiotic resistance genes: tetM – confers tetracycline resistance, TnA – ampicillin resistance transposon, β -lactamase genes
Chromosomal mutation/transformation	Chromosomally mediated high-level penicillin resistance; antibiotic efflux pump genes

3 Gonococcal Infection in Newborns

Newborn infants acquire GC via passage through an infected birth canal. As the prevalence of gonorrhoea among pregnant women varies widely, so does the risk of neonatal infection. Factors that influence maternal infection rates include prevalence of gonorrhoea within a region and within specific populations (see Partners, Condoms and Sexual Networks), sexual practices and access to health care, and STI diagnosis and treatment during pregnancy. In Africa, an estimated 0.5–22% of pregnant women are infected with gonorrhoea (World Health Organization 2001; Laga et al. 1989; Foster and Klauss 1995; Apea-Kubi et al. 2004), in Nepal 2.3% (Christian et al. 2005), in Fuzhou China 0.8% (Chen et al. 2006), while in the United States the range in 2004 was from a high of 3.1% in prenatal clinics in Mississippi to a low of <0.0% in Iowa. (Centers for Disease Control and Prevention 2005). Of pregnant women with gonorrhoea, 23% have premature deliveries (associated with a 33% mortality rate), 29% have premature rupture of membranes and in 13% pregnancy ends with a septic abortion (Woods 2005). In neonates born to mothers with gonorrhoea, 26–40% will have orogastric colonization, while 35% have oropharyngeal colonization (Woods 2005).

4 Gonococcal Ophthalmia Neonatorum

Ophthalmia neonatorum (ON) is the most common presentation of neonatal gonococcal infection. At the turn of the last century, the prevalence of gonococcal ON among live births in maternity hospitals in Europe exceeded 10%, with 20% of these infants experiencing corneal damage and 3% becoming blind. (Schaller and Klauss 2001). With the overall decrease in gonorrhoea rates among pregnant women in industrialized countries, the prevalence of gonococcal ON has dropped to 0.04 per 1,000 live births in Belgium and to 0.3 per 1,000 in the United States). (Schaller and Klauss 2001). In contrast, gonococcal ON remains a serious problem in many developing countries. For example, the rate of gonococcal ON in Nairobi Kenya in the 1980s was 3–4% among live births (Laga et al. 1989). If a pregnant woman has gonorrhoea, 2–48% of exposed infants who have not received ocular prophylaxis will develop ON. This rate drops to 0–10% if prophylaxis is given (Woods 2005).

The typical presentation of gonococcal ON includes bilateral conjunctival discharge, hyperaemia, and eyelid edema appearing 2–7 days after birth. In some instances, onset of symptoms may be delayed into the second week after birth. The discharge may initially be watery but then progresses to become mucopurulent and, in some, blood tinged. Even with treatment, exudative conjunctivitis may continue for a week or two, further contributing to corneal scarring. Untreated, permanent corneal damage and blindness may occur secondary to corneal inflammation and ulceration. Current estimates of the risk of scarring and blindness vary from 1% to 16% (Laga et al. 1989).

Chlamydia trachomatis, another STI that can be passed from mother to newborn, is another cause of ophthalmia neonatorum. ON due to *Chlamydia* tends to be less fulminant, have a later onset (5–10 days of age) and a less severe course.

5 Neonatal Gonococcal Scalp Abscess and Disseminated Gonococcal Infection

Although less prevalent, scalp abscesses and disseminated infection are other clinical entities seen in neonates born to infected mothers. Scalp abscesses typically occur when fetal monitors have been used in a mother with an infected birth canal. Neonatal disseminated gonococcal infection (DGI) may arise from a scalp abscess or as an invasive sequelae of oropharyngeal colonization. Septic arthritis is the most common manifestation of DGI and typically presents as polyarticular disease. Other less common manifestations of DGI include bacteremia and meningitis. Strains that cause DGI tend to be less susceptible to the bactericidal action of sera and not to express Opa proteins (See The Microbe) (Woods 2005).

6 Diagnosis of Neonatal Gonococcal Infections

Diagnosis of gonococcal infection in a newborn requires a high index of suspicion so that appropriate tests are obtained (See Diagnostic Testing). Given that maternal co-infection with other STIs is common, newborns with suspected gonococcal infection also need testing for concomitant infection with *Chlamydia trachomatis*, congenital syphilis, HIV and Hepatitis B.

For suspected gonococcal ocular disease, swabs of ocular discharge and the oropharynx are cultured; for scalp abscesses, aspirates of the lesion or swabs of drainage fluid; and for DGI synovial fluid, blood, cerebrospinal fluid (and, if indicated conjunctival swabs) for culture. Culture of the joint fluid usually yields the organism, but polymerase chain reaction (PCR) targeting the gene coding for bacterial 16S ribosomal RNA has become a useful adjunct to culture (Rosey et al. 2006). Given that *N. gonorrhoea* is a fastidious organism, swabs must be placed immediately in transport media and taken to the laboratory for Gram stain and culture. (See Diagnostic Testing). Culture has the benefit of being inexpensive and allows antimicrobial sensitivity testing. Additionally, the mother and her partner(s) need examination and management for *N. gonorrhoea* infection and for other possible STIs.

7 Treatment of Neonatal Gonococcal Infections

Antimicrobial treatment depends on the clinical syndrome (See Table 2). Penicillin is not recommended, due to high resistance rates. Hospitalization is recommended for all infants with clinical evidence of gonococcal infection (American Academy of Pediatrics 2006; Public Health Agency of Canada 2006a).

In addition to antimicrobial therapy, gonococcal ON treatment includes frequent irrigation of the eyes with sterile saline to eliminate discharge and minimize local corneal inflammation and damage. Therapeutic failures may be due to co-infection with *C. trachomatis* which should always be tested for in these settings.

Table 2 Treatment of neonatal gonococcal infections (American Academy of Pediatrics 2006; Public Health Agency of Canada 2006a; Centers for Disease Control and Prevention 2006c)

Disease	Antimicrobial treatment
Asymptomatic: born to mother with gonorrhea	Ceftriaxone 25–50mg/kg IV or IM not to exceed 125 mg (single dose) or Cefotaxime 100mg/kg IV or IM (single dose)
Gonococcal ophthalmia neonatorum	As above
Focal site of colonization e.g. oropharynx	As above
Disseminated gonococcal infection e.g. septic arthritis meningitis bacteremia or Scalp abscess	Ceftriaxone 25–50mg/kg once daily IV or IM for 7 days or cefotaxime 100mg/kg in 3 divided doses per day IV or IM for 7 days

8 Prevention of Neonatal Gonococcal Infection

Primary prevention requires modification of maternal behaviour to lessen the risk of contracting gonorrhoea. The difficulty in achieving this has led to current recommendations for screening and treatment of pregnant women. Screening of all pregnant women is cost effective if the prevalence of gonorrhoea in the population is $\geq 5\%$ (Laga et al. 1989). If the prevalence is lower, selective screening based on risk factors is indicated. The risk factors are the same as for nonpregnant women, i.e. a history of previous gonococcal infection, a history of previous STI, new or multiple sexual partners, inconsistent condom use, working in the sex industry or drug use (Public Health Agency of Canada 2006b).

Secondary prevention has been known to be effective for over 100 years. Crede noted in 1881 that application of 1% silver nitrate solution to the eyes of newborns born to women with vaginal discharge decreased the rate of ON from 30 to 35 per year to only 1 in his hospital in Leipzig. (Schaller and Klauss 2001). Current prophylaxis options include 1% silver nitrate drops, 1% tetracycline drops or 0.5% erythromycin drops applied to newborns eyes within the first hours of life (American Academy of Pediatrics 2006). Povidine-iodine 2.5% solution has been shown to be effective (Isenberg et al. 1995) but is unavailable in many countries. In choosing between the available products, antibiotic ointments are less likely to cause chemical conjunctivitis than silver nitrate and hence maybe more acceptable to parents. Single dose ampoules are recommended for all products to minimize contamination and cross infection between infants. In regions of the world where gonococcal ON remains a significant risk, ocular prophylaxis is part of the recommended routine care of the neonate (Whitcher et al. 2001). None of the current prophylactic agents provide protection against *C. trachomatis* ON.

9 Gonococcal Infection in Adolescents

While gonococcal infection in newborns in industrialized countries is now very uncommon, the same cannot be said for adolescents (Risser et al. 2005). As Fig. 1 shows, in the United States, adolescent women – aged 15–19 years – had the highest notification rates of gonorrhoea ($>6/1,000$) in 2004; the next highest were women aged 20–24 years (Centers for Diseases Control and Prevention 2005). For males, adolescent males 15–19 years had the third highest rate, $2.5/1,000$ (Centers for Diseases Control and Prevention 2005) (Fig. 2).

Lest one mistakenly thinks that these notification rates only reflect infection rates in “special populations” from STI clinics, population based estimates of gonococcal infection based on nucleic acid amplification testing (NAAT) (see Diagnostic Testing) of 18–24 year olds in the United States are reported to be $4/1,000$ (Miller et al. 2004). Similarly, high notification rates of gonococcal infection in adolescents are reported for Australia (Australian Government, Department of Health and Aging 2004). Canada

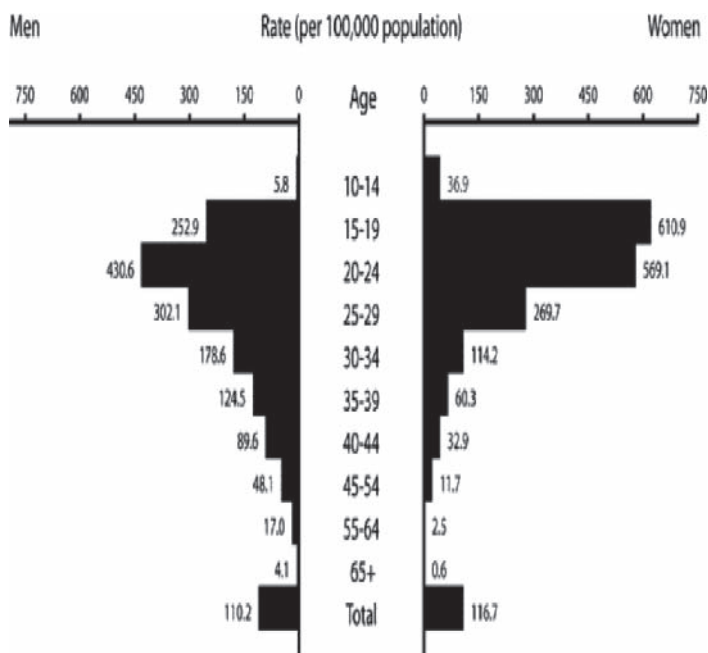


Fig. 2 Gonorrhoea age specific rates in the United States, 2004 (Centers for Disease Control and Prevention, 2005) (From Centers for Diseases Control and Prevention. STD Surveillance 2004. National Profile. Figure 16. Gonorrhea – Age- and sex-specific rates: United States, 2004 www.cdc.gov/std/stats/gonorrhea3.htm)

(Public Health Agency of Canada 2005), the United Kingdom (Brown et al. 2004) and other industrialized countries.

Figure 3 puts gonococcal notification rates in the United States over the last 30 years in perspective with respect to those for *C. trachomatis*. Gonorrhoea notification rates have been falling for a number of years while the rates for chlamydia have been rising. The latter trend may be due in part to increased testing and the availability of better diagnostic tools for *C. trachomatis*. Similar trends for gonorrhoea rates with respect to chlamydia rates have been seen in other industrialized countries.

10 Why Are Adolescent STI Rates so High?

There are a number of factors that sustain an STI epidemic whether in adolescents or adults. These are summarized by the formula $R = B \times C \times D$ where R is the reproductive rate of the STI, B is the transmissibility of the organism, C is the number of partners and D is the duration of infectivity (May and Anderson 1987). What sets adolescent STI risk apart from adult STI risk are the adolescent

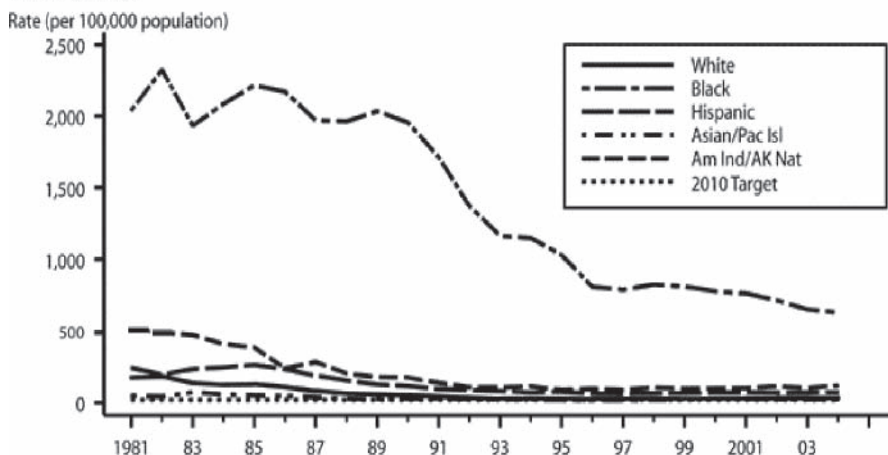
Gonorrhoea**Chlamydia**

Fig. 3 Reported rates of gonococcal infection and chlamydial infection in the United States: 1970 to 2003 (Centers for Diseases Control and Prevention, 2005). (From Centers for Diseases Control and Prevention. STD Surveillance 2004. National Profile. Figure 15. Gonorrhea – Rates by race and ethnicity: United States, 1981–2004 and the Healthy People 2010 target www.cdc.gov/std/stats/gonorrhea3.htm) (From Centers for Diseases Control and Prevention. STD Surveillance 2004. National Profile. Figure 5. Chlamydia – Rates by sex: United States, 1984–2004 www.cdc.gov/std/stats/chlamydia3.htm)

behavioural and social factors which influence these three factors (Table 3) (MacDonald et al. 1990, 1994; Ford et al. 2002; Shafii and Burstein 2004; Robertson et al. 2005; Brown et al. 2006; Centers for Disease Control and Prevention 2006a; Joesoef et al. 2006; Lofy et al. 2006; Public Health Agency of Canada 2006c).

The *transmissibility* (*B*) of the organism is relatively similar for adolescents and adults although cervical immaturity in young female adolescents does put them at higher risk for infection with STI agents like human papilloma virus (Kahn et al. 2002). For gonorrhoea, the sexual transmissibility rates are higher from a male to a female (50–70%) than from a female to a male (20%) (Holmes

Table 3 Risk factors for sexually transmitted infection in adolescents (MacDonald et al. 1990, 1994; Ford et al. 2002; Shafii and Burstein 2004; Robertson et al. 2005; Brown et al. 2006; Lofy et al. 2006; Joesoef et al. 2006; Centers for Disease Control and Prevention 2006a; Public Health Agency of Canada 2006c)

Physical factors

- Younger age at puberty
- Cervical ectopy (esp. increase risk of HPV infection)
- Smaller introitus leads to traumatic sex
- Asymptomatic STI

Behavioural factors

- Early adolescence: poor abstract thinking
- Middle adolescence: belief in invulnerability
- Inconsistent condom use
- Age discordance of sexual partners

Social factors

- Limited access to adolescent friendly healthcare
 - Adolescent health seeking behaviour: denial
 - Concern for confidentiality
 - Older male sexual partners of young adolescent females
 - “Sexy” media (television, movies, music, magazines)
 - Alcohol use, drug use
 - Sexual abuse and violence
 - Homelessness, street youth, incarceration
-

et al. 1970; Hooper et al. 1978). Condom use does decrease transmission of gonorrhoea (Steiner and Cates 2006; Warner et al. 2006) but adolescents do not consistently use condoms (Centers for Disease Control and Prevention 2006a) (See Partners, Condom Use and Sexual Networks) so adolescent transmission rates remain high.

The *duration of infectivity* (*D*) depends on a number of factors such as whether an STI can be spontaneously cleared, whether the STI causes symptoms and hence sends a signal to seek health care, whether diagnosis and curative treatment are available and whether compliance with treatment is high. Even though gonorrhoea can be readily diagnosed and effectively treated, prolonged genital infection can occur without treatment, as it is not spontaneously cleared. Also gonococcal infection is also not always symptomatic (Bozicevic et al. 2006). Males often develop symptoms after 3–5 days but some male infections are asymptomatic. Female infection is often asymptomatic. Even if adolescents have symptoms or suspect that they may be at risk for infection, they are more likely to deny that there is a problem compared to an adult and hence seek medical help less readily. Furthermore, adolescent-friendly STI treatment clinics are not common, compounding adolescent reluctance to seek care. In some locales, adult STI clinics require an appointment and may be overbooked leading to marked delays for appointment times (Ward and Robinson 2006). Given that many adolescents live in the “now” not

being able to be seen quickly can further discourage care seeking. All of these factors can contribute to prolongation of the duration of gonococcal infectivity in adolescents.

11 Partners, Condom Use and Sexual Networks

Adolescents and youth have frequent partner change (MacDonald et al. 1990, 1994; Ford et al. 2002; Shafii et al. 2004; Robertson et al. 2005; Centers for Disease Control and Prevention 2006a; Public Health Agency of Canada 2006c). In the 2005 National Youth Risk Behavior Survey in the United States, 47% of grade 9 (age 14 years) to grade 12 (age 17 years) students had had sexual intercourse at least once, 34% were currently sexually active i.e. sexual intercourse with ≥ 1 person in the last 3 months, 14% already had had ≥ 4 lifetime sexual partners and 7.5% had been forced to have sexual intercourse (Centers for Disease Control and Prevention 2006a). While some of these factors have decreased modestly from a decade ago e.g. 1991-ever had sexual intercourse, 54.1%; ≥ 4 lifetime sexual partners 18.7%, (Centers for Disease Control and Prevention 2006b), the numbers of partners and on going lack of consistency of condom use remain serious risk issues. Among the currently sexually active adolescents, 37% reported that neither they nor their partner had used a condom during last sexual intercourse thus increasing the risk of transmission of STI such as gonorrhoea. Alcohol and/or drug use also influenced condom use and partner choice (Centers for Disease Control and Prevention 2006a).

For persistence of STI, the core group must not only consist of individuals with a high rate of partner change but also a high degree of connectivity (Doherty et al. 2005). Figure 4 shows two typical patterns of social networks; linear and radial (Wylie and Jolly 2001). Adolescents fit the “core group criteria” as they have high rates of partner change, high connectivity within their cohort of “friends and acquaintances,” and furthermore do not always use condoms. Hence when an STI is introduced into an adolescent sexual network, it can spread quickly through the group (Ford et al. 2002). Detection and treatment of a few cases of gonorrhoea in the network will not eradicate infection from the network. New infections are introduced into the network when a member of the network meets other new sexual partner(s) through social, work or educational activities. Each provides an opportunity for introduction or reintroduction of an STI back into the former social/sexual network if the adolescent stays connected to the first network i.e. bridge partners (Ford et al. 2002). Very large partner networks are common among adolescents who are homeless, live on the street (MacDonald et al. 1994; Public Health Agency of Canada 2006c) are involved in the sex industry and/or use of drugs (Centers for Disease Control and Prevention 2006a) as well as those who are or have been incarcerated (Robertson et al. 2005; Joesoef et al. 2006; Lofy et al. 2006).

Adolescent sexual behaviour (frequent partner change, limited use of condoms) and their sexual networks (mixing patterns and bridge partners) all help to fuel the STI epidemic.



Fig. 5 Urethritis. (Public Health Agency of Canada, 2002) (Reproduced with permission Dr Marc Steben from the Public Health Agency of Canada. Self-Learning Module. STD Slide Gallery 2002. Urethritis. With permission. <http://www.phac-aspc.gc.ca/slm-maa/slides/other/pages/u203.html>)

symptoms may include intermenstrual bleeding, dysuria, lower abdominal pain or heaviness and dyspareunia. Fifty percent are asymptomatic. As with urethritis, cervicitis may be caused by several STI organisms. Gonococcal salpingitis can be asymptomatic or be associated with symptoms like cervicitis with or without fever. Pelvic inflammatory disease and infertility are significant sequelae of salpingitis.

DGI, disseminated gonococcal infection, is an uncommon presentation of gonococcal infection in adolescents. Strains causing DGI seem to differ from those that do not (see *The Microbe*). DGI occurs more commonly in those with complement deficiencies, particularly terminal components (Ellison 1987). The clinical presentation usually involves skin lesions (Fig. 7), arthralgias, tenosynovitis and polyarticular arthritis (Fig. 8) (Rice 2005). Potential complications include hepatitis, myocarditis, endocarditis and meningitis. Gonorrhoea may also cause monoarticular septic arthritis without skin and other lesions (Rice 2005).

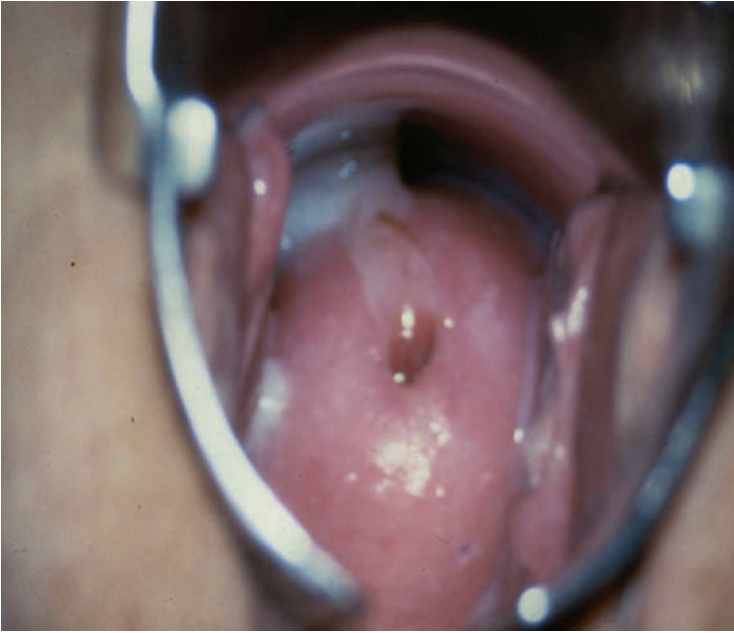


Fig. 6 Mucopurulent Cervicitis. (Public Health Agency of Canada, 2002) (Reproduced with permission Dr Marc Steben from the Public Health Agency of Canada. Self-Learning Module. STD Slide Gallery 2002. Mucopurulent cervicitis. With permission <http://www.phac-aspc.gc.ca/slm-maa/slides/other/pages/stdx003.html>)



Fig. 7 Skin lesions in disseminated gonococcal infection. (Public Health Agency of Canada, 2002) (Reproduced with permission Dr Marc Steben from the Public Health Agency of Canada. Self-Learning Module. STD Slide Gallery 2002. Skin Lesions in Disseminated Gonococcal Infection. With permission <http://www.phac-aspc.gc.ca/slm-maa/slides/other/pages/r909.html>)



Fig. 8 Polyarticular Arthritis in Disseminated Gonococcal Infection (Public Health Agency of Canada, 2002) (Reproduced with permission Boehringer Ingelheim (Canada) Ltd. From the Public Health Agency of Canada. Self-Learning Module. STD Slide Gallery 2002. Polyarticular arthritis in Disseminated Gonococcal Infection. <http://www.phac-aspc.gc.ca/slm-maa/slides/other/pages/8r931.html>)

13 Diagnostic Testing for *N gonorrhoea* Infections in Adolescents

Gram stain of urethral or cervical discharge is the point-of-care test for *N. gonorrhoea* (See Fig. 1 and Table 4). Laboratory based tests for *N. gonorrhoea* include culture, non-amplified tests such as enzyme immunoassay, nucleic acid hybridization and nucleic acid genetic transformation and nucleic acid amplified tests (NAAT) (Centers for Disease Control and Prevention 2002). Culture is necessary for antibiotic testing and has been the standard for medicolegal cases. NAAT testing is often more acceptable to adolescent males as a urine specimen may be submitted, but NAAT is less useful in low prevalence populations where even highly specific tests may have unacceptably high false positive rates. Table 4 provides an overview of the advantages and disadvantages of these different tests. For DGI, in adolescents, in about 50% the blood culture will be positive. As with neonates, culture of the joint fluid usually yields the organism, but polymerase chain reaction (PCR) targeting the gene coding for bacterial 16S ribosomal RNA has become a useful adjunct to culture (Rosey et al. 2006).

14 Treatment of Adolescent Gonococcal Infections

Gonococcal antimicrobial resistance has become a serious problem. Figure 9 shows *N. gonorrhoea* resistance rates for penicillin and tetracycline in 1990–1997 in a number of South American countries (Dillon et al. 2006). Neither of these inexpensive

Table 4 Comparison of tests for detection of *Neisseria gonorrhoeae* infection (Centers for Diseases Control and Prevention 2002; Gaydos 2005, 2006; Rosey et al. 2006)

Test	Advantages	Disadvantages/ Concerns	Comments
Gram stain test	Urethritis Symptomatic: sensitivity >95%, specificity >99% Asymptomatic: sensitivity 50% Cervicitis Sensitivity 50%, specificity >95% Rectal infection Blind anorectal swab sensitivity: 40–60%; anos- copy swab 79%	Positive predictive value varies with rate of gonococcal infection in the population Pharynx: not useful	Gram stained smear is positive if polymorpho- nuclear neutrophils and Gram negative intracellu- lar diplococci (see Fig. 1)
Culture	Approved for all sites sensitivity Male urethra: 95% Cervix: 95% Rectum: 70–90% Pharynx: 70–90% Antibiotic testing possible Useful for blood, CSF and joint fluid specimens Test of choice if medico legal case	Need careful trans- port and selective media Need a separate specimen for chlamydia testing	This is a fastidious organism and if not carefully han- dled during transport and culture may not grow
Non-amplified tests			
Enzyme immu- noassay (EIA)	Less sensitive to handling than culture	No antibiotic sensitiv- ity testing	Not recommended for neonates
Nucleic acid hybridization	Potential for more timely results than culture	Not approved for rectal or throat specimens	Not able to confirm result: a problem in low prevalence populations e.g. neonates
Nucleic acid genetic transfor- mation	Some test for both gonorrhoea and for chlamydia Sensitivity: 85–90% Specificity 95%	Lower sensitivity in asymptomatic populations Not approved for medico legal cases	
Nucleic acid ampli- fication tests	Approved for ure- thral and cervical specimens	No antibiotic sensitiv- ity testing	Not recommended for neonates

(continued)

Table 4 (continued)

Test	Advantages	Disadvantages/ Concerns	Comments
Polymerase chain reaction (PCR)	TMA approved for vaginal specimens		
Transcription-mediated amplification (TMA)	All approved for urine except PCR in females	Not approved for throat or rectal specimens	
Standard displacement amplification (SDA)	Can also use samples for chlamydia testing	Not approved for medico legal cases	Not able to confirm result: a problem in low prevalence populations eg neonates

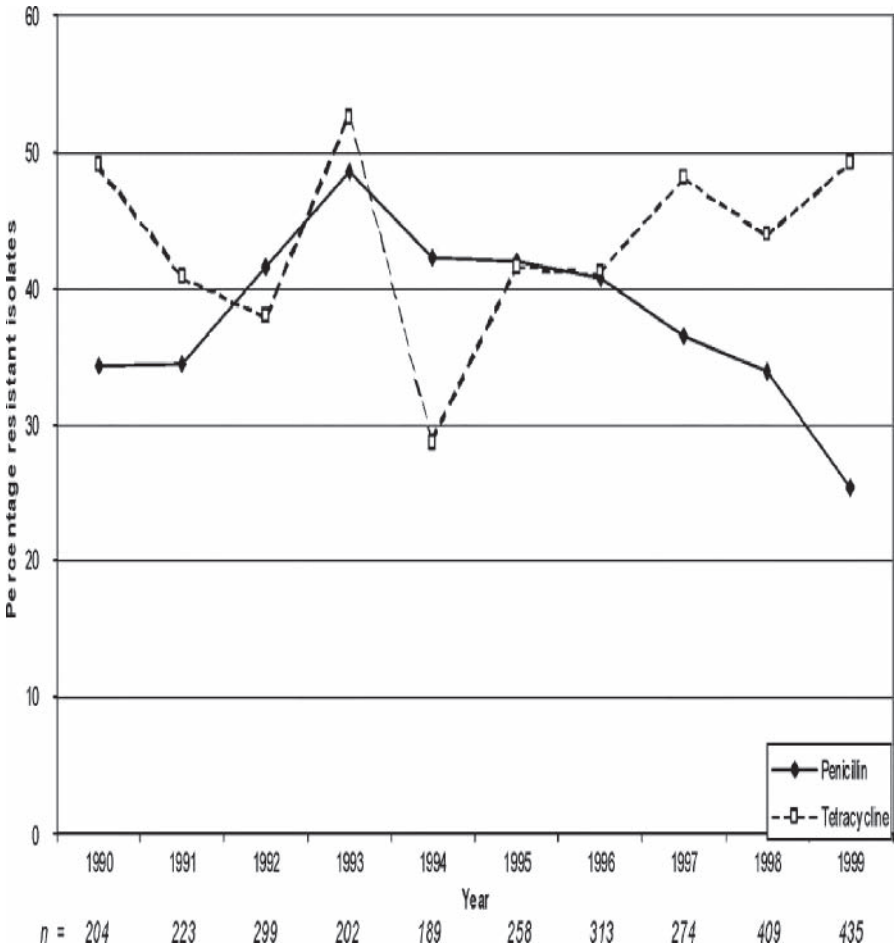


Fig. 9 Gonococcal Penicillin and Tetracycline Resistance Rates in Selected South American Countries (Argentina, Chile, Columbia, Peru, Venezuela, Uruguay) (Dillon et al., 2006). (Reprinted from Sexually Transmitted Diseases vol 33, Dillon JA, Ruben M, Li H, Borthagaray G, Marquez C, Fiorito S, Galarza P, Portilla JL, Leon L, Agudelo CI, Sanabria OM, Maldonado A, and Prabhakar P. (2006). Challenges in the control of gonorrhea in South America and the Caribbean: monitoring the development of resistance to antibiotics. Figure 1 Page 90. Copyright 2001, with permission from Lippincott, Williams and Wilkins.)

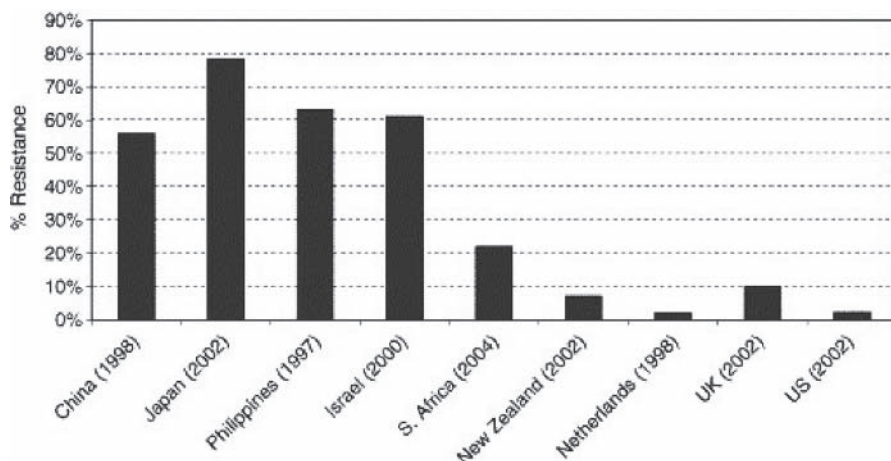


Fig. 10 Fluoroquinolone-resistance *N. gonorrhoea* in Selected Countries. (Ghanem et al., 2005) (Reprinted from Infectious Diseases Clinics of North America Vol 19, Ghanem KG, Giles JA, Zenilman JM; Fluoroquinolone-resistant *Neisseria gonorrhoeae*: the inevitable epidemic, page 358, Copyright 2005, with permission from Elsevier.)

drugs is useful to treat gonorrhoea anymore, as the resistance rates are too high. This has serious implications, as the currently effective antimicrobial agents are less widely available and more expensive. The resistance problem is not limited to penicillin and tetracycline. Fluoroquinolone resistance is a growing problem, particularly in Asia (Fig. 10) but also in the United Kingdom and some parts of the United States (Ghanem et al. 2005). In Canada, fluoroquinolone therapy for gonococcal infection is not recommended if the infection was acquired in Asia, the Pacific Islands, India, Israel, Australia, New Zealand, Hawaii, California, Washington State, Arizona and Michigan (Mann et al. 2004.).

Table 5 provides an outline of recommended antimicrobial treatment regimens when fluoroquinolone resistance is not suspected and the treatment options when it is suspected (Bignell 2001; American Academy of Pediatrics 2006; Public Health Agency of Canada 2006a; Centers for Disease Control and Prevention 2006c). Cefixime is not available in all countries but when available does provide an oral treatment option in areas where fluoroquinolone resistance is a problem. For DGI, intravenous antigonococcal therapy is recommended for 24–48 h followed by oral therapy to complete a 1 week course.

In all instances where an adolescent has a gonococcal infection, regardless of the site of infection, treatment for *Chlamydia trachomatis* should be given concurrently.

Beyond the selection of antimicrobial therapy for gonorrhoea and the concurrent treatment of *C. trachomatis*, other components of treatment must be addressed (Table 6). For adolescents, compliance with treatment is a serious problem, hence single dose therapy for *N. gonorrhoea* with single dose therapy for *C. trachomatis* all administered on site is the preferred regimen. (American Academy of Pediatrics 2006; Centers for Disease Control and Prevention 2006c).

Table 5 Antimicrobial treatment of gonococcal infections in adolescents (Bignell, 2001; American Academy of Pediatrics 2006; Public Health Agency of Canada 2006a; Centers for Disease Control and Prevention 2006c)

No suspected fluoroquinolone resistance	Suspected fluoroquinolone resistance
Treatment of urogenital, rectal and pharyngeal infection	
Cefixime 400 mg orally or ceftriaxone 125 mg IM or ciprofloxacin 500 mg orally or ofloxacin 400 mg orally	Cefixime 400 mg orally or ceftriaxone 125 mg IM
If allergic to cephalosporins: use fluoroquinolone	If allergic to cephalosporins: use azithromycin 2 gm orally or spectinomycin 2 gm orally
Treatment of disseminated gonococcal infection	
Intravenous therapy for 24–48 h, then complete 7 day course	Intravenous therapy for 24–48 h, then complete 7 day course
Concurrent treatment for <i>Chlamydia trachomatis</i> recommended for all adolescents with gonococcal infection	Concurrent treatment for <i>Chlamydia trachomatis</i> recommended for all adolescents with gonococcal infection
Azithromycin 1 gm orally or doxycycline 100 mg twice daily for 7 days	Azithromycin 1 gm orally or doxycycline 100 mg twice daily for 7 days

Table 6 Treatment of adolescents with gonococcal infection (American Academy of Pediatrics 2006; Public Health Agency of Canada 2006a)

Antimicrobial therapy (see Table 5)
Directly observed therapy
Least complex regime
Check for other STIs
Partner notification and treatment
Report to public health
Prevention counseling
Abstinence
Safer sex
Consistent condom use
Partner selection
Abstinence
Immunization: HBV, HPV
Follow up testing: routine retesting for gonorrhoea not indicated

Adolescents with gonorrhoea also need to be tested for other STIs such as *C. trachomatis*, syphilis, HIV and HBV infections. Partner notification and treatment is a key component to treatment in order to limit the risk of reinfection and as a major step towards decreasing infection within the adolescent's sexual network. Compliance with partner notification is a difficult issue for adults with gonorrhoea but is especially problematic for adolescents where the male partner may be considerably older than the female and the partner networks very complex (Ford et al. 2002). As a strategy to increase partner treatment, Kahn and colleagues explored patient delivered partner therapy in adults at an urban STI clinic in Indianapolis (Khan et al. 2005). A substantial

number of the partners had infections different from or in addition to the infection in the index partner. Many of these would not have been diagnosed and treated if only patient delivered therapy was used. Furthermore, 26% of the partners were not infected with an STI – more common with *C. trachomatis* in the index partner-and hence would have received medication unnecessarily. Hence partner-delivered therapy has problems. Beyond partner notification, in most industrialized countries, cases of gonorrhoea must be reported to public health, i.e. as a notifiable disease. This is important for tracking the ongoing epidemic.

Treatment of gonococcal infection also includes counselling about safer sexual practices including abstinence, consistent condom use and partner selection (see Prevention of Gonococcal Infections). As the recommended antimicrobial therapies (Table 5) are effective if taken, routine follow up testing is not warranted if the adolescent has received treatment (American Academy of Pediatrics 2006; Public Health Agency of Canada, 2006a). However, given that the adolescent has already had one STI, i.e. gonorrhoea, he/she is at increased risk for recurrence or infection with this or another STI unless sexual practices are changed or abstinence is practiced. A 9 month study in adults diagnosed with gonorrhoea at a Baltimore STI clinic in 2003–2004 noted that the rate of reinfection with gonorrhoea was 13.8/100 person years (Bernstein et al. 2006), a rate far higher than in the unselected population. High-risk individuals or “core transmitters” contribute disproportionately to the gonorrhoea epidemic. Encouragement to seek regular testing may be important for this group.

15 Prevention of Gonococcal Infections in Adolescents

For adults, although many interventions have been found to be effective, few have been widely implemented or replicated (Manhart and Holmes 2005), (Golden and Manhart 2005). Studies of STI intervention and prevention strategies for adolescents are even more meagre (DiClemente and Crosby 2006). Behavioural change programs incorporating information, education and communication (including skills for condom use negotiation with a partner) are the most studied. Varying approaches have been taken including one-on-one, groups, school based, community based, as well as self-help. All work to some extent – but none are brilliant. The Society for Adolescent Medicine has noted that providing “abstinence only” or “abstinence until marriage” messages as a sole option for adolescents is flawed from both scientific and medical ethics viewpoints (Santelli et al. 2006). Scientific evidence supporting effectiveness of abstinence only programs are lacking. Oral antimicrobial prophylaxis has been shown to be a risky intervention as the risk of selection of antimicrobial resistant organisms outweighs the prevention benefit (Manhart and Holmes 2005) and it is not recommended.

A major problem for control of gonococcal infection in adolescents and adults is the lack of a vaccine. The microbe lacks the polysaccharide capsule of its cousin *Neisseria meningitidis* making vaccine development more difficult.

16 Summary

Gonococcal infections are now an uncommon problem in newborns in industrialized countries but remain a serious problem in developing countries due to ongoing high infection rates in pregnant women. Prompt diagnosis in the newborn with appropriate treatment can minimize sequelae. The mother and her partner(s) also require investigation and treatment.

Adolescents are a core group fuelling the ongoing gonococcal epidemic in industrialized countries. This is unlikely going to change unless sexual behaviour changes substantially. Education is a critical step along with access to more youth friendly STI care. As noted in the 2001 Institute of Medicine Report, learning about sex, sexuality and prevention of STI is a basic human right of adolescents (DiClemente and Crosby 2006).

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Management of Severe Dengue in Children

Christopher Moxon and Bridget Wills

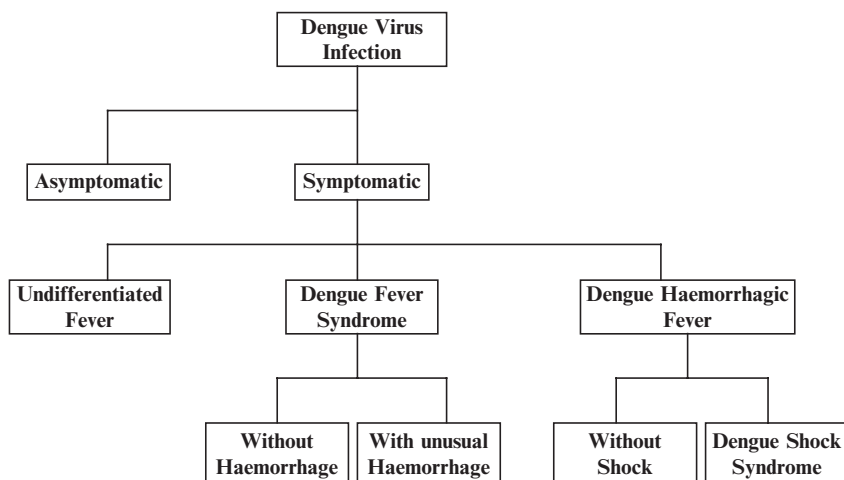
1 Introduction

Although recognised for over two centuries, during the last 50 years dengue has emerged as an important burden on global health, with significant increases in the number of cases reported from many sites in the tropics together with the spread of major disease epidemics across Asia and South America.

Dengue is a single stranded RNA virus of the *flavivirus* family. There are four closely related serotypes (DENV 1–4); infection with one serotype typically resulting in life-long immunity to that serotype but not to the remaining serotypes. Dengue is one of several arthropod-borne viral (arboviral) diseases. However, in contrast to most other arboviral infections (which are zoonotic i.e. they affect animal species predominantly), humans are the primary vertebrate hosts in the dengue transmission cycle. Although several mosquito species from the *Aedes* family can spread infection, the principal vector is *Aedes aegypti*, a day-biting, urban-adapted mosquito widely distributed throughout the tropics.

Three clinical syndromes are typically described: undifferentiated fever; dengue fever (DF); and a more serious form, dengue haemorrhagic fever (DHF), diagnosed using specific criteria (WHO 1997) (Fig. 1). Dengue is estimated to affect some 50–100 million people each year, with up to 500,000 cases of DHF reported to WHO annually (Gubler 1998). While previously regarded as distinct entities, a growing body of evidence implies that these syndromes are part of a continuum of mild to severe disease (Harris et al. 2000; Rigau-Perez and Bonilla 1999; Phuong et al. 2004; Deen et al. 2006). Although mild infection may be temporarily disabling, it is usually a self-limiting illness that is rarely life-threatening. Conversely patients with DHF/severe disease may progress to shock and in this group mortality may be high. No specific drug treatment is available and at present there is no effective vaccine.

In hyperendemic regions, particularly southeast Asia, children and adolescents are most likely to experience symptomatic disease and require hospitalisation, while in South America and other areas with lower transmission potential all age groups can be affected. In this chapter we review the clinical features of severe dengue infections in children and discuss important aspects of management, with the major focus on fluid resuscitation for shock. At the end of the chapter an algorithm is presented



Criteria necessary for diagnosis:-

Dengue Haemorrhagic Fever (DHF)

- 1) Fever or history of fever lasting 2-7 days
- 2) A haemorrhagic tendency shown by a positive tourniquet test or any spontaneous bleeding
- 3) Thrombocytopenia with a platelet count of 100×10^9 or less
- 4) Evidence of plasma leakage shown either by relative haemoconcentration or by the development of pleural effusions or ascites.

Dengue Shock Syndrome (DSS)

- 1) All the above features of DHF
- 2) Plus evidence of cardiovascular compromise due to leakage, indicated by narrowing of the pulse pressure to ≤ 20 mm Hg or hypotension for age with signs of impaired perfusion.

Fig. 1 Schematic diagram illustrating the major clinical manifestations of dengue infection as suggested by WHO (WHO, 1997)

describing a practical schedule for volume replacement for dengue shock syndrome (DSS) that is particularly relevant for use in resource limited environments.

2 Clinical Features of Severe Dengue Infection in Children

In children severe disease is usually characterised by increased vascular permeability and altered haemostasis resulting respectively in intravascular volume depletion and haemorrhage. In addition, children with dengue may present with severe hepatic or neurological disorders, but such presentations are relatively rare and will not be discussed further here.

The onset of illness in severe dengue is similar to that seen during uncomplicated infections. Symptoms begin around 5–8 days after an infected mosquito bite, typically with high fever, non-specific flu-like symptoms, vomiting, and sometimes rash and lymphadenopathy. Differentiation to severe disease usually occurs at around day 4–5 of

illness, often coinciding with defervescence; the development of increasingly severe upper abdominal pain and tender hepatomegaly are warning signs that the patient is likely to deteriorate. Depletion of intravascular plasma volume becomes apparent at this time, manifested initially by a rising plasma haematocrit without signs of cardiovascular decompensation. It is likely that a slow plasma leak has been present for some days, balanced by increased lymphatic return and compensatory cardiovascular, renal and adrenal mechanisms acting together in a coordinated response to maintain normal plasma volume. Eventually, these processes result in peripheral vasoconstriction and a rise in the diastolic pressure towards the systolic pressure so that the pulse pressure narrows; when this falls below ≤ 20 mmHg the patient is defined as having DSS (WHO 1997) (Fig. 1). A rapid weak pulse, impaired peripheral perfusion and lethargy or restlessness are usually also apparent by this stage, although interestingly some patients do not develop a significant tachycardia despite profound volume depletion. Thus the severity of plasma leakage may be underestimated if there is failure to monitor the pulse pressure, peripheral perfusion and haematocrit carefully during the critical period around the time of defervescence. If fluid resuscitation is not instituted promptly when the pulse pressure first narrows the signs of cardiovascular decompensation progress rapidly and irreversible shock and death may follow. However with appropriate volume replacement and good general supportive care most patients do well. Clinically detectable accumulation of leaked fluid in the pleural/peritoneal cavities and interstitium is rarely significant unless shock is profound or large volumes of parenteral fluid have been administered.

Haemorrhagic manifestations are said to be universal in patients with DHF, but are often limited to the presence of skin petechiae or minor bruising, or else a positive tourniquet test (defined as more than 20 petechiae visible in a 2.5 cm² area of skin on the volar aspect of the forearm after inflation of a standard sphygmomanometer cuff to between systolic and diastolic pressures for 5 min) (WHO 1997). Mucosal bleeding (usually epistaxis or gum bleeding) may occur but is rarely severe. Thrombocytopenia and minor haemostatic abnormalities are common, but the pattern observed in most cases is not suggestive of disseminated intravascular coagulation (DIC) (Wills et al. 2002), and thrombotic events are not seen despite quite marked haemoconcentration. Severe mucosal bleeding may be seen among the small number of children with very profound or prolonged shock, and in these cases there is usually evidence of associated multi-organ failure, metabolic acidosis and DIC. In adults mucosal bleeding is a more prominent feature, and rarely massive gastrointestinal bleeding causing haemorrhagic shock (as distinct from shock due to vascular leak) has been reported (Tsai et al. 1991; Chiu et al. 2005). The mechanisms underlying the bleeding seen in dengue, as well as the differences noted between infected adults and children have yet to be elucidated.

The increased vascular permeability and abnormal haemostasis are transient and usually resolve within 48–72 h of becoming clinically apparent. Spontaneous reabsorption of the excess fluid from the interstitial compartment begins at about day 6–8 of illness and progresses rapidly. A maculopapular rash may appear at around this time particularly in older children and adults. Sometimes this convalescent rash is extremely florid, with an intensely erythematous appearance interspersed with islands of pale skin. (Fig. 2) The rash fades after several days, although desquamation and pruritus may follow.



Fig. 2 Convalescent rash in a Vietnamese patient recovering from dengue

3 Pathogenesis

The pathogenesis of dengue is poorly understood and at present no suitable animal model exists to investigate the underlying disease mechanisms *in vivo*. Although dengue virus has been shown to infect and replicate in a wide range of cells of endothelial and epithelial origin *in vitro*, few data are available to establish the major sites of dengue virus replication *in vivo*. Cells of the macrophage/monocyte lineage do support infection, as do dendritic cells, but there is no evidence that the virus infects endothelial cells and only minor non-specific changes have been demonstrated in histopathological studies of the microvasculature (Bhamarapravati 1997; Rosen et al. 1999; Wu et al. 2000). However, an increase in microvascular permeability has been confirmed in children with DHF using strain gauge plethysmography (Bethell et al. 2001), an established non-invasive measurement technique (Gamble et al. 1993).

DHF/severe dengue typically occurs with second or subsequent infections, possibly as a result of antibody dependent enhancement; low levels of non-neutralising antibodies from a primary infection with one serotype are thought to cross-react with a new viral serotype during a subsequent infection, increasing uptake and viral replication within cells of the macrophage/monocyte lineage, and setting in train a cascade of immunopathological events (Halstead et al. 1970; Halstead and O'Rourke 1977). Mobilisation of serotype cross-reactive memory T cells has been suggested as an alternative mechanism triggering the inflammatory cascade and releasing vasoactive molecules (Mongkolsapaya et al. 2003). Other possible pathogenic mechanisms for which there are supporting data include differences in viral virulence (Rico-Hesse et al. 1997; Leitmeyer et al. 1999), molecular mimicry (Markoff et al. 1991; Chungue et al. 1994), and immune complex and/or complement mediated dysregulation (Bokisch et al. 1973; Petchclai and Saelim 1978; Wang et al. 2003). It seems likely that a complex interaction of several of these factors, together with host genetic factors, contributes to the overall disease phenotype. However, although a range of markers of immune activation have been found in association with severe disease during secondary infections (Bethell et al. 1998; Green et al. 1999), these response patterns do not differ significantly from those occurring in other viral infections which do not progress to a haemorrhagic shock syndrome. Secondly, as yet no specific pathway has been identified linking any of these immunopathogenic events with the profound derangements in microvascular permeability and haemostatic control that are seen.

In recent years considerable advances have been made towards understanding the mechanisms governing normal microvascular permeability, resulting in the currently accepted concept that intrinsic permeability is regulated by the endothelial surface glycocalyx as much as by endothelial cells themselves (Michel and Curry 1999). This highly anionic proteoglycan matrix is located on the luminal surface of the vascular endothelium, anchored in the plasma membrane of the endothelial cells, and forms an electrostatic barrier repelling negatively charged plasma proteins away from the endothelial surface and thus effectively restricting access to underlying cellular transport mechanisms. Structural examination of this barrier layer has proved to be extremely difficult and as a result its importance in regulating microvascular fluid flux has been rather neglected.

There is preliminary evidence to suggest that a transient disruption in the function of the endothelial glycocalyx layer occurs during dengue infection. A marked reduction in plasma proteins is seen and the severity of the hypoproteinaemia correlates with the severity of vascular leak (Wills et al. 2004). Molecular size and charge characteristics appear to determine which proteins are preferentially lost from the circulation; small proteins up to and including the molecular weight of albumin (MW 69,000 Da) are lost together with uncharged particles, consistent with a small but crucial change in the selective properties of the glycocalyx. In the few studies of dengue infected patients in which endothelial architecture has been examined, the absence of detectable abnormalities at the cellular level supports the idea that the pathological process occurs elsewhere. It is possible that the dengue virus, one of the dengue non-structural proteins produced during acute infection, or one of the many components of the immune response to infection, interacts directly with the

glycocalyx layer in such a way as to alter the characteristics of the fibre-matrix temporarily. Heparan sulfate, an important constituent of the structure to which the virus is known to adhere, may be involved in this process and there does appear to be a relative increase in urinary heparan sulfate excretion in children with severe dengue infection (Wills et al. 2004). New techniques to visualise the surface glycocalyx layer are becoming available and may prove interesting in this context.

4 Management – Theoretical Considerations

The only treatment currently available for symptomatic dengue infections is supportive. Desmopressin acetate has been used in a handful of patients with DSS with a view to preventing haemostatic abnormalities, but carries a risk of aggravating fluid overload due to its anti-diuretic properties (Pea et al. 2001). Corticosteroids have not been shown to improve either morbidity or mortality when given to patients with shock (Sumarmo et al. 1982; Tassniyom et al. 1993), nor has heparin even in cases of suspected disseminated intravascular coagulation (Srichaikul and Nimmannitya 2000). Similarly carabazochrome, an agent thought to influence haemostasis and vascular function, did not improve the severity of plasma leakage in a randomised trial (Tassniyom et al. 1997). However, in most of these studies formal evaluation was limited and the number of patients recruited small.

For patients with DSS prompt restoration of an adequate circulating plasma volume is essential. If appropriate fluid resuscitation is started at an early stage shock is usually reversible and, once the capillary leak has resolved, most patients recover rapidly. However, significant fluid overload with respiratory compromise is a well-recognised complication and one of the major contributors to mortality. Many issues need to be considered when choosing an appropriate fluid for resuscitation. Parenterally administered fluids distribute rapidly throughout the three main fluid compartments of the body, intravascular, interstitial and intracellular, according to specific physicochemical properties of the individual solutions (Griffel and Kaufman 1992). In essence, the sodium content determines the efficacy of crystalloid solutions for intravascular volume replacement and only isotonic crystalloid solutions (such as 0.9% saline or Hartmann's/Ringers Lactate preparations), which distribute primarily between the intravascular and interstitial compartments, should be used. Theoretically, colloid solutions offer advantages over crystalloid solutions, particularly in patients with increased vascular permeability (Haupt et al. 1992). Immediate distribution of colloid solutions is primarily within the intravascular compartment limited by the permeability of the capillary wall to the particular colloid molecules. In addition the colloid molecules increase plasma oncotic pressure thereby altering the balance of fluid flux across the endothelium and providing volume expansion in excess of the actual volume of fluid infused. In general small colloid molecules exert a relatively greater osmotic effect than larger molecules at the same concentration. However large molecules remain within the circulation for longer than small molecules and might be expected to have a more sustained effect.

The side effect profile of the different fluids available must also be considered; all colloid solutions can cause allergic reactions, most have adverse effects on haemostasis, an important consideration in patients with dengue, and small molecular weight preparations have been associated with the development of renal failure when used in patients with severe hypovolaemia.

In clinical practice a clear benefit of colloids over crystalloids for volume resuscitation has never been demonstrated and the issue remains contentious (Schierhout and Roberts 1998; Alderson et al. 2000; Webb 2000). However, recently, in a large and well conducted trial, albumin and 0.9% saline were shown to be equally effective for volume replacement in a heterogeneous population of adults requiring fluid resuscitation in an intensive care setting (Finfer et al. 2004). Post-hoc analysis also suggested a possible advantage associated with albumin use in the subgroup of patients with underlying sepsis. Despite the fact that fluid resuscitation is the critical intervention for most children with severe dengue, there have been very few studies addressing the issue of optimal fluid management. Three randomised blinded trials have been published comparing the effects of a variety of isotonic crystalloids and synthetic colloids in Vietnamese children with DSS (Dung et al. 1999; Ngo et al. 2001; Wills et al. 2005). To date the use of albumin preparations has not been evaluated in dengue, largely because they are expensive and rarely available in the parts of the world where dengue is common.

Collectively these three studies have shown that while early resuscitation with colloid solutions results in more rapid improvement in cardiovascular parameters and haematocrit levels, this effect is transient and in general does not alter the likelihood of a recurrence of shock during the critical period of ongoing leakage. The study by Ngo and colleagues did suggest that early treatment with colloid solutions might influence overall recovery in those with profound shock, but was statistically underpowered (Ngo et al. 2001). The largest study, involving more than 500 children with DSS, confirmed that for those with moderately severe shock Ringers Lactate was as effective as colloid therapy (Wills et al. 2005), but use of the crystalloid was not evaluated in the group with very profound shock because of safety concerns. Since there is evidence of a preferential leak of small molecules in DSS, larger molecular weight colloid preparations might be expected to offer advantages for resuscitation but in this study 6% Dextran 70 (average molecular weight 70,000Da, i.e. similar in size to albumin) and 6% HES (average molecular weight 200,000Da) performed equally well. However the dextran preparation was associated with significantly more allergic reactions.

From these studies and knowledge of the intrinsic properties of different fluid solutions, we can conclude that the majority of children with DSS can be managed successfully with isotonic crystalloid solutions. If a colloid is judged to be necessary (as discussed in the following section) a medium molecular weight preparation which combines good initial plasma volume support with good intravascular persistence and an acceptable side effect profile, particularly in relation to haemostasis, is probably the preparation of choice. Clinicians should become familiar with the use of one or two of the suitable preparations available locally. Further research is needed to determine whether early treatment with a colloid confers any advantage over crystalloid in those with severe or refractory shock.

5 Management – Practical Guidelines

For most patients hospital admission is unnecessary during the first 2–3 days of illness. Fever should be controlled with fans, tepid sponging and paracetamol. Aspirin and non-steroidal anti-inflammatory drugs are contraindicated because of the potential to cause gastritis and bleeding (and Reye's syndrome in the case of aspirin), as well as the established anti-platelet effects of these drugs in patients likely to have significant disease-related thrombocytopenia. For children with mild disease oral rehydration is usually sufficient, although for those unable to tolerate oral fluid, judicious use of parenteral fluids may be necessary. Persistent vomiting or severe abdominal pain, mucosal bleeding or severe skin bleeding/bruising, a rapidly rising haematocrit or a marked drop in the platelet count indicate the need for close observation on a high dependency ward where vital signs and haematocrit can be checked frequently particularly during the critical period around defervescence. Ideally children who develop shock should be managed in an intensive care facility, as should those with major gastrointestinal or other mucosal bleeding.

5.1 Fluid Resuscitation (*See the Suggested Algorithm in Fig. 5*)

For the majority of children with DSS, resuscitation should be started with an isotonic crystalloid solution at a rate of 10–20 mL/kg over 1 h. If the patient's clinical condition has stabilised after this time (wider pulse pressure, warm peripheries, a reduction in heart rate) the rate of fluid administration may be reduced to 10 mL/kg/h, and then gradually reduced to maintenance levels over the next 6–8 h. In most cases intravenous therapy can be stopped after approximately 24 h. Some children become transiently hypertensive in the first few hours after resuscitation, probably reflecting the degree to which intrinsic adrenal and other mechanisms have been stimulated by the severity of volume depletion prior to resuscitation (Fig. 3). Parenteral fluid therapy should not be stopped as these children are still relatively under-filled; if the rate of fluid administration is reduced the blood pressure settles spontaneously after a few hours.

If there is evidence of ongoing cardiovascular compromise after the first hour of treatment (no improvement in pulse pressure or pulse rate, persisting peripheral shutdown, a rising haematocrit) a colloid solution (see above for discussion about which colloid to use) should be substituted for the crystalloid solution, at an initial rate of 10–15 mL/kg over 1 h. After 1 h of colloid infusion, treatment should revert to the reducing schedule of isotonic crystalloid, provided the patient's condition has improved. Subsequently some patients may experience further episodes of cardiovascular decompensation and may require supplementary treatment with small infusions of 5–10 mL/kg of colloid (Fig. 4). However it must be remembered that all colloids influence coagulation to some degree, although clinically significant effects are unlikely with infused volumes of less than 20–25 mL/kg/day. In addition

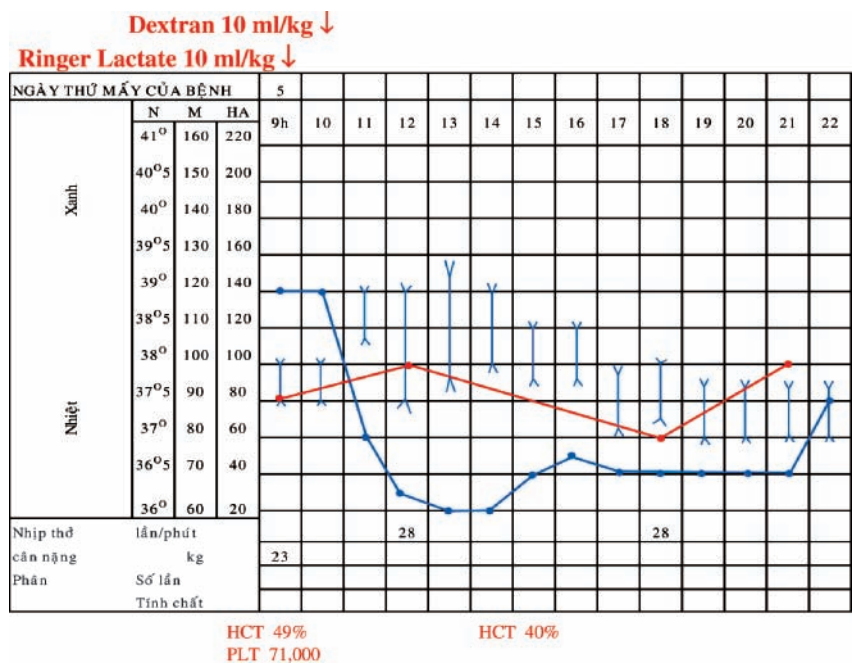


Fig. 3 Observation chart for an 11 year old boy Vietnamese boy admitted with DSS on day 5 of illness. Note the narrow pulse pressure (20mm Hg), tachycardia and haemoconcentration at admission. After 20mL/kg of parenteral fluid over 2 h he improved rapidly but note that his blood pressure was high for several hours immediately after the infusion. Subsequently he received maintenance IV fluid for 24h but required no further resuscitation

fluid overload exacerbated by leakage of intravenous therapy is a major contributor to mortality, particularly in endemic areas with limited access to ventilatory support. Frequent observations of vital signs, mental state and urine output, together with immediate access to repeated haematocrit measurements, are essential to assess the response to treatment and decide on further fluid therapy. In general the volume of parenteral fluid given should be kept to the minimum required to maintain critical organ perfusion during the phase of active leakage, and as soon as re-absorption begins the intravenous infusion should be stopped.

Patients with very profound shock at presentation (no recordable pulse or blood pressure with cold clammy skin) frequently receive a colloid initially although there are no formal research data to support this practice at present. Notwithstanding the initial severity, most of these patients do improve with aggressive initial volume replacement, and can be managed subsequently as suggested above. Supplementary treatment with one or two further small boluses of colloid is quite likely to prove necessary but a conservative approach to decision making is still essential. The small number of children who fail to improve despite these measures require high-level intensive care and management is likely to be complex and difficult. Inotropic

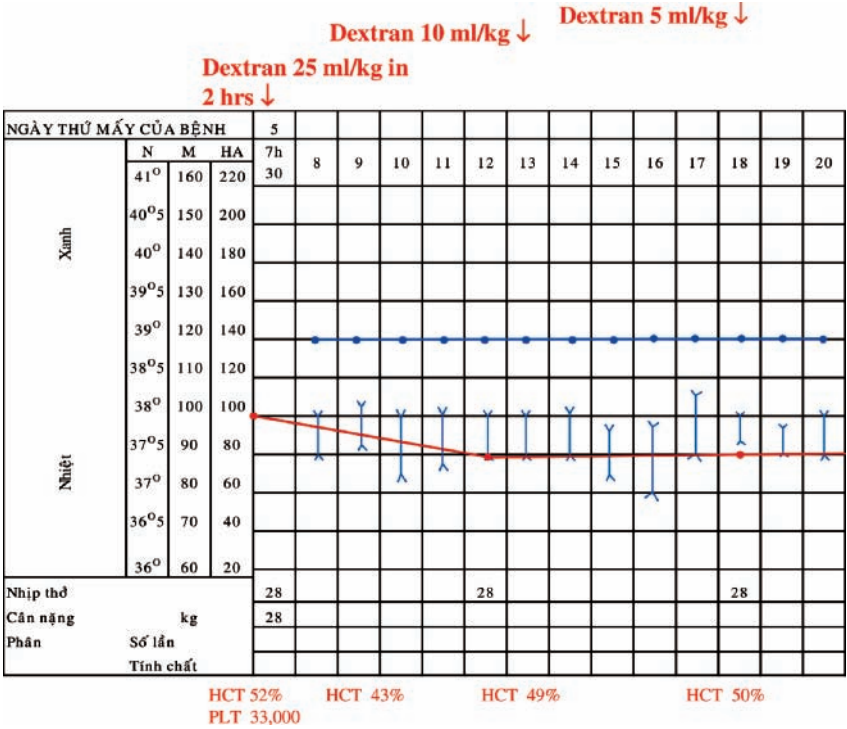
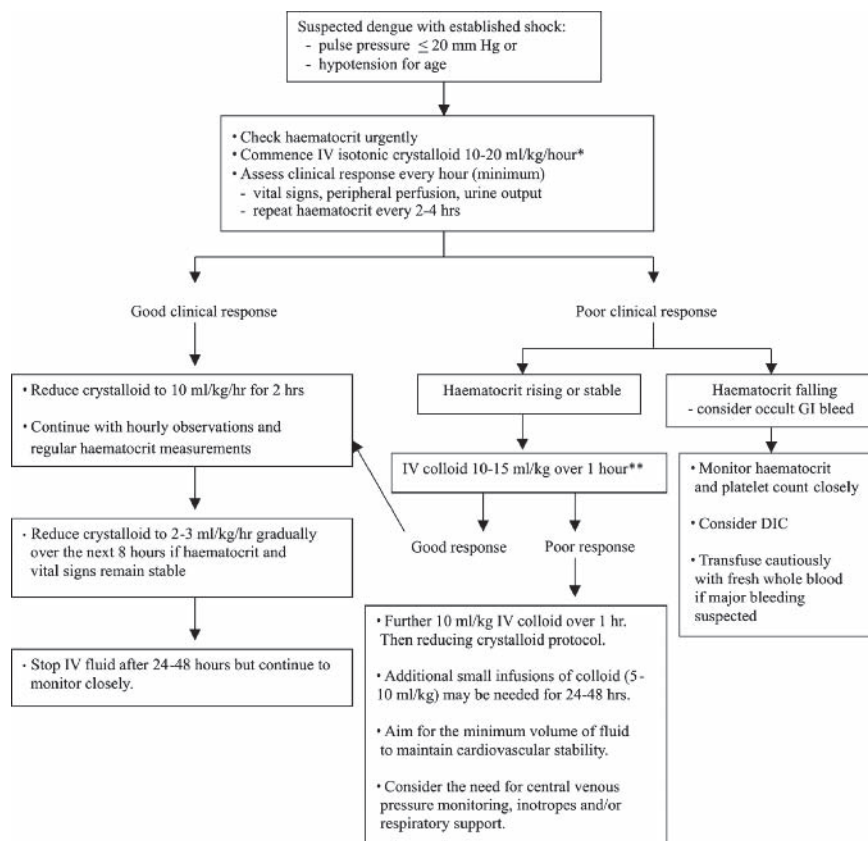


Fig. 4 Observation chart for a 10 year old Vietnamese girl admitted with severe DSS on day 5 (unrecordable pulse and blood pressure and marked haemoconcentration). She required repeated small boluses of colloid after the initial resuscitation but eventually made a good recovery and was discharged on day 8. After the initial resuscitation ongoing fluid therapy should be limited to the minimum necessary to maintain critical organ perfusion until the onset of re-absorption, since aggressive attempts to normalize cardiovascular and haematological indices are likely to result in significant fluid overload.

support is often necessary in addition to volume support. Significant pleural effusions and respiratory compromise are likely to develop and pleural and ascitic drainage, together with respiratory support in the form of nasal CPAP or assisted ventilation, may all prove to be necessary. Metabolic and electrolyte derangements are common and should be actively sought and corrected.

5.2 Management of Bleeding

For most patients routine inpatient observation with regular monitoring of the platelet count is all that is necessary, although nasal packing may be required for persistent epistaxis. Children with profound thrombocytopenia but without major bleeding should be managed expectantly with bed rest and protection from trauma.



* Use only isotonic crystalloid solutions such as Ringer's Lactate or normal saline.

** Use a medium molecular weight iso-oncotic colloid preparation e.g. 6% Dextran 70 or 6% Hydroxyethylstarch MW 200,000.

Fig. 5 Flow chart for suggested volume replacement for patients with dengue shock syndrome

Prophylactic platelet transfusions are not indicated and are likely to be harmful. Rapid recovery in the platelet count occurs during the second week of illness.

Major bleeding is almost always associated with very severe or prolonged shock, and is usually from the gastrointestinal tract. Contributing factors include profound thrombocytopenia, tissue hypoxia, metabolic acidosis and disseminated intravascular coagulation. Internal bleeding may not become apparent for many hours until the first melaena stool is passed. It should be considered in all those with DSS who fail to improve clinically after appropriate fluid resuscitation (see the suggested algorithm in Fig. 5), particularly if the haematocrit is stable or falling and the abdomen is distended and tender. Transfusion should be undertaken with extreme care because of the risk of fluid overload in these circumstances. In the event that major internal bleeding is suspected in a child with severe DSS, a small volume of fresh whole blood (5–10 mL/kg) should be given

over 1–2 h and the response observed. Further small transfusions may be given subsequently if there is a good clinical response and significant bleeding is confirmed. Use of platelet concentrates, fresh frozen plasma and other blood products should be guided by the platelet count and coagulation profile. Two small recent studies indicate that recombinant activated factor VII may be helpful in controlling life threatening bleeding, but further research is needed (Chuansumrit et al. 2004, 2005).

6 Summary/Conclusion

Dengue is a major global disease which, in its severe form, affects up to 500,000 people worldwide each year, most of whom are children. The development of a safe and effective vaccine is a clear priority, together with public health measures to prevent the spread of infection. However, while major epidemics continue to occur, clinicians must also focus on optimising management. Although no specific treatment is available at present, with good supportive care, mortality for children with DHF can be reduced to well below 1%. In patients without signs of shock, fluid replacement can be attempted orally, but in children with DSS parenteral treatment is essential. Very careful titration of fluid therapy is necessary combined with frequent reassessment for signs of worsening shock or the development of fluid overload. In most DSS cases isotonic crystalloid solutions are as effective as colloid solutions, but the question whether early intervention with colloid solutions improves outcome in more advanced shock requires further investigation. The outcome of studies to address this question, together with further research to examine the pathophysiological mechanisms underlying the plasma leakage, will hopefully result in better management of children with severe dengue but may also provide useful insights into other diseases that affect endothelial function.

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11

Human Papillomavirus Vaccines: Who Should Get Them and Why?

Stéphane Paulus and Simon Dobson

1 Introduction

In 2006, the first of two vaccines for human papillomavirus (HPV) prevention was approved for use in several countries. This has prompted great attention to be paid to HPV infections and their consequences. As the genital HPV types are acquired sexually, acceptability to the public of a vaccine to prevent a sexually transmitted infection (STI) will be an important issue in ensuring good uptake of the vaccine by those who would most benefit.

The HPV family consists of over 100 different double-stranded DNA viruses enclosed in a protein shell. Of these, approximately 40 can infect the genital tract (Stanley et al. 2006). HPV infections are transmitted sexually by direct epithelial (skin or mucosa) to epithelial contact and vertically to an infant exposed to the virus in the maternal genital tract; as well, transmission from oral mucosal contact in head and neck infections is likely.

HPV is capable of causing benign and cancerous anogenital disease as well as benign and malignant head and neck lesions. HPV infection is a necessary cause of cervical cancer. Globally, HPV-16 and -18 are associated with 70% of squamous cell carcinoma of the cervix and 86% of adenocarcinoma of the cervix. Six HPV genotypes (-31, -33, -35, -45, -52, and -58) account for an additional 20% of cervical cancers world wide (Clifford et al. 2006). Infection from HPV-16 and -18 is also implicated in cancers of the penis, anus, vulva and vagina as well as in squamous cell cancers of the conjunctiva, mouth, oropharynx, tonsils and larynx (Munoz et al. 2006).

HPV infection is followed in most cases by a slow onset cell mediated immune response. Low level neutralizing antibodies are detected in the serum coincident with lesion regression. Most HPV infections resolve spontaneously within 5–6 months for low-risk HPV types and 8–14 months for high-risk HPV types. A persistent infection is established if the immune response fails to clear or control the virus (Stanley 2006).

Cervical cancer appears to develop in a progressive fashion: usually mild dysplastic changes (cervical intraepithelial neoplasia 1 or CIN 1) evolve into severe dysplastic changes (CIN 2 and 3) and ultimately into carcinoma in situ (CIS) and,

if untreated, invasive squamous cell carcinoma (SCC). It can take anywhere from 1 to 10 years post infection for the development of precancerous lesions, and then another ten years for the development of invasive cervical cancer. Thus, there is a long latency period from infection to disease (Moscicki et al. 2006). The ability of Papanicolaou (Pap) smear screening to detect cervical dysplastic changes prior to the development of carcinoma has led to dramatic reductions in invasive cancer in the developed world (Kitchener et al. 2006). Even with effective vaccine programs, until close to 100% coverage can be achieved for all oncogenic HPV types this ability to detect pre-invasive disease will remain critically important.

While HPV infection is a necessary cause of cervical cancer, other cofactors have been implicated in cervical cancer pathogenesis. These include smoking, other sexually transmitted infections (*Chlamydia trachomatis*, Herpes Simplex Viruses and HIV), and hormonal factors, such as pregnancy and long term OCP use (Cogliano et al. 2005; Munoz et al. 2006).

Infection with HPV-6 and -11 is responsible for over 90% of the genital warts (Lacey et al. 2006). A more infrequent complication of HPV-6 and -11 infection is recurrent respiratory papillomatosis (RRP). It is characterized by recurrent warts or papillomas in the upper respiratory tract, particularly the larynx. Malignant transformation occurs in 3–5% of RRP patients and is associated with the presence of HPV-16 and -18. RRP can result in substantive morbidity as the papillomas can enlarge and result in respiratory compromise. This is managed by repeat laryngoscopy and bronchoscopy for wart removal/debulking every few weeks to months (Shykhon et al. 2002).

2 HPV Epidemiology

HPV is often described as the most common sexually transmitted infection (STI). Acquisition of HPV occurs rapidly following sexual debut. While the overall prevalence of HPV infection ranges from 11% to 29%, the peak prevalence tends to occur in adolescents and young adults (25 years of age or younger) (Clifford et al. 2006).

A prevalence study in British Columbia (BC) conducted in 2004–2005 provides the most recent Canadian data (Dobson et al. 2007). The overall prevalence of the HPV-16 and -18 was 11.6% while in women less than 20 years of age, the prevalence of these two genotypes was 16.7%. Women less than 20 years of age also had the highest prevalence rates of any HPV (26.9%), and any of high risk HPV (20.6%). HPV incidence rates have been examined in Ontario, and the highest rates were found in women of ages 15–19 years (25.0%), followed by those in 30–34 year age group (14.7%) (Sellors et al. 2002).

HPV prevalence estimates for women in countries around the world range from 2 to 44%, depending on the geographic region, population sampled and testing methodology (Bosch and de Sanjosé 2003). A peak prevalence of HPV infection in women <25 years of age has been demonstrated consistently, with a decreasing

prevalence with age thereafter. Herrero et al. (2000) found that among women <25 years of age, oncogenic HPV types predominated, whereas in women >55 years, non-oncogenic and uncharacterized types were the most common. A second peak in HPV prevalence among older women has also been found in some studies (Herrero et al. 2000; Sellors 2002), but this has not been seen consistently.

Other risk factors for HPV infection include behavioural factors that increase probability of exposure to HPV (eg, number of sexual partners, early age of first intercourse, never being married, never being pregnant), endogenous factors (immunosuppression secondary to HIV, transplant, etc), and factors that relate to the cervical microenvironment (sexually transmitted infections) (Winer et al. 2003).

3 Cervical Cancer Epidemiology

Cervical cancer is estimated to be the second most common malignancy affecting women worldwide. In 2005, approximately 1 million women were estimated to have cervical cancer and more than 250,000 deaths were attributed to the condition world wide (WHO 2005). Older women in developing countries suffer disproportionately from cervical cancer, with an estimated incidence rate in 2005 of 70 per 100,000, and an estimated mortality rate of 60 per 100,000, among women aged 70 years and older. Canadian cervical cancer incidence and mortality rates have declined since the 1970s (Fig. 1). Declines can be attributed to the success of pap cytology screening efforts beginning in the 1960s.

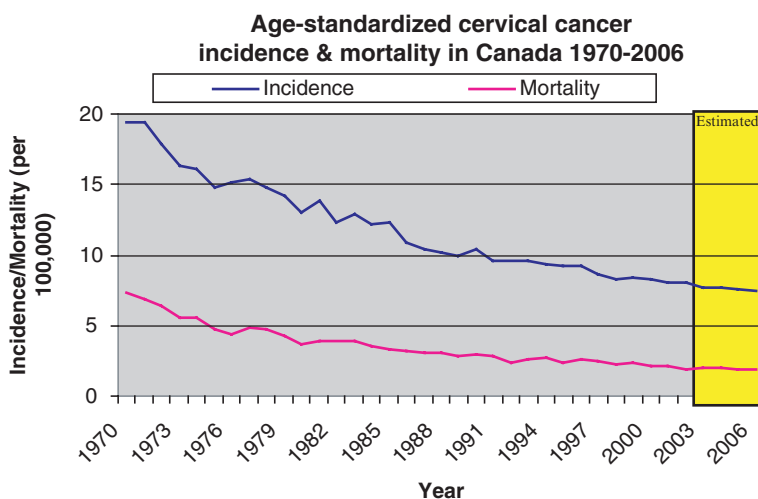


Fig. 1 Age-standardized cervical cancer incidence and mortality in Canada 1,970–2,006 (Source: Centre for Chronic Disease Prevention and Control, Public Health Agency of Canada)

Table 1 Sexual activity by age

Age	Grade	Males (%)	Females (%)	Reference (Dobson et al. 2007)
≤11	6	1.1	1.1	Canadian Community Health Survey
		4	1	Health Behaviours in School Aged Children
12	7	3.7	3.3	Canadian Community Health Survey
13	8	9.0	9.0	Canadian Community Health Survey
14	9	23	19	Canadian Youth, Sexual Health and HIV/AIDS Study
		19	17	Health Behaviours in School Aged Children
15	10	27	25	Health Behaviours in School Aged Children
16	11	40	46	Canadian Youth, Sexual Health and HIV/AIDS Study

4 Age of Sexual Debut

As HPV is a sexually transmitted infection with the highest incidence and prevalence rates documented in people younger than 20 years of age, it is important to consider the age of sexual debut in order to choose the age for program implementation. Several Canadian surveys have determined that a significant proportion of children are sexually active by the time they reach high school. Table 1 synthesizes the results from these surveys.

5 Education/School Leaving

Another important consideration for selecting the age group for a school based vaccination program is the age at which children leave school. While the proportion of children who leave junior high school at each age strata is unclear, a survey of street youth reveals that 9.6% of females and 12% of males left school before grade 7, and 20–21% of street youth left school by grade 8 (Dobson et al. 2007).

6 HPV Vaccines: Nature and Characteristics of Immunizing Agent

The current HPV vaccines consist of L1 proteins of HPV manufactured with the use of recombinant technology. The L1 proteins self assemble into empty non infectious virus-like particles (VLPs).

Gardasil™ is a quadrivalent HPV-6, -11, -16, -18 L1 VLP vaccine developed by Merck and Co. This vaccine targets two high-risk oncogenic HPVs that are associated with the majority of cervical cancers and two low-risk HPVs that are associated with over 90% of genital warts.

Cervarix™ is a bivalent HPV-16, -18 L1 VLP candidate vaccine developed by GlaxoSmithKline Biologicals. This vaccine targets two high-risk HPVs that are associated with causing 70% of the world's cervical cancers.

Both vaccines are prophylactic vaccines and produce a virus neutralization antibody response; thus they are indicated for prevention of infection from respective HPV serotypes and their associated diseases. They are not therapeutic vaccines and are not effective in disease modification once the HPV infection has been acquired.

Gardasil™ is approved for use in females 9–26 years of age. It is recommended to be administered using a 3 dose schedule at 0, 2 and 6 months. Cervarix™, in its pre-licensure studies is also administered on a 3 dose schedule at 0, 1 and 6 months.

Antibodies are the primary mode of protection from HPV infection. HPV VLPs are highly immunogenic even in the absence of adjuvants (Stanley et al. 2006). Both vaccines induce a robust humoral response in the vaccine trials with antibody levels that are sustained at or above those induced by natural infection. Post vaccination, neutralising antibodies have also been detected in cervical secretions (Harper et al. 2006).

Immunogenicity and 5 year efficacy data are available for Gardasil™ (Villa et al. 2005, 2006a). 100% of the subjects in the vaccine arm seroconverted at month 7 with GMTs that were approximately 7-fold (HPV-11), 11-fold (HPV-6), 19-fold (HPV-18) and 105-fold (HPV-16) higher than unvaccinated women with prior natural infection at day 1. Antibody levels dropped from a peak of 7 months and reached a plateau at 18 months. The antibody titres at 18 months were sustained to approximately the same levels at 5 years of follow up. A report documenting HPV immunogenicity outcomes over a 5.5 year follow-up for Cervarix™ should be available in the near future.

Immunogenicity bridging data are available for boys and girls 10–15 years of age (Dubin et al. 2005; Block et al. 2006). The boys and girls mounted a strong immune response to all four HPV VLPs of the quadrivalent vaccine. The GMTs for girls were 1.7–2.0 times higher than the control group, and the GMTs for boys were 1.8–2.7 times that of the control.

At 5 years, Gardasil is highly effective (95.6%, CI, 83-98) against preventing persistent infection from the genotypes covered in the vaccine (Villa et al. 2006b). Vaccine is also highly effective against prevention of dysplastic lesions that are precursors to cervical carcinoma in situ. Over the 5 years of follow up, no cases of CIN or condyloma were identified in the vaccinees while 3 cases of CIN 1–3 and 3 cases of condyloma were identified in the placebo group yielding a disease rate of 0.8/100 women-years at risk and a vaccine efficacy against all disease of 100% (95% CI, 12.4-100) (Villa et al. 2005, 2006b). Similarly highly effective results are obtained for Cervarix™ against HPV-16 and HPV-18 persistent infection (100%, CI, 52.2-100) and cancer precursors CIN2 + (100% CI, 7.7-100) ((Harper et al. 2004, 2006). In the Cervarix™ trial, significant cross-protection has been documented against infection from HPV-45 (vaccine efficacy of 94%), and some protection against HPV-31 (vaccine efficacy of 54%).™. (Harper et al. 2006) HPV-45 and -31 are estimated to cause 7–10% of cervical cancers (Clifford et al. 2006).

Phase three studies enrolling thousands of participants and of much longer duration are now underway to investigate vaccine efficacy against more severe disease outcomes (CIN 2+).

7 Vaccine Safety

Gardasil™ is safe and well tolerated. Local injection site reactions included pain, redness or swelling and were reported with a slightly higher frequency (6–8%) among vaccine recipients than among the placebo group. The majority (94%) of injection-site reactions reported in female recipients of Gardasil™ were mild to moderate in intensity. Systemic adverse events such as headache or fatigue were reported by a similar proportion of subjects in the vaccine and placebo arm (Villa et al. 2005). Cervarix was also well tolerated with a similar profile of safety (Harper et al. 2004).

8 HPV Vaccine Implementation

HPV vaccines offer an opportunity to prevent cervical cancer in women. Their usefulness will be maximized by the provision of vaccine before the age of sexual debut. The rationale behind the implementation of mass vaccination varies depending on the part of the world in which it is implemented. In the developed world, the use of the vaccine would:

- Further reduce incidence of cervical cancer
- Reduce medical, psychological, economic costs of abnormal Pap tests, and the dysplastic changes that are the precursors of cervical cancer
- Reduce the incidence of genital warts

In the developing world where there is less access to systematic cervical cancer screening, the impact of the vaccine would even be greater through:

- Substantial reduction in rates of cervical cancer
- Reduced morbidity from genital warts
- Bypassing the need for complex medical protocols

Efficacy of HPV vaccines in males is still under study. It seems unlikely that recommendations will be made for immunizing males until such information is available. This would inform mathematical modeling to determine how quickly differing immunization strategies would alter HPV transmission dynamics and cervical cancer rates.

Recent recommendations that females between the ages of 9 and 13 years in Canada (Dobson et al. 2007) receive HPV vaccines will likely be implemented in a school setting as there is a long history of school-based immunization programs.

Critical to the success of such programs will be the attitudes of physicians and parents toward the vaccine. Several studies have been published looking at the perception of both health care providers and the public towards vaccines against HPV. Kahn et al. looked at the attitudes of a random sample of American Academy of Pediatrics (AAP) members ($N = 513$) and found that 74% intend to recommend HPV vaccines overall (Kahn et al. 2005). The intention to immunize was higher for: younger versus older teens, female versus male teens, cervical cancer/genital wart versus cervical cancer vaccine. The factors predicting intention were related to:

- Older age, male gender of the Pediatrician
- Knowledge about HPV
- Clinical experience with young adolescents
- Attitudes: endorsement by professional organizations, fewer perceived barriers

Similarly, Riedesel et al. (2005) asked for the opinion of a random sample of American Academy of Family Practice (AAFP) members ($N = 145$). They found that 74% intend to recommend HPV vaccines, but that they would be reluctant to immunize younger, male teens. The factors predicting intention were:

- Female provider
- Knowledge about HPV
- Attitudes: endorsement, fewer barriers to administration

When it comes to the general population 74–89% would agree to vaccination (Davis et al. 2004). The key determinants of intention are related to knowledge about HPV and beliefs about vaccines in general, as well as recommendations made by the medical profession. Davis et al. have also demonstrated that after an educational intervention, a significant number of undecided parents would become willing to give the vaccine. The key factors for a parent to give vaccines to their child include:

- Desire to protect their child from harm
- Beliefs about HPV-related disease: HPV-related disease is serious and child will be susceptible to HPV
- Attitudes about vaccines in general and HPV vaccine in particular
- Physician recommendation
- Personal experience with STIs, abnormal Pap tests and genital warts

Education will be a key in the process of implementing the vaccine programs. For health care providers, the education about HPV-related diseases and vaccines will be critical to help them provide effective counseling. For the parents and teens, educational resources will be needed to help them understand HPV and the benefits and limitations of vaccination. Public health policy will need to address the need for education. It will need to work closely with Pediatricians, Gynecologists and Family Practitioners to achieve this. The statements on the use of HPV vaccines produced by the Societies representing these physician groups should be as consistent as possible. The focus of education should be on modifiable predictors of intention to vaccinate and will aim to increasing knowledge about HPV and HPV

vaccines, addressing perceived barriers and endorsement by professional organizations.

Since the current vaccines can only prevent approximately 70% of the HPV-caused cervical cancers, the message to immunized females should be that they still need to partake in cervical cancer screening programs in the same way as they would have done if they were unimmunized. How widespread use of the vaccines might allow screening programs to change will be a question requiring careful study in the years to come.

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12

Antimicrobial Resistance among Enteric Pathogens

Larry K. Pickering

1 Introduction

Each year diarrheal disease results in significant morbidity and mortality worldwide. In the United States diarrhea is associated with 1.5 outpatient visits, 200,000 hospitalizations and approximately 300 deaths per year. Worldwide in children under 5 years of age, over 1.5 billion diarrhea episodes and approximately 2 million deaths occur annually, although mortality due to diarrhea has been reduced significantly worldwide by oral rehydration therapy (King et al. 2003). Enteric infections generally are self-limited conditions that require fluid and electrolyte therapy, but with certain bacterial and protozoal agents, antimicrobial therapy should be considered. Antimicrobial agents should be prescribed with an appreciation of their limitations, specifically the potential for development of bacterial resistance which occurs following widespread use of antimicrobial agents in humans and animals (Johnson et al. 2006; Gallay et al. 2007). Because resistance among enteric pathogens can spread rapidly, constant monitoring of susceptibility patterns of bacterial isolates is critical for selection of appropriate antimicrobial agents for therapy when indicated (Centers for Disease Control and Prevention, NARMS 2006). The purpose of this article is to review limitations of and indications for antimicrobial therapy of enteric infections with emphasis on antimicrobial resistance.

2 Advantages of Antimicrobial Therapy

The use of antimicrobial agents in humans for many conditions is widespread including therapy of children with diarrheal disease. Antimicrobial agents will not benefit children with acute infectious diarrhea associated with viral enteropathogens, which are a major cause of diarrhea and dehydration in young children. In children and adults with other forms of acute infectious gastroenteritis, use of specific antimicrobial therapy should be limited to well-defined bacterial and protozoal

Table 1 Categories of antimicrobial therapy for bacterial enteric pathogens

Categories	Organisms
Established benefit	<i>Clostridium difficile</i>
	ETEC and EIEC
	Salmonella species
	Shigella species
	<i>Vibrio cholerae</i>
Limited or unknown benefit	Aeromonas
	<i>Campylobacter jejuni/coli</i>
	Intestinal salmonellosis
	<i>Yersinia enterocolitica</i>

ETEC = enterotoxigenic *Escherichia coli*; EIEC = enteroinvasive *E. coli*

Table 2 Indications for antimicrobial therapy for children and adults with acute infectious gastroenteritis

Reduce signs, symptoms and duration of disease
Prevent mortality
Prevent sequelae
Eradicate fecal shedding of the causative organism
Prevent transmission of enteric pathogens

agents (Table 1). Indications for use of antimicrobial agents for treatment of children with acute infectious gastroenteritis are shown in Table 2.

3 Limitations and Challenges of Antimicrobial Therapy

Although benefits of antimicrobial therapy can be achieved for treatment of people infected with certain bacterial enteric pathogens, antimicrobial therapy is not always appropriate or beneficial and may be associated with certain limitations. These include complexity and cost of identifying causative organisms; safety and tolerability of antimicrobial agents, particularly in young children, the elderly, immunocompromised people and during pregnancy; potential for enhancement of virulence properties; prolongation of the carrier state; and development of resistance. Therapy of people with gastroenteritis due to Salmonella may result in an increase rate of relapse, increase in risk for a positive culture after 3 weeks, and increase risk for adverse drug reactions with no decrease in length of illness (Sirinavin and Garner 2000). Antimicrobial therapy of infants and children with hemorrhagic colitis due to shiga toxin producing *E. coli* (STEC) has been reported to increase development of hemolytic uremic syndrome (HUS), although this association is debatable for STEC (Ohara et al. 2002; Safdar et al. 2002) as well as *Shigella dysenteriae* type 1 (Bennish et al. 2006).

4 Reasons for Mechanisms of Resistance

The reason for increasing resistance among enteric bacterial pathogens is multifactorial. Excessive and inappropriate use of antimicrobial agents in humans for proven or presumed infections of many organ systems including the gastrointestinal tract has been well documented. This use occurs in part because of inappropriate prescribing practices and in part because of misguided beliefs and expectations of patients and parents who often lack an awareness of the dangers of antimicrobial use (Eng et al. 2003.) A well-documented risk factor for infection or colonization with resistant bacterial pathogens is recent antibiotic use, particularly within 4 weeks before exposure (Magee et al. 1999; Eng et al. 2003). The emergence and spread of resistant enteric organisms occurs because of mutations in common resistance genes, exchange of genetic information among microorganisms, proliferation and spread of multiply resistant bacterial clones, and selective pressure in communities and hospitals that facilitate development and spread of resistance.

Many classes of antimicrobial agents used as antimicrobial growth promoters in diets of livestock and poultry also are used in humans (Angulo et al. 2004). Studies have demonstrated an association between antimicrobial growth promoters and resistant enteric pathogens in humans. Because of the potential for harm to humans, use of antibiotic growth promoters that belong to an antimicrobial class used in humans should be regulated (World Health Organization 1999; Wedel et al. 2005; Gallay et al. 2007).

5 Resistance Patterns of Enteric Pathogens

There has been a progressive increase in antimicrobial resistance patterns of major bacterial enteric pathogens associated with enteric disease in many countries. These resistance patterns are influenced by geographic location, year isolate obtained, class of antimicrobial agent, pressure exerted by antimicrobial agent use, and source of the isolate tested. Studies from both economically developed and developing countries have reported resistance to bacterial enteric pathogens including *Campylobacter jejuni/coli*, *Shigella* species, *Salmonella* species, STEC and *Clostridium difficile*. In many geographic areas, resistance patterns have demonstrated a consistent increase over time and have exhibited resistance to several classes of antimicrobial agents. Antimicrobial resistance patterns of *C. jejuni/coli*, *Shigella* species, and *Salmonella typhi* and non-typhi *Salmonella* are shown below.

5.1 *Campylobacter jejuni/coli*

Most patients with *Campylobacter* infections of the intestinal tract do not require antimicrobial therapy since infections generally are self limited. However, there are specific clinical circumstances in which antimicrobial therapy should be considered

including extraintestinal infection, infection in an immunocompromised host, bloody stools, high fever and prolonged illness. Although *C. jejuni* strains generally are susceptible to a wide variety of antimicrobial agents, erythromycin, azithromycin or fluoroquinolones represent the agents of choice for therapy.

Erythromycin resistance in economically developed countries including the United States is generally stable at less than 5% (Gaudreau and Gilbert 1998; Talsma et al. 1999; Engberg et al. 2001; Feierl et al. 2001; Gupta et al. 2004), whereas higher resistance has been reported from some countries including Nigeria (Coker and Adefeso 1994), Thailand (Hoge et al. 1998; Sanders et al. 2002), Spain (Saenz et al. 2000), Taiwan (Li et al. 1998) and Canada (Gaudreau and Gilbert 2003). Strains of *C. jejuni* and *C. coli* that show high level resistance to erythromycin appear also to be resistant to clarithromycin and erythromycin (Taylor and Chang 1991; Hoge et al. 1998;). A cluster of 11 erythromycin and ciprofloxacin resistant *C. jejuni* cases was reported from Quebec, Canada (Gaudreau and Michaud 2003).

Studies of human isolates of *C. jejuni/coli* have shown a marked increase over time in resistance to fluoroquinolones in countries throughout the world (Table 3) (Gaudreau and Gilbert 1998; Smith et al. 1999; Talsma et al. 1999; Feierl et al. 2001; Melby and Mannsäker 2001; Nachamkin et al. 2001; Gaudreau et al. 2003; Hakanen et al. 2003; Krausse and Ullmann 2003). In several countries the increase in resistance to fluoroquinolones coincided with initiation of administration of a

Table 3 *Campylobacter jejuni* resistance to ciprofloxacin by time and country

Country	Period	Resistance %		Difference %	
		Initial	Year 2000	%	
Germany	1980–1982	0			
Krausse and Ullmann (2003)	1977–2001		30	30	
Austria	1996–2000	25	40	15	
Feierl et al. (2001)					
USA	1995–2000	<10	36	26	
Nachamkin et al. (2001)					
Norway	1988–2000	6	36	30	
Melby and Mannsäker (2001)					
Finland ^a	1995–1997	40			
Hakanen et al. (2003)	1998–2000		60	20	
Canada	1985–1986	0			
Gaudreau and Gilbert (1998)	1992–1993	4			
	1995–1997		13	9	
Canada	1998–2001	10	47	37	
Gaudreau et al. (2003)					
USA	1992–1998	1	10	9	
Smith et al. (1999)					
Netherlands ^b	1994–1997	11	29	18	
Talsma et al. (1999)					

^aForeign isolates

^bTested against ofloxacin

fluoroquinolone to animal food or use in veterinary animals (Smith et al 1999; Talsma et al. 1999; Engberg et al 2001; Gupta et al. 2004; Gallay et al. 2007). A high frequency of cross-resistance has been detected among the fluoroquinolones (Gaudreau and Gilbert 1998; Smith et al. 1999; Gaudreau et al. 2003; Krausse and Ullmann 2003).

In strains isolated from 1980–1990 in the US, there was no *C. jejuni/coli* resistance to fluoroquinolones (Centers for Disease Control and Prevention, NARMS 2006), but in 2001 ciprofloxacin resistance was 19% (Gupta et al. 2004). A study conducted in Minnesota reported an increase in fluoroquinolone resistance from 1% in 1992 to 10% in 1998 (Smith et al. 1999). Data from the National Antimicrobial Resistance Monitoring System (NARMS) (Centers for Disease Control and Prevention, NARMS 2006) from 1997 to 2003 showed a slight increase in resistance to ciprofloxacin in *C. jejuni* isolates (Centers for Disease Control and Prevention, NARMS 2006). The percentage of *C. jejuni/coli* isolates resistant to ciprofloxacin was 13% in 1997 and 18% in 2003. In 2003 less than 1% of the isolates were resistant to erythromycin or azithromycin. *C. coli* strains generally are less susceptible to antimicrobial agents than *C. jejuni* (Saenz et al. 2000; Engberg et al. 2001; Gallay et al. 2007).

5.2 *Shigella* Species

Shigella strains have become progressively resistant to multiple antimicrobial agents, initially to sulfonamides, shortly after they became commercially available, then to tetracycline, chloramphenicol, and streptomycin less than 10 years after each was introduced, and subsequently to ampicillin, kanamycin, and TMP-SMX (Murray 1989; Griffin et al. 1989; Replogle et al. 2000; Jain et al. 2005). In Oregon 59% of *Shigella* isolates were resistant to trimethoprim-sulfamethoxazole (TMP-SMX) and 63% were resistant to ampicillin (Replogle et al. 2000). Similar resistance patterns have been reported from England and Wales (Cheasty et al. 1998), Canada (Harnett 1992) and Germany (Aleksic et al. 1993).

In 1999 through 2003 in the NARMS data, resistance of *Shigella* isolates to ampicillin was approximately 80% each year and to TMP-SMX resistance was approximately 40% per year. Of the *Shigella* isolates tested in 2003, 88% were *S. sonnei* and 10% were *S. flexneri*. Among the 495 *Shigella* isolates, 91% were resistant to one or more antimicrobial agents and 23% to 5 or more antimicrobial agents. Ampicillin resistance occurred in 79% of isolates, TMP-SMX in 38% and, tetracycline in 29%. None of the isolates were resistant to ceftriaxone, imipenem, gentamicin or ciprofloxacin. Susceptibility testing against azithromycin was not performed. Interpretation of in vitro susceptibility for azithromycin is difficult because there are inadequate data correlating azithromycin minimal inhibitory concentration (MIC) to clinical efficacy for treatment of shigellosis as well as difficulty caused by the dual-zone phenomenon that occurs with the E test and disk diffusion methods (Jain et al. 2005). Neither ampicillin nor TMP-SMX should be

considered appropriate empiric therapy for shigellosis because of high levels of resistance. Recommended therapy of people infected with *Shigella* includes fluoroquinolones, azithromycin and extended cephalosporins (Guerrant et al. 2001; American Academy of Pediatrics 2006).

5.3 *Salmonella* Species

The type of syndrome produced by *Salmonella* spp. dictates selection and duration of antimicrobial therapy. Antimicrobial agents have been shown to prolong symptoms and increase risk of complications among people who are non-typhoidal *Salmonella* carriers or in patients who have mild gastroenteritis. Randomized studies have demonstrated no difference between treated and untreated patients (Sanchez et al. 1993). Antimicrobial therapy may convert intestinal carriage to systemic disease with bacteremia, produce a bacteriologic and symptomatic relapse, encourage development or selection of resistant strains, or prolong fecal excretion (Sirinavin and Garner 2000). Antimicrobial resistance to *Salmonella* strains is common (Threlfall and Ward 2001; Vahaboglu et al. 2001; Molbak et al. 2002; Villa et al 2002; van Duikeren et al. 2003;). Food animals may be the primary reservoir of multidrug-resistant *S. typhimurium* (Wedel et al. 2005)

Table 4 shows the five most commonly identified non-typhi *Salmonella* serotypes identified by NARMS in 2003. The two most commonly identified serotypes were typhimurium (22%) and enteritidis (14%). The 20 most common serotypes accounted for 83% of isolates that were serotyped. Of the 1873 isolates tested for susceptibility, 23% were resistant to one or more antimicrobial agents and 11% were resistant to five or more agents with 30% of typhimurium and 22% of Newport isolates showing resistance to 5 or more antimicrobial agents. The antimicrobial agents to which the isolates showed the highest prevalence of resistance were tetracycline (16%), sulfamethoxazole (15%), streptomycin (15%) and ampicillin (14%), and chloramphenicol (10%). Only 2% of isolates were resistant to TMP-SMX, and 8 isolates (0.4%) were resistant to ceftriaxone and 3

Table 4 The five most common non-typhi *Salmonella* serotypes, NARMS, 2003

Serotype	Number (%)	Resistant (%) ≥ 5 drugs
Typhimurium	403 (22)	30
Enteritidis	257 (14)	0.4
Newport	222 (12)	22
Heidelberg	96 (5)	5
Javiana	85 (5)	0

$\geq 85\%$ of isolates from stool

(0.2%) to ciprofloxacin. From 1996 to 2003 in the United States, resistance to nalidixic acid increased from 0.4% to 2.3% (Stevenson et al. 2007)

There were 334 *Salmonella typhi* isolates tested as part of NARMS in 2003. Of these isolates, 26% were resistant to one or more antimicrobial agents with the most common resistance being to chloramphenicol (17%), tetracycline (16%), TMP-SMX (17%), ampicillin (16%) and streptomycin (14%). One of the *S. typhi* isolates was resistant to amoxicillin-clavulanic acid, ceftriaxone and ciprofloxacin.

6 Effect of Resistance on Clinical Manifestations and Treatment Options

Clinical manifestations of enteric infections include signs and symptoms involving the gastrointestinal tract, neurologic manifestations, dissemination of organisms to sites outside of the gastrointestinal tract and immune mediated sequelae. People infected with enteric pathogens which are resistant to frequently used antimicrobial agents may manifest as either clinical or bacteriologic treatment failures with longer duration of diarrhea and increase in adverse events (Helms et al. 2002, 2005; Nelson et al. 2004; Varma et al. 2005) and may have an extended duration of excretion of viable organisms (Bhutta et al. 2000; Sirinavin and Garner 2000).

7 Future Directions

Resistance of bacterial enteric pathogens has shown a progressive increase over time in many areas of the world. Approaches to minimize development of antimicrobial resistance and optimize therapy include educational interventions for physicians and parents, appropriate use of antimicrobial agents, reducing use of growth promoters in animal feed, optimizing infection control measures to prevent spread of resistant organisms, development and use of rapid diagnostic techniques to optimize therapy, use of non-absorbable antimicrobial agents, use of non-antibiotic therapeutic and preventive measures including receptor blockers, and development and use of enteric vaccines. Table 5 shows web sites containing information on antimicrobial resistance.

The findings and conclusions in this paper are those of the author and do not necessarily represent the view of the Centers for Disease Control and Prevention.

Table 5 Antimicrobial resistant web sites

www.who.int/topics/drug_resistance/en

Antibiotic resistance by region

www.cdc.gov/narms

Summary of data from the National Antimicrobial Resistance Monitoring System (NARMS)

www.cdc.gov/drugresistance/community

Appropriate antimicrobial use in the community

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What the Paediatrician Needs to Know When Pandemic Influenza Arrives in Clinical Practice

Nicole Ritz and Nigel Curtis

1 Introduction

1.1 *The Media Outbreak*

Avian (H5N1) influenza or “bird ‘flu” has received considerable attention in both the medical literature and the mass media in the last few years. Despite the tabloids’ portrayal of an imminent threat, to date there have been relatively few cases in humans in spite of large numbers of infected poultry (Hien et al. 2004). However, this may be falsely reassuring. Most indications suggest that it is just a matter of time until the next influenza pandemic occurs (Osterholm 2005). In the words of the UK Chief Medical Officer: “most experts believe that it is not a question of whether there will be another severe influenza pandemic but when” (Department of Health 2005). Although experts are agreed that a future influenza pandemic is almost inevitable, its timing is unpredictable and it is uncertain whether the virus responsible will be H5N1 or another, novel, influenza strain (Osterholm 2005). A recent editorial described avian influenza as a “predicament of extraordinary proportions” (Anonymous 2006). The next influenza pandemic will have a dramatic impact on all levels of health care including the everyday work of doctors. This chapter focuses on the clinical aspects of pandemic influenza about which paediatricians need to be familiar.

2 The Impact of Pandemic Influenza

2.1 *How Many People will be Affected?*

There have been ten influenza A pandemics in the past 300 years, of which the last three have been the best studied. The pandemic of 1918 (H1N1) “Spanish Influenza” killed 50–100 million people, with more than half of deaths occurring in healthy people between 18 and 40 years of age (Osterholm 2005). In the

following two pandemics – in 1957 (H2N2; “Asian influenza”) and 1968 (H3N2; “Hong Kong influenza”) – the mortality was strikingly lower, with each pandemic killing approximately one million people (Hien et al. 2004). This highlights the association between the virulence of virus subtype and mortality. Death rates are also determined by various other factors including clinical attack rates, R_0 (basic reproduction number), vulnerability of affected populations and the effectiveness of preventive measures. It is therefore impossible to predict with accuracy the impact of the next pandemic. Best-case scenarios, modelled on the mild pandemic of 1968, predict global deaths in the range of 2–7.4 million (World Health Organization 2005a). Should a virulent H5N1 become the next pandemic strain, evidence suggests that this strain would mimic the 1918 pandemic with estimates up to 360 million deaths globally (Osterholm 2005). As of September 2007, there have been 327 confirmed cases (with 199 deaths) of H5N1 avian influenza (World Health Organization 2006b). The mortality rate of 60% is remarkably high, but may decrease in a pandemic. The 1918 Spanish influenza which had an estimated mortality rate of 2.5% (Hien et al. 2004).

2.2 How Fast will Pandemic Influenza Spread?

In a pandemic situation it is likely that influenza would strike in several waves, each lasting approximately 15 weeks with a cumulative clinical attack rate of up to 25% of the population (Department of Health 2005). In the first wave, it is expected that the number of cases would rise exponentially within a few weeks. Second and third waves, which may be weeks or months apart, with possible increased virulence may occur, as has been the case during past pandemics (Department of Health 2005; World Health Organization 2005a). Previous pandemics spread around the globe in 6–9 months. Given the pace and dimensions of international travel today, it is likely that pandemic influenza will spread more rapidly, reaching all continents in less than 3 months (World Health Organization 2005a).

The World Health Organisation (WHO) has defined stages in the evolution of an influenza pandemic ranging from phase 1 (inter-pandemic) to phase 6 (pandemic). During 2006 and 2007, WHO declared a phase 3 (pandemic alert) stage, which is defined as “no or very limited human-to-human transmission.” The next phases are “evidence of increased” (phase 4), “significant” (phase 5) and “efficient and sustained” (phase 6) “human-to-human transmission” (World Health Organization 2005b).

2.3 Is Human-to-Human Transmission Likely to Occur?

In birds there has been a substantial rise in the number of cases of H5N1 influenza during the past few years, with an expanding range of infected avian species, (Perkins and Swayne 2002). In mammals the broadening of the host

range including infection of felids, mice, pigs and ferrets has also been documented (Chen et al. 2004; Kuiken et al. 2004). To date, human H5N1 avian influenza has occurred almost exclusively as a result of bird-to-human transmission (Tran et al. 2004; Beigel et al. 2005). Human-to-human transmission has been associated with two family clusters of H5N1 avian influenza (Tran et al. 2004). The first documented human-to-human transmission occurred in September 2004 in Thailand. An 11-year-old girl infected her mother and aunt, who both provided unprotected nursing care and subsequently developed respiratory symptoms. Autopsy tissue from the mother and nasopharyngeal and throat swabs from the aunt were positive for H5N1 by reverse transcriptase-polymerase chain reaction (RT-PCR). No other routes of transmission could be identified (Ungchusak et al. 2005).

At the time of the most recent pandemic, which emerged in China in 1968, the population of that country comprised 790 million humans, 5.2 million pigs and 12.3 million poultry. Today these populations have increased to 1.3 billion, 508 million and 13 billion respectively (Osterholm 2005). This potent mix of people, pigs and poultry creates the perfect conditions for genetic reassortment to create a novel influenza virus strain (antigenic shift). However, recent evidence suggests that reassortment is probably less dangerous than expected in the case of H5N1 avian influenza. In an animal model in ferrets – who have a similar α -2,6 sialic acid receptor predominance on respiratory epithelial cells as humans – transmission of H5N1 reassorted influenza virus was poor (Maines et al. 2005).

Nevertheless, the properties of an influenza virus that increase transmissibility are poorly understood and it is also possible that, without re-assortment, a mutation of an influenza virus such as H5N1 could produce a strain adapted to humans. For example, the receptor binding specificity of influenza H5N1 virus can be altered through a change of one amino acid in the H5 protein (Gambaryan et al. 2006). There is evidence that the change of preferred binding of the influenza H5N1 virus to the specific receptor on human respiratory epithelial cells (sialic acid α -2,6) could be the critical event in the evolution of a human-to-human transmissible strain (Matrosovich et al. 2000, Wong and Yuen 2006).

3 Diagnosis and Clinical Features of H5N1 Avian Influenza

3.1 Differences Between Pandemic Influenza and Seasonal Influenza

Important differences between annual and pandemic influenza are summarised in Table 1. The remainder of this section relates to H5N1 avian influenza specifically.

Table 1 Key differences between seasonal and pandemic influenza

	Seasonal influenza	Pandemic influenza
Seasonality	Winter	Unpredictable; not always in winter
Clinical attack rate	Up to 10% of population	Up to 25% of population
Incubation	1–3 days	2–4 days (up to 10 days) ^a
Pattern of illness	“Typical” influenza illness	Wide spectrum of presentation and illness
Excretion of virus	Peak 1–3 days (up to 10 days) (Frank et al. 1981)	Up to 18 days (Beigel et al. 2005) ^a
Viral titres	Higher viral titre in nose	Higher viral titre in throat ^a
Highest mortality	<5 years and >65 years	All ages (in previous pandemics >50% deaths in healthy adults 18–40 years)
Case fatality rate	2.5% (Hien et al. 2004)	60% (World Health Organization 2006b) ^a
Antiviral treatment	For any person likely to have a life-threatening influenza-related illness; for persons >65 years of age	Likely to be an important component of treatment but efficacy uncertain
Prophylactic antiviral treatment	For persons who live or work in institutions in case of an institutional outbreak; exposed high risk persons within a family; persons with immuno-suppressive conditions who are not likely to mount adequate immune response to vaccination (Centers for Disease Control and Prevention 2006)	Optimal strategy for use of antivirals uncertain but likely to be used to protect health care workers and in strategy to contain initial outbreaks
Vaccine	Highly effective vaccine available and recommended annually	Will probably take up to 6 months to develop and distribute in best case scenario

^aSpecifically for H5N1 avian influenza

3.2 Clinical Features

3.2.1 Clinical Features at Presentation

More than half of H5N1 avian influenza cases have been in individuals under 18 years of age and a quarter have occurred in children under 10 years of age (Fig. 1). In the first published series of ten case of H5N1 avian influenza, of which eight subsequently died, the mean age was 13.2 years (range 5–24 years) (Tran et al. 2004). Significantly, none of these patients had any known pre-existing medical condition. Nine had clear evidence of either handling poultry or exposure to sick poultry the week before the onset of illness.

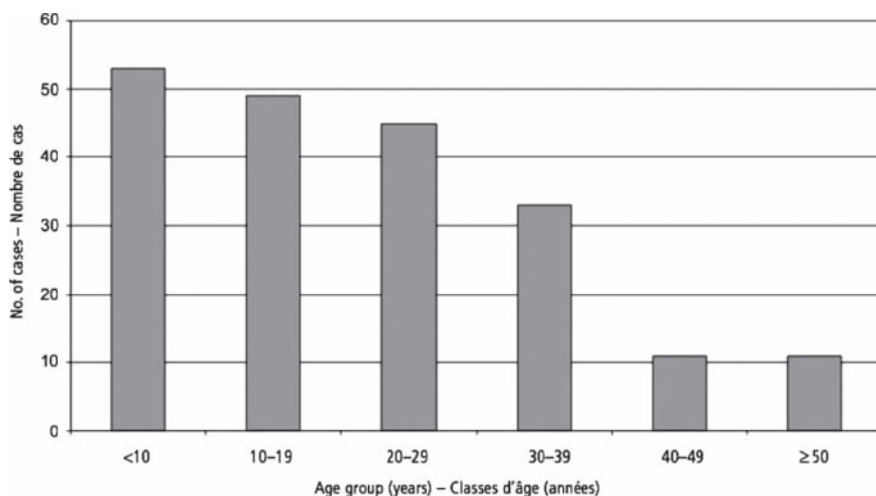


Fig. 1 Distribution of H5N1 cases by age group, as of April 2006 ($n = 202$) (World Health Organization 2006c)

The incubation period in H5N1 avian influenza is 2–10 days (Ungchusak et al. 2005). Patients present with initial symptoms of fever ($38.5\text{--}40^\circ\text{C}$), an influenza-like illness (headache, myalgia, malaise) and lower respiratory tract symptoms (non-productive cough, shortness of breath). Upper respiratory tract symptoms and conjunctivitis are rare, in contrast to other types of influenza A. Diarrhoea (seven of ten patients in the first series (Tran et al. 2004)), vomiting, abdominal pain, pleuritic pain and bleeding from the nose or gums have all been reported early in the course of illness (Beigel et al. 2005). Dyspnoea develops a median of 5 days after the onset of symptoms (range 1–16 days) (Chotpitayasunondh et al. 2005) and respiratory distress, tachypnoea and inspiratory crackles are common. Most patients have required ventilatory support within 48h of admission (Tran et al. 2004; Chotpitayasunondh et al. 2005).

3.2.2 Presentation with Delayed Respiratory Features

Of major importance is the observation that the clinical presentation of H5N1 avian influenza has a wide spectrum. One report from Vietnam documented siblings (a 9-year-old girl and her 4-year-old brother) who died following a presentation with severe diarrhoea, seizures and coma. Notably, both of them lacked any respiratory symptoms and both had normal chest radiographs on admission to hospital (de Jong et al. 2005a). H5N1 avian influenza virus was isolated from throat, stool, serum and cerebrospinal fluid. A presentation with watery diarrhoea (without blood or inflammation) preceding respiratory symptoms by 1 week has also been described (Apisarnthanarak et al. 2004).

3.2.3 Complications

Complications of H5N1 avian influenza include renal and liver dysfunction, cardiac compromise, supraventricular tachyarrhythmia (due to dilatation), myocarditis, pulmonary haemorrhage, pneumothorax, pancytopenia, Reye's syndrome, encephalopathy and sepsis syndrome (Tran et al. 2004; Beigel et al. 2005). Death is associated with acute respiratory distress syndrome (ARDS) and multi-organ failure due to a virus-induced "cytokine storm" (Osterholm 2005).

Death has occurred an average of 9–10 days after the onset of illness (range 6–30 days) (Beigel et al. 2005) and, similar to the 1918 pandemic, most patients have died of progressive respiratory failure associated with ARDS and a "cytokine storm" (Beigel et al. 2005; Osterholm 2005). The fatality rate among hospitalised patients is between 33% and 100%. In contrast to the H5N1 outbreak in 1997, in which most deaths occurred in patients older than 13 years of age, recent H5N1 avian influenza outbreaks have caused high rates of death among infants and young children with a case fatality rate of 89% reported among those younger than 15 years of age in Thailand (Chotpitayasunondh et al. 2005).

3.3 Radiology

Radiographic abnormalities in H5N1 avian influenza are present a median of 7 days after the onset of fever in almost all patients. Chest x-ray findings are very variable and include diffuse, multifocal or patchy infiltrates, interstitial infiltrates, and segmental and lobular consolidation with air bronchograms. Pleural effusions are uncommon (Beigel et al. 2005). Progression to respiratory failure and ARDS is associated with diffuse, bilateral, ground-glass infiltrates (Beigel et al. 2005).

3.4 Laboratory Features

3.4.1 Routine Investigations

Abnormalities detected on laboratory tests in H5N1 avian influenza include significant lymphopenia (median count 700/mm³) and mild thrombocytopenia (median count 75,000/mm³) (Tran et al. 2004), hyperglycaemia (Beigel et al. 2005). Lymphopenia and thrombocytopenia have been associated with a poor prognosis (Tran et al. 2004; Chotpitayasunondh et al. 2005).

3.4.2 Laboratory Confirmation

The following specimens from the upper respiratory tract are suitable for the diagnosis of H5N1 avian influenza: nasal swab, nasopharyngeal swab, nasopharyngeal

aspirate, nasal wash and throat swab. In addition, diagnosis can be made from other specimens including tracheal aspirate, bronchoalveolar lavage fluid, lung biopsy tissue, cerebrospinal fluid and faeces. H5-specific RNA is not detected in urine (Beigel et al. 2005). Viral excretion is prolonged and can be detected in throat-swabs up to 18 days after illness onset and in nasopharyngeal isolates from 1 to 16 days after onset. Viral loads detected in H5N1 avian influenza from pharyngeal swabs are at least 10 times higher than in H3N2 or H1N1 influenza (Beigel et al. 2005). Throat samples may have a better yield than nasal samples but the sensitivity and specificity of different samples and assays is not well defined (Beigel et al. 2005). Procedures for specimen collection, especially those involving potential aerosol generation, should be performed with appropriate precautions. WHO has produced detailed guidelines for the safe collection of specimens (World Health Organization 2005c).

H5N1 avian influenza can be confirmed in several different ways: rapid antigen test, viral isolation from culture, and the detection of H5-specific RNA with RT-PCR assays. Rapid antigen tests are less sensitive than RT-PCR. WHO laboratory criteria for confirmation require one or more of the following: a positive viral culture, a positive PCR assay, a positive immunofluorescence test for antigen (monoclonal antibody against H5), and at least a fourfold rise in H5-specific antibody titre in paired serum samples (Beigel et al. 2005).

3.5 When Should H5N1 Avian Influenza be Considered?

The possibility of H5N1 avian influenza should be considered in all patients with severe acute respiratory illness and those who present with serious unexplained illness (e.g. encephalitis or diarrhoea), who have had possible exposure to H5N1 avian influenza in the previous 2 weeks (i.e. all individuals who either live in or who have visited areas where H5N1 avian influenza has been identified in birds or other animals (Fig. 2)).

4 The Treatment of H5N1 Avian Influenza

The primary strategy for treatment of H5N1 influenza should be prevention. However, currently no commercially available vaccine against H5N1 avian influenza is available. In any case, the preparation of a vaccine against a novel strain will require several months and in the course of a pandemic, it is likely to take a minimum of 6 months before adequate supplies of vaccine are available. Therefore, effective antiviral agents are of major importance. There are two classes of drugs currently available for treatment and prophylaxis of influenza: the adamantanes (amantadine and rimantadine) and the neuraminidase inhibitors (zanamivir and oseltamivir). Other drugs, such as peramivir, which is highly effective in vitro and in animal models are subject to further studies (McCullers 2006).

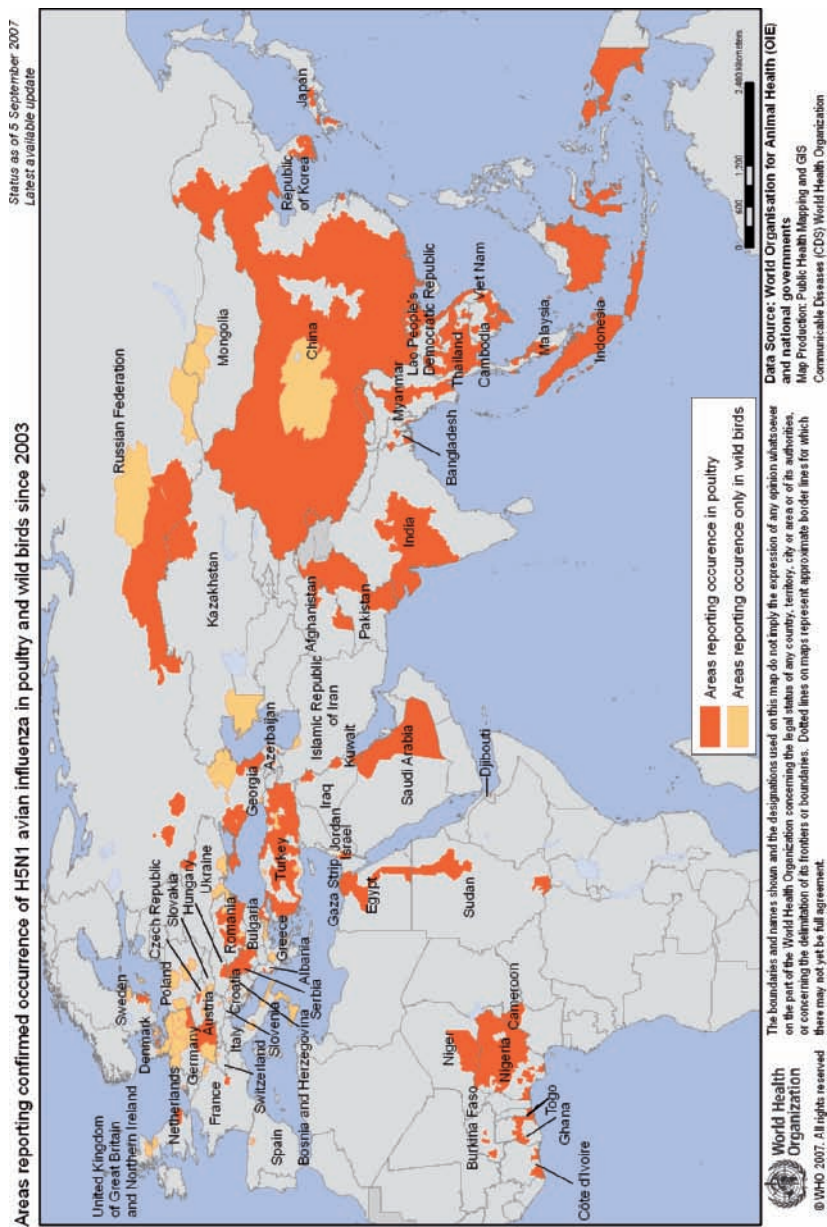


Fig. 2 Areas reporting confirmed occurrence of H5N1 avian influenza in poultry and wild birds since 2003 (World Health Organization 2006c)

4.1 *Adamantanes (M2 blockers)*

Influenza virus enters host respiratory cells by endocytosis and it is then enclosed within endosomes in the cell. Subsequent acidification, through influx of H^+ ions through the M2 protein channel, is the precondition for release of the viral nucleic acid from the endosome into the cell (Fig. 3). At low concentrations, the adamantanes block the influx of H^+ ions through the M2 protein channel. This inhibits the uncoating of the virus (McKimm-Breschkin 2005; Hayden 2006; Pinto and Lamb 2006). At very high concentrations the adamantanes prevent the fusion of the virus and cell membrane by interfering with binding to hemagglutinin (McKimm-Breschkin 2005). Adamantanes may be cheaper than neuraminidase inhibitors, but they have significant limitations. They are only effective against influenza A viruses, as they exclusively block the A/M2 channel, which is not present on influenza B virus. The B/M2 channels on influenza B viruses are not affected by adamantanes (Pinto and Lamb 2006). Adamantanes are associated with gastrointestinal (nausea) and central nervous system (nervousness, anxiety, difficulty concentrating, insomnia and hallucinations (Harper et al. 2005; Jefferson et al. 2006)) adverse effects in 10–30% of patients (McKimm-Breschkin 2005). The greatest problem with this class of anti-influenza drugs is the rapid emergence (as early as day two of treatment) of resistance in up to 30% of patients (McKimm-Breschkin 2005). Furthermore, adamantane-resistant isolates can be transmitted to susceptible contacts and are pathogenic (Moscona 2005a; Wong and Yuen 2006).

4.2 *Neuraminidase Inhibitors*

4.2.1 Mechanism of Action

The neuraminidase inhibitors interfere with the release of influenza virus from infected host cells and thereby limit the spread of the infection (Moscona 2005a).

The neuraminidase enzyme – the target molecule of the neuraminidase inhibitor – is present on the cell surface of all influenza viruses. It cleaves the bond by which the surface viral protein hemagglutinin attaches to the host cell-surface receptor, sialic acid. Cleavage is essential for both viral entry into the host cell but more importantly for exit of viral progeny after replication within the host cell (Fig. 4) (McKimm-Breschkin 2005; Moscona 2005a; McCullers 2006). The neuraminidase inhibitors mimic the sialic acid cell-surface receptor preventing neuraminidase from cleaving host-cell receptors and, as a result, from releasing newly-replicated virus.

4.2.2 Administration

Neuraminidase inhibitors need to be taken as early as possible in the course of the illness and ideally within 72 h, as replication of influenza virus in the respiratory tract reaches its peak between 24 and 72 h after onset of symptoms. However, a recent report suggests that treatment may still be beneficial later, if there is evidence

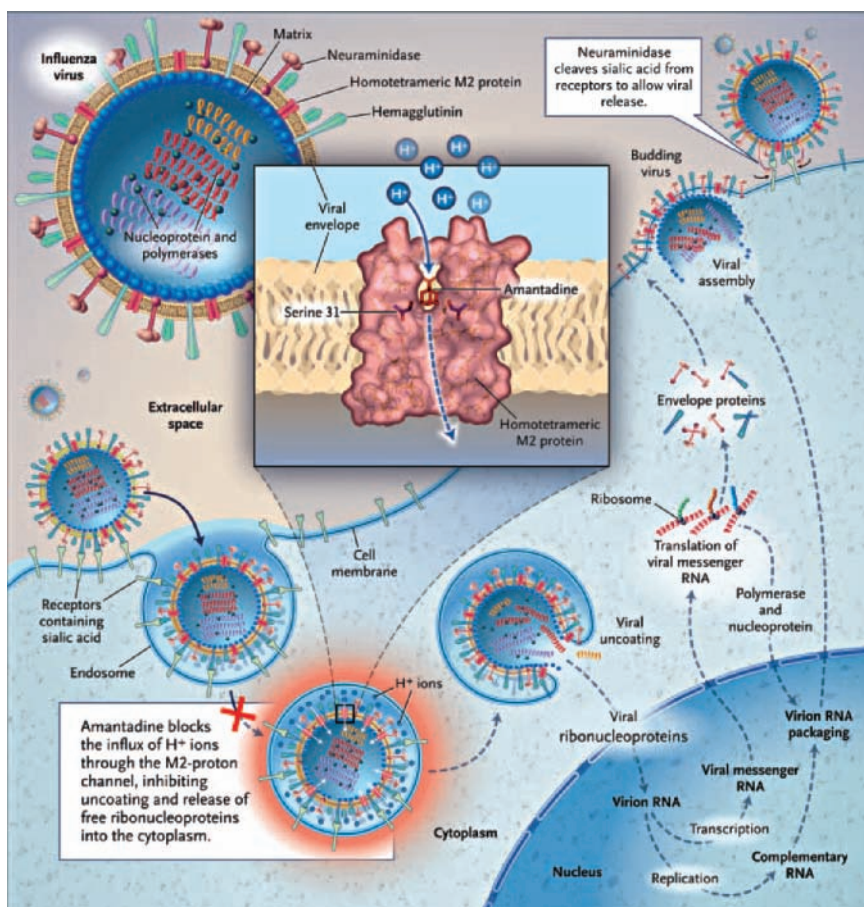


Fig. 3 Mechanism of action and development of resistance to M2 inhibitors. In the absence of amantadine, the proton channel mediates an influx of H^+ ions into the infecting virion early in the viral replication cycle, which facilitates the dissociation of the ribonucleoproteins from the virion interior and allows them to be released into the cytoplasm and transported into the cell nucleus. In highly pathogenic avian viruses (H5 and H7), the M2-proton channel protects the hemagglutinin from acid-induced inactivation in the trans-Golgi network during transport to the cell surface. In the presence of amantadine, the channel is blocked and replication is inhibited. The serine at position 31 lies partially in the protein-protein interface and partially in the channel (see inset). Replacement of serine by a larger asparagine leads to the loss of amantadine binding and the restoration of channel function. Depending on the particular amino acid, other mutations at position 26, 27, 30, or 34 may inhibit amantadine binding or allow binding without the loss of ion-channel function. Inset courtesy of Rupert Russell, Phillip Spearpoint, and Alan Hay, National Institute for Medical Research, London (Hayden 2006) (with permission from the publisher, Copyright © 2006 Massachusetts Medical Society)

of ongoing viral replication. This was shown in four patients with H5N1 avian influenza who had a rapid decline in viral load, and who all subsequently survived, despite oseltamivir being initiated later than 72 h after illness onset (de Jong et al. 2005b).

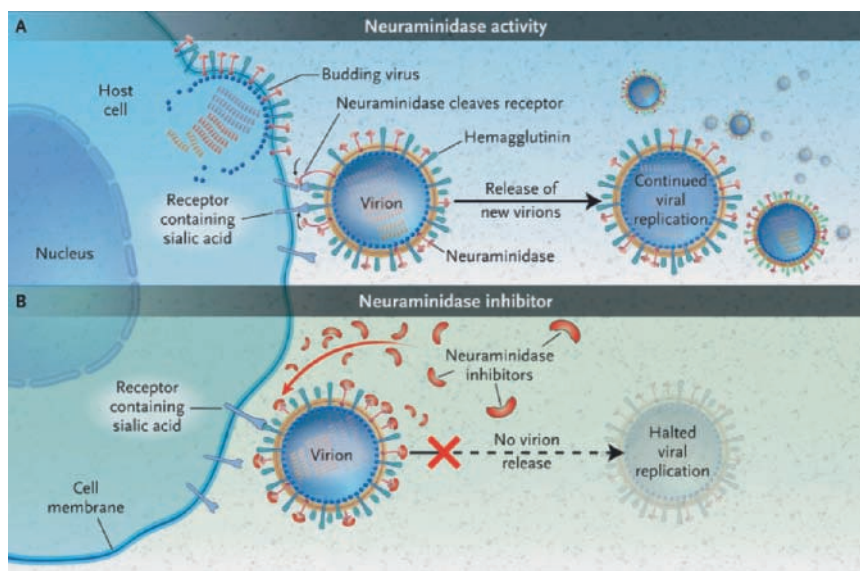


Fig. 4 Mechanism of action of neuraminidase inhibitors. Panel A shows the action of neuraminidase in the continued replication of virions in influenza infection. The replication is blocked by neuraminidase inhibitors (Panel B), which prevent virions from being released from the surface of infected cells (Moscona 2005a) (with permission from the publisher, Copyright © 2005 Massachusetts Medical Society)

4.2.3 Formulations

Oseltamivir is available as a capsule or powder for liquid suspension with good oral bioavailability. After absorption and conversion through hepatic esterases, the active form (oseltamivir carboxylate) is widely distributed in the body with a half-life of 6–10h. The drug is excreted primarily through the kidneys thus requiring dosing modifications in patients with renal insufficiency (Moscona 2005a) (Table 2). Zanamivir is not bioavailable orally and is directly delivered to the respiratory tract through inhalation of a dry powder from a specially-designed device. Between 10% and 20% of the active component reaches the lungs and the rest is deposited in the oropharynx. Bioavailability in serum reaches a maximum of 2% (Moscona 2005a).

4.2.4 Effectiveness

Neuraminidase inhibitors are effective against all strains of influenza and their efficacy has been subject to numerous trials (Hayden et al. 1999; McKimm-Breschkin 2005; Moscona 2005a; Jefferson et al. 2006). Children with clinically-diagnosed influenza who received oseltamivir within 48h of onset of symptoms had the duration of their illness reduced by 36h (Whitley et al. 2001).

Table 2 Recommended daily doses for the treatment and prophylaxis of influenza in children (modified after Moscona 2005a; Harper et al. 2005)

	<1 year	1–6 years	7–11 years	>12 years	Renal insufficiency
Treatment Zanamivir		Not licensed for use under 7 years of age	10 mg (two inhalations) twice daily for 5 days	10 mg (two inhalations) twice daily for 5 days	No dose adjustment
Oseltamivir	Not licensed for use under 1 year of age	<15 kg: 30 mg twice daily for 5 days 15–23 kg: 45 mg twice daily for 5 days >23–40 kg: 60 mg twice daily for 5 days >40 kg: 75 mg twice daily for 5 days	<15 kg: 30 mg twice daily for 5 days 15–23 kg: 45 mg twice daily for 5 days >23–40 kg: 60 mg twice daily for 5 days >40 kg: 75 mg twice daily for 5 days	75 mg twice daily for 5 days	If creatinine clearance <30 mL/min: 75 mg once daily ^a
Prophylaxis Oseltamivir			Not approved for prophylaxis under 13 years of age	75 mg once daily for 7–10 days (Beigel et al. 2005)	If creatinine clearance <30 mL/min: 75 mg once every other day ^a

^aOnly evaluated in adults

Clinical trials on the efficacy of neuraminidase inhibitors for the treatment of H5N1 avian influenza have not been undertaken. In an animal model of H5N1 avian influenza-infected mice, both zanamivir and oseltamivir improved survival (Leneva et al. 2001). However, the predominant sialic acid on the cell surface receptor in mice is α -2,3 (in contrast to primarily α -2,6 in humans), which limits the interpretation of this study. In vitro studies in human survivors of H5N1 avian influenza have showed that the virus can generally no longer be cultured 2 or 3 days after starting oseltamivir (Beigel et al. 2005).

4.2.5 Adverse Effects

Neuraminidase inhibitors are associated with a low risk of adverse effects. Transient nausea, vomiting and abdominal pain occurs in up to 10% of patients treated with oseltamivir (Moscona 2005a). Cough and bronchospasm have been reported following treatment with zanamivir (Freund et al. 1999).

4.2.6 Dosing in Children

Table 2 details dosing of neuraminidase inhibitors, including dose adjustment in renal insufficiency.

Higher doses (150 mg twice daily) and longer treatment (7–10 days) may be considered in severe H5N1 influenza infections but no data have yet been published (Beigel et al. 2005).

The safety of oseltamivir in infants under 1 year of age has not been established yet. Of concern is the observation that juvenile rats accumulate high levels of oseltamivir in the central nervous system. Although the immature blood–brain barrier in infants could similarly lead to high levels of oseltamivir in the central nervous system, there have been no reports of adverse effects from oseltamivir use in infants. In addition, a retrospective study in Japan, in which 103 children younger than 1 year of age were treated with 4 mg/kg for 4 days, did not show any encephalopathy (Okamoto et al. 2005). Concerns about potential toxicity in pregnant women and breast-feeding mothers have also been raised (Moscona 2005a).

4.3 Resistance to Anti-Influenza Drugs

Resistant influenza virus can be isolated from approximately 1% of adults and 5% of paediatric patients treated with oseltamivir (Whitley et al. 2001; McKimm-Breschkin 2005). The emergence of oseltamivir-resistant H5N1 avian influenza can result from the substitution of a single amino acid in the N1 neuraminidase (tyrosine for histidine

at position 274: His274Tyr) (Ward et al. 2005). In a report of eight patients with H5N1 avian influenza in Vietnam, two had high-level resistance to oseltamivir with the His274Tyr mutation. This may have been associated with disease progression as both patients died. In the other two patients who died, one revealed wild-type 274H and in the other patient no sequences could be obtained from the specimen. In the four surviving patients none showed oseltamivir-resistant H5N1 virus (de Jong et al. 2005b).

Factors which may favour the development of resistance include: the chemical structure of oseltamivir (Moscona 2005b), altered pharmacokinetics in severely ill patients, inadequate dosing, and reduced bioavailability resulting from diarrhoea. Prolonged therapy, higher doses or combination therapy may be of benefit (de Jong et al. 2005b). Murine studies indicate that, compared with a strain from 1997, the H5N1 avian influenza virus strain from 2004 requires higher doses and longer administration (8 days) to induce similar antiviral effects and survival rates (Yen et al. 2005). The transmissibility of oseltamivir-resistant H5N1 avian influenza strains is not yet known.

No influenza strains resistant to zanamivir have yet been isolated from immuno-competent patients after therapy (Moscona 2005b). The His274Tyr mutation does not lead to cross-resistance as the binding of zanamivir is not prevented by this mutation (McKimm-Breschkin 2002). Treatment regimens combining the two different neuraminidase inhibitors might be of benefit but to date there is insufficient evidence (Gupta and Nguyen-Van-Tam 2006).

Amantadine resistance in H5N1 avian influenza is associated with the presence of several mutations (Ser31Asn, Val27Ala, Leu26Ile), which result in loss of binding to M2 ion channel blockers. The distribution of amantadine-resistant H5N1 virus appears to be largely limited to Thailand, Vietnam and Cambodia. Most H5N1 isolates from China, Indonesia (Cheung et al. 2006), Mongolia, Russia and Turkey appear to be sensitive to amantadine (Hayden 2006). However, susceptible strains rapidly develop resistance. In addition, WHO states that current isolates of H5N1 avian influenza, in contrast to isolates from the 1997 outbreak, are highly resistant to amantadine and rimantadine, and that consequently these drugs should not be used in treatment (Beigel et al. 2005). The only role for amantadine may be in combination with neuraminidase inhibitors. In vitro studies suggest that combination chemotherapy with adamantanes and neuraminidase inhibitors reduces the emergence of drug-resistant influenza variants (Ilyushina et al. 2006).

4.4 Prophylaxis

Both zanamivir (Monto et al. 2002) and oseltamivir (Moscona 2005a) are effective for post-exposure prophylaxis in seasonal 'flu with a protective efficacy of 80% in children older than 1 year. In the 1968 pandemic, adamantanes were found to have a protective efficacy of 70%. The protective efficacy of neuraminidase inhibitors in a pandemic is expected to be at least as high (Moscona 2005a), but current data on the effectiveness of neuraminidase prophylaxis in a pandemic situation are lacking. It is thought that prophylactic use of neuraminidase inhibitors does not prevent

infection but efficiently limits viral replication and shedding. This is important because children are the main source of dissemination of influenza within the community, since they usually have higher viral loads and excrete viruses for longer periods (Moscona 2005b). As a result, children who receive oseltamivir for prophylaxis will be able to mount an immune response due to sub clinical infection, but will not be the hub for infectious spread (Dolin 2005; Smith et al. 2006).

4.5 Who Should be Treated?

Recommendations for the use of antiviral drugs in seasonal influenza are detailed elsewhere (Jefferson et al. 2006, Centers for Disease Control and Prevention 2006). During an influenza pandemic, depending on the number of cases, current supplies of neuraminidase inhibitors may be inadequate for any proposed strategy of prevention (e.g. around a localised outbreak or post exposure) and may not be sufficient for the treatment of even those with disease (Hayden 2004). One approach that has been proposed to maximise supplies is to reduce the required dose of neuraminidase inhibitors through the co-administration of probenecid. By reducing the renal clearance of oseltamivir, probenecid has the capacity to increase plasma levels by 50% (Howton 2006).

4.6 Additional and Other Treatments

Secondary bacterial infection is a common and serious complication of seasonal influenza. Rates of secondary bacterial infections in H5N1 avian influenza have not been defined, but *Staphylococcus aureus* and *Haemophilus influenzae* have been isolated from tracheal aspirates in patients with H5N1 influenza (Tran et al. 2004). WHO recommends that empirical treatment with broad spectrum antibiotics should be considered in patients with suspected H5N1 avian influenza (Beigel et al. 2005). Other drugs that have been used but for which there is currently no evidence of efficacy in the treatment of H5N1 avian influenza include ribavirin, corticosteroids, interferon alpha and intravenous immunoglobulin (Beigel et al. 2005; Wong and Yuen 2006).

4.7 Personal Stockpiling of Antiviral Drugs

A benefit of having a supply of antivirals at home is that treatment can be started soon after onset of symptoms, without any delay through access to medical services. However, if oseltamivir were dispensed in advance of an outbreak, it is likely that patients would misuse their stockpiles, possibly wasting it on illnesses other than influenza. Insufficient dosing and inadequate courses are of further concern. High rates of resistance (16%) have been shown in H1N1 influenza A virus isolated from

patients in Japan as a result of under-dosing (Ward et al. 2005). Patients' requests for a personal stockpile of oseltamivir place the physician in a difficult position in between the obligation to an individual patient and the demands of public health. Currently, there is no evidence of a benefit from personal stockpiling of antivirals and therefore an individual physician has no obligation to prescribe (Brett and Zuger 2005), and moreover, has an obligation from a public health viewpoint not to prescribe. Therefore, because personal stockpiles of oseltamivir will lead to improper use and shortages of supply they should be strongly discouraged.

5 Limiting the Spread of Influenza During a Pandemic

H5N1 avian influenza is transmitted through inhalation of respiratory droplets and droplet nuclei (dry droplets), by direct or indirect contact, or by contact with fomites. The relative efficiency of these different routes has not been defined (Beigel et al. 2005), but it is highly likely that the major mode of spread is through respiratory droplets expelled when coughing or sneezing (Bridges et al. 2003).

The possibility of person-to-person transmission of avian H5N1 influenza is of great concern since the case in Thailand, described above, in which there was apparent transmission to the child's relatives who provided unprotected care. Further transmission to health care workers did not occur in this case (Ungchusak et al. 2005). The current low risk of nosocomial transmission to health care workers is reassuring. However, in the advent of a pandemic, precautions to prevent human-to-human transmission would be critical for individuals caring for affected patients. In addition to standard and droplet precautions, the WHO recommends eye protection and, where possible, airborne precautions (World Health Organization 2006a).

Furthermore, respiratory hygiene, so-called "cough etiquette," is recommended by several health organisations, though it is of unproven efficacy. It involves covering coughs and sneezes with a disposable tissue, the use of masks if coughing and sneezing, and personal hand hygiene after contact with respiratory secretions (Centres for Disease Control and Prevention 2003; World Health Organization 2006a).

Contacts of a patient with proven or suspected virus should monitor their temperature and self-quarantine for a period of 1 week after their last exposure (Beigel et al. 2005). Household contacts of individuals with confirmed H5N1 avian influenza should receive post-exposure prophylaxis as described above (Beigel et al. 2005).

Travel restrictions and quarantine were not very effective in the previous three pandemics. However, banning of public gatherings and closure of schools may be effective in preventing the spread that is associated with close contact and crowding. Such measures may not need to be in place for prolonged periods, as pandemic influenza peaks have generally been short-lived. Preventing spread may lead to cases occurring over a longer time frame by flattening the epidemiological peak. The resulting fewer cases in any time period would decrease the burden on medical and other essential services (World Health Organization 2005a).

6 The Prevention of Pandemic Influenza

Controlling avian influenza in birds is a key tactic in preventing the emergence of pandemic influenza. Active surveillance in animals and humans is needed to monitor the evolution of potentially threatening avian viruses (Hien et al. 2004). In Hong Kong, for example, surveillance of influenza in poultry, recognition of early outbreaks and active surveillance in humans helped keep Hong Kong free of H5N1 avian influenza virus in humans for 7 years after the 1997 outbreak (Hien et al. 2004).

Vaccination remains the primary strategy for prevention of influenza and is beyond the scope of this chapter.

7 Conclusion

The world has its first opportunity to be prepared for the next influenza pandemic (Shortridge 2006). Understanding the epidemiology, clinical and laboratory features, and treatment and prophylactic strategies might provide a head start that will prevent the repetition of the mistakes in previous pandemics (Anonymous 2006).

8 Addendum

Subsequent to the completion of this manuscript in September 2006, new information about pandemic influenza had continued to be published at a high rate. Amongst the most interesting new developments are:

- Concerns about abnormal neuropsychiatric behaviour in adolescents receiving oseltamivir (2007)
- WHO recommendations on treatment and prophylaxis of H5N1 avian influenza, including a once daily dose of oseltamivir for the prophylactic treatment of children 1 to 12 years of age (Schunemann et al., 2007)
- The description of clades and subclades of H5N1 avian influenza virus with implications for resistance patterns and vaccine production (Webster and Govorkova, 2006)
- Confirmation that H5N1 avian influenza is predominantly a paediatric disease possibly explained by the presence of α -2,3 sialic acids in the upper airway tract in children (Goicoechea, 2007)

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Key web resources for pandemic and H5N1 avian influenza

Of the tens of millions of web sites with information about pandemic and H5N1 avian influenza available on the internet, many happy hours can be spent browsing the following sites that are amongst the most useful:

World Health Organisation

http://www.who.int/csr/disease/avian_influenza/en/index.html

<http://www.who.int/csr/disease/influenza/nationalpandemic/en/index.html> (other countries' guidelines)

UK Health Protection Agency

www.hpa.org.uk/infections/topics_az/influenza/avian

www.hpa.org.uk/infections/topics_az/influenza/avian/microbiological_guidance.htm

UK Department of Health

<http://www.dh.gov.uk/en/PandemicFlu/index.htm>

UK National Health Service

<http://www.nhsdirect.nhs.uk/articles/article.aspx?articleId=1565§ionId=10>

<http://www.nhsdirect.nhs.uk/articles/article.aspx?articleId=1303§ionId=10>

European Centre for Disease Prevention and Control

<http://www.ecdc.eu.int>

US National Library of Medicine and National Institute of Health

www.nlm.nih.gov/medlineplus/flu.html

US Department of Health and Human Services

<http://www.pandemicflu.gov>

Centers for Disease Control and Prevention

<http://www.cdc.gov/flu>

<http://www.cdc.gov/flu/avian/index.htm>

International Society of Infectious Diseases

<http://www.promedmail.org>

Miscellaneous

<http://www.medscape.com/resource/influenza>

<http://www.influenzareport.com>

<http://www.fluwikie.com>

<http://www.fluwire.com>

<http://www.connotea.org/tag/AvianFlu>

<http://pandemicnews.blogspot.com/>

Eugene D. Shapiro

1 Introduction

1.1 Etiology and Epidemiology

Lyme disease is caused by the spirochete, *Borrelia burgdorferi* sensu lato, a fastidious, microaerophilic bacterium that replicates slowly and requires special media to grow in the laboratory (Shapiro and Gerber 2000). The organism has been subclassified into several genomospecies, including *B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii* and others. Different genomospecies seem to be associated with an increased likelihood of certain specific manifestations of Lyme disease – for example, *B. burgdorferi* sensu stricto seems to have a predilection to cause arthritis (if not treated early), while *B. garinii* seems to be associated with an increased risk of neurological manifestations of Lyme disease. In the United States, only *B. burgdorferi* sensu stricto has been isolated from humans. In contrast, there is substantial variation in the genomospecies of *B. burgdorferi* sensu lato isolated from humans in Europe.

Lyme disease occurs throughout the world. In Europe, most cases occur in the Scandinavian countries and in central Europe (especially in Germany, Austria and Switzerland), although cases have been reported from throughout the region, including the United Kingdom (where many cases occur in the South Downs or New Forest areas). Most cases of Lyme disease in the United States occur in southern New England, southeastern New York, New Jersey, eastern Pennsylvania, eastern Maryland, Delaware, and parts of Minnesota, Wisconsin and Michigan. More than three-quarters of these cases occur in fewer than 70 counties, an indication of the geographic limitation of the disease (Centers for Disease Control 2004). The incidence of Lyme disease varies substantially from region to region and even within local areas. Information about the true incidence of the disease is complicated by reliance on passive reporting of cases as well as by the high frequency of misdiagnosis (Steere et al. 1993; Reid et al. 1998). In the most highly endemic areas of the United States, such as Connecticut, the incidence is about 0.5 cases/1,000, but can be substantially higher in local areas. The incidence is highest in children 5–10 years of age – nearly twice as high as the incidence among adults.

B. burgdorferi is transmitted by Ixodid ticks – in the United States, primarily by *Ixodes scapularis*, the deer tick (Shapiro and Gerber 2000). Other vectors include *Ixodes ricinus* (the sheep tick), *Ixodes persulcatus* and *Ixodes pacificus* in Europe, Asia and the Pacific coast of the United States, respectively. Ixodid ticks have a 2-year, three-stage life cycle. The larvae hatch in the early summer and are usually not infected with *B. burgdorferi*. The tick may become infected at any stage of its life cycle by feeding on a host that is a natural reservoir for *B. burgdorferi*, usually a small mammal such as the white-footed mouse (*Peromyscus leucopus*). The larvae overwinter and emerge the following spring in the nymphal stage, which is the stage of the tick that is most likely to transmit the infection (Nadelman et al. 2001). Nymphs molt to become adults in the fall. Adult females, which often spend the winter attached to large animals such as deer or sheep (hence the names – deer or sheep tick), lay their eggs the following spring before they die, and the 2-year life cycle begins again.

A number of factors are associated with the risk of transmission of *B. burgdorferi* from ticks to humans. First, the tick must be infected. The proportion of infected ticks varies greatly both by geographic area and by the stage of the tick in its life cycle. Lyme disease is uncommon in the Pacific states since few *Ixodes pacificus* ticks are infected with *B. burgdorferi*, in part because one of its major hosts, lizards, have a factor in their serum that kills *B. burgdorferi* (Ullmann et al. 2003). By contrast, in highly endemic areas of southern New England rates of infection of *I. scapularis* are, approximately, 2% for larvae, 15–30% for nymphs and 30–50% for adult ticks. There has been substantial variation in rates of infection of *Ixodes ricinus* in reports from various European countries, but approximate averages are 10% for nymphs and 20% for adult ticks (Rauter and Hartung 2005).

Based on studies with experimental animals, to transmit *B. burgdorferi*, an infected tick generally must feed for 48–72 h or longer (Piesman et al. 1987). These experimental findings were confirmed in a study in humans in which the risk of transmission from ticks (for which the duration of feeding could be assessed) to humans was 25% for nymphal ticks that had fed for at least 72 h and 0% for nymphal ticks that had fed for ≥ 72 h (Nadelman et al. 2001). The bacteria live in the mid-gut of the tick, which needs to become engorged with blood before the bacteria migrate to the salivary glands and the saliva, through which the organism is injected into the host. Persons with occupational, recreational or residential exposure to tick-infested fields, yards or woodlands in endemic areas are at increased risk of developing Lyme disease.

2 Clinical Manifestations

The clinical manifestations of Lyme disease are classified into stages – early localized disease, early disseminated disease, and late disease (Steere 2001; Shapiro and Gerber 2003). Erythema migrans, the manifestation of early localized disease, appears at the site of the tick bite, 3–30 days (typically within 7–14 days) after the

bite. Erythema migrans begins as a red macule or papule and expands for days to weeks to form a large, annular, erythematous lesion that is at least 5 cm and as much as 70 cm in diameter (median of 15 cm) (Fig. 1). Most often, the rash is uniformly erythematous or it may appear as a target lesion with variable degrees of central clearing. It can vary greatly in shape, and, occasionally, may have vesicular or necrotic areas in the center. Erythema migrans is usually asymptomatic but may be pruritic or painful, and it may be accompanied by systemic findings such as fever, malaise, headache, regional lymphadenopathy, stiff neck, myalgia, or arthralgia.

The most common manifestation of early disseminated Lyme disease in the United States is multiple erythema migrans. In the United States, erythema migrans (single or multiple) is found in about 90% of patients with objective evidence of infection with *B. burgdorferi* (Gerber et al. 1996; Nadelman and Wormser 1998). The secondary skin lesions, which usually appear from 3 to 5 weeks after the tick bite, consist of multiple annular erythematous lesions similar to, but usually smaller than, the primary lesion. Other common manifestations of early, disseminated Lyme disease are cranial nerve palsies, especially facial nerve palsy, and meningitis (sometimes accompanied by papilledema and increased intracranial pressure). Systemic symptoms such as fever, myalgia, arthralgia, headache, and fatigue are also common in this stage of Lyme disease. Carditis, which usually is manifest as heart block, is a rare manifestation of early, disseminated disease. Although it occurs only rarely in the United States, borreliolymphocytoma (Fig. 2), an inflammatory infiltrate that typically occurs in the ear lobe or the breast, is seen with some frequency in patients with Lyme disease in Europe. Likewise, meningoradiculoneuritis (Bannwarth's syndrome), a sometimes painful radiculopathy due to Lyme disease, is far more common in Europe than in the United States.



Fig. 1 Erythema migrans (See Color Plates)

Fig. 2 Borrelial lymphocytoma of the ear lobe (See Color Plates)



The most common manifestation of late Lyme disease, which occurs weeks to months after the initial infection, is arthritis. The arthritis is usually monoarticular or oligoarticular and affects the large joints, particularly the knee. Although the affected joint is typically swollen and somewhat tender, the intense pain associated with a septic arthritis usually is not present. However, Lyme arthritis can occasionally mimic septic arthritis. Encephalitis, encephalopathy, and polyneuropathy are also manifestations of late Lyme disease, but they are very rare in children. Acrodermatitis chronica atrophicans (Fig. 3), a chronic sclerosing dermatitis, is an uncommon manifestation of Lyme disease in Europe but is virtually unknown in the United States.

Ixodes ticks may transmit other pathogens in addition to *B. burgdorferi*, including Babesia, Anaplasma, other Borrelia species, and viruses (Shapiro and Gerber 2000; Wormser et al. 2006). These agents may be transmitted either separately from or simultaneously with *B. burgdorferi*. However, the frequency with which coinfection occurs is unknown and its impact on both the clinical presentation and the response to treatment of Lyme disease is not well defined.

3 Diagnosis

The diagnosis of Lyme disease, especially in the absence of the characteristic rash, may be difficult, since the other clinical manifestations of Lyme disease are not specific. Even the diagnosis of erythema migrans sometimes may be difficult, since the rash initially may be confused with nummular eczema, granuloma annulare, an insect bite, ringworm or cellulitis. The relatively rapid and prolonged (untreated, it lasts for weeks) expansion of erythema migrans helps to distinguish it from these other conditions.

Fig. 3 Acrodermatitis chronica atrophicans
(See Color Plates)



The sensitivity of culture for *B. burgdorferi* is only fair and special media are required; moreover, it is necessary for patients to undergo an invasive procedure to obtain appropriate tissue or fluid for culture. Consequently, such tests are indicated only in rare circumstances. Likewise, diagnostic tests that are based on the identification of antigens of *B. burgdorferi*, including the polymerase chain reaction (PCR), have not been shown to be sufficiently accurate to be clinically useful under non-experimental conditions. Although studies in research laboratories suggest that the PCR test is promising, contamination is a potential problem in commercial laboratories and an invasive procedure is still necessary to obtain appropriate material to test. Consequently, the confirmation of Lyme disease by the laboratory usually rests on the demonstration of antibodies to *B. burgdorferi* in the patient's serum.

It is well documented that the sensitivity and specificity of antibody tests for Lyme disease vary substantially. The accuracy and reproducibility of pre-packaged commercial kits is much poorer than that of tests performed by "reference" laboratories that maintain tight quality control and regularly prepare the materials that are used in the test. Official recommendations from the Second National Conference on Serologic Diagnosis of Lyme Disease and from the CDC are that clinicians use a two-step procedure when ordering antibody tests for

Lyme disease – first, a sensitive screening test, such as an enzyme-linked immunosorbent assay (ELISA) and, if that result is positive or equivocal, a Western immunoblot (a more specific test than the ELISA) to confirm the result (Centers for Disease Control and Prevention 1995). If the ELISA result is negative, an immunoblot is not necessary. Immunoblots should not be ordered without a simultaneously ordered ELISA. The ELISA provides a quantitative estimate of the concentration of antibodies against *B. burgdorferi*. The immunoblot provides information about the specificity of the antibodies; positive “bands” mean that antibodies against specific protein antigens of *B. burgdorferi* are present. Most authorities require the presence of antibodies against at least either 2 (for IgM) or 5 (for IgG) specific proteins of *B. burgdorferi* for the immunoblot to be considered positive (Centers for Disease Control and Prevention 1995). Antibody tests are not useful for the diagnosis of early localized Lyme disease, since only a minority of patients with single erythema migrans will have a positive test because the rash usually develops before the antibodies are detectable. A diagnosis of Lyme disease should not be based on a positive IgM result alone in patients who have had symptoms for ≥ 4 weeks (Steere 2001).

It is critically important to understand that the predictive value of antibody tests, even of very accurate tests, is highly dependent on the prevalence of the infection among patients who are tested. Antibody tests for Lyme disease should *not* be used as screening tests (Seltzer and Shapiro 1996; Tugwell et al. 1997). Unfortunately, because many lay persons (as well as physicians) have the erroneous belief that chronic, nonspecific symptoms alone (e.g., fatigue or arthralgia) may be manifestations of Lyme disease, parents of children with only nonspecific symptoms frequently demand that the child be tested for Lyme disease (and some physicians routinely order tests for Lyme disease on such patients). Lyme disease will be the cause of the nonspecific symptoms in very few such children, if any. However, because the specificity of even the best antibody tests for Lyme disease is nowhere near 100%, some of the test results in children without specific signs or symptoms of Lyme disease will be positive; the vast majority of these (>95%) will be false-positive results (Seltzer and Shapiro 1996; Tugwell et al. 1997). Nevertheless, an erroneous diagnosis of Lyme disease, based on the results of these tests, frequently is made and such children often are treated unnecessarily with antimicrobials.

Clinicians should realize that even though a symptomatic patient has a positive serological test result for antibodies to *B. burgdorferi*, it is possible that Lyme disease may not be the cause of that patient's symptoms. In addition to the possibility that it is a false-positive result, the patient may have been infected with *B. burgdorferi* previously, and the patient's current symptoms may be unrelated to that previous infection. Once serum antibodies to *B. burgdorferi* do develop, they may persist for many years despite adequate treatment and clinical cure of the illness (Feder et al. 1992; Kalish et al. 2001). In addition, because some people who become infected with *B. burgdorferi* never develop symptoms, in endemic areas there will be a background rate of seropositivity among patients who have never had clinically apparent Lyme disease. Physicians should not routinely order

antibody tests for Lyme disease either for patients who have not been in endemic areas or for patients who only have nonspecific symptoms.

4 Treatment

A panel of experts from the Infectious Diseases Society of America has recently updated recommendations in a very comprehensive document that provides practice guidelines for the management of patients with Lyme disease, Anaplasmosis and Babesiosis (Wormser et al. 2006). These are available on the website of the society (www.idsociety.org) under Practice Guidelines.

5 “Chronic” Lyme Disease

The long-term prognosis for children who are treated with appropriate antimicrobial therapy for Lyme disease, regardless of the stage of the disease, is excellent. The most common reason for a lack of response to appropriate antimicrobial therapy for Lyme disease is misdiagnosis (i.e., the patient actually does not have Lyme disease). Nonspecific symptoms (such as fatigue, arthralgia or myalgia) may persist for several weeks even in successfully treated patients with early Lyme disease; their presence should not be regarded as an indication for additional treatment with antimicrobials. These symptoms usually respond to non-steroidal anti-inflammatory agents. Within a few months of completing the initial course of antimicrobial therapy, these vague, nonspecific symptoms will usually resolve without additional antimicrobial therapy. For those unusual patients who have persistent symptoms more than 6 months after the completion of antimicrobial therapy, an attempt should be made to determine if these symptoms are the result of a post-infectious phenomenon or of another illness.

Klempner and coworkers recently reported the results of two controlled trials of antibiotic treatment for adult patients with chronic musculoskeletal pain, neuro-cognitive symptoms, or both that persisted after antibiotic treatment for Lyme disease (Klempner et al. 2001). One study included patients who were seropositive for IgG antibodies to *B. burgdorferi* at the time of enrollment; the other study included patients who were seronegative. In both studies, patients were randomly assigned to receive either ceftriaxone administered intravenously for 30 days followed by doxycycline orally for 60 days or matching regimens with intravenous and oral placebos. There were no significant differences in the outcomes of patients treated with antibiotics compared with those treated with placebo among either the seropositive or the seronegative patients. Of note, nearly 40% of subjects treated with placebo improved. These findings support earlier recommendations that such patients are best treated symptomatically rather than with prolonged courses of antibiotic therapy, which have been associated with serious adverse side effects (Ettestad et al. 1995).

6 Congenital Lyme Disease

Congenital Lyme Disease Much of the initial information about the potential for transplacental infection with Lyme disease was alarming. However, this information came from a small number of case reports, most of which involved women with unrecognized and untreated Lyme disease during their pregnancies. There has also been considerable skepticism about these cases because in none was there evidence of inflammation and there was no consistent pattern of disease. In addition, although spirochetes compatible with *B. burgdorferi* were seen in pathological specimens, *B. burgdorferi* was never isolated in culture from any of these cases.

Several subsequent studies, designed to assess the potential link between Lyme disease during pregnancy and congenital infection with *B. burgdorferi*, found no consistent pattern of disease and no clearly documented *B. burgdorferi* infections of either the fetus or the infant. In addition, the obstetric outcomes were similar among women who had documented Lyme disease during their pregnancies and those who did not. Moreover, in a survey of 162 neurologists practicing in areas endemic for Lyme disease, the investigators were unable to identify any well-documented cases of prenatally-acquired neuroborreliosis (Gerber and Zolneraitis 1994). Although there has been a temporal relationship between Lyme disease during pregnancy and adverse outcomes, a causal relationship has not been established. There is no evidence of increased risk of abnormal outcomes with Lyme disease during pregnancy (Silver 1997). Transmission of Lyme disease via breast-feeding has not been documented.

7 Prevention of Lyme Disease

Reducing the risk of tick bites is one obvious strategy to prevent Lyme disease. In endemic areas, clearing brush and trees, removing leaf litter and woodpiles, and keeping grass mowed may reduce exposure to ticks. Application of pesticides to residential properties is effective in suppressing populations of ticks, but may be harmful both to other wildlife and to people.

Tick and insect repellents that contain *n,n*-diethylmetatoluamide (DEET) applied to the skin provide additional protection, but require reapplication every 1–2 h for maximum effectiveness. Serious neurological complications in children from either frequent or excessive application of DEET-containing repellents have been reported, but they are rare and the risk is low when these products are used according to instructions on their labels. Use of products with concentrations of DEET greater than 30% is not necessary and increases the risk of adverse effects. DEET should be applied sparingly only to exposed skin, but not to a child's face, hands, or skin that is either irritated or abraded. After the child returns indoors, skin that was treated should be washed with soap and water. Permethrin (a synthetic pyrethroid) is available in a spray for application to clothing only and is particularly effective because it kills ticks on contact.

Because most persons (approximately 75%) who recognize that they were bitten by a tick remove the tick within 48 h, the risk of Lyme disease from recognized deer

tick bites is low – approximately 1–3% in areas with a high incidence of Lyme disease. Indeed, the risk of Lyme disease is higher for unrecognized bites (since such ticks will feed for a longer time). Persons should be taught to inspect themselves and their children's bodies and clothing daily after possible exposure to Ixodid ticks. An attached tick should be grasped with medium-tipped tweezers as close to the skin as possible and removed by gently pulling the tick straight out. If some of the mouth parts remain embedded in the skin, they should be left alone, since they usually are extruded eventually; additional attempts to remove them often result in unnecessary damage to tissue and may increase the risk of local bacterial infection. Analysis of ticks to determine whether they are infected is not indicated because the predictive values of results of such tests for the development of disease in humans is unknown. No vaccine for Lyme disease is currently available.

A study of antimicrobial prophylaxis for ticks bites among adults found that a single, 200mg dose of doxycycline was 87% effective in preventing Lyme disease, although the 95% confidence interval around this estimate of efficacy was wide (the lower bound was 25% or less, depending on the method used) (Nadelman et al. 2001). In that study, the only persons who developed Lyme disease had been bitten by nymphal stage ticks that were at least partially engorged; the risk of Lyme disease in this group was 9.9% (among recipients of placebo), while it was 0% for bites by all larval and adult deer ticks. Unfortunately, the expertise to identify the species, stage and degree of engorgement of a tick, and thereby to assess the degree of risk, is rarely available to persons who are bitten. Consequently, routine use of antimicrobial agents to prevent Lyme disease in persons who are bitten by a deer tick, even in highly endemic areas, is not generally recommended because the overall risk of Lyme disease is low (1–3%), only doxycycline (which is not recommended for children <8 years of age) has been shown to be effective and treatment for Lyme disease, if it does develop, is very effective (Shapiro 2001).

Serological testing for Lyme disease after a recognized tick bite also is not recommended. Antibodies to *B. burgdorferi* that are present at the time that the tick is removed would probably be due either to a false-positive test result or to an earlier infection with *B. burgdorferi* rather than to a new infection from the recent bite. Likewise, in this setting the predictive value of a positive result is very low.

8 Lyme Hysteria

A panel of experts that developed clinical guidelines for managing patients with Lyme disease for the Infectious Diseases Society of America concluded that there is no such diagnostic entity as “chronic Lyme disease” (Wormser et al. 2006). This contention is supported by the clinical trials that found that long-term treatment with antimicrobials was not effective for patients who believed that they had chronic Lyme disease (Klempner et al. 2001).

In some of the original reports of Lyme disease, the proportions of patients who also had nonspecific symptoms such as arthralgia, myalgia, headache or fatigue were substantial. Of course, all of the patients in those reports also had specific

objective signs (such as erythema migrans, facial nerve palsy or arthritis) that are associated with Lyme disease. Nevertheless, some have drawn the erroneous inference that nonspecific symptoms alone often could be the sole manifestations of Lyme disease. The nonspecific symptoms sometimes attributed to Lyme disease are highly prevalent in the general population. The nonspecific symptoms also can be caused by common ailments such as viral illnesses or may be manifestations of either anxiety or depression. Nevertheless, the idea that Lyme disease might be the cause of nonspecific symptoms alone (without any objective signs of the illness) has been publicized by patient-advocate groups and augmented by extensive misinformation in the lay press and on the internet (Cooper and Feder 2004). In some instances anxious (often misinformed) parents are driven by the fear that their child's nonspecific complaints may be a manifestation of Lyme disease which, if not detected and treated, could lead to serious chronic disability. There is a large body of evidence that Lyme disease rarely causes long-term problems (Seltzer et al. 2000). Although long-term problems due to Lyme disease have been documented, they are extremely rare and have occurred almost exclusively in adults with objective evidence of Lyme disease, most of whom either were not treated with antimicrobials or received treatment only many years after the onset of Lyme disease.

Nevertheless, physicians in referral centers who specialize in Lyme disease continue to be deluged by patients who are thought either to have (or who believe they have) chronic Lyme disease. Reports from such centers indicate that in the great majority of instances the patients either never had Lyme disease or the symptoms that led to the referral were not due to Lyme disease (Steere et al. 1993; Reid et al. 1998). The challenge for clinicians who are faced with such patients (or with the parents of such patients) is to be able to address their concerns without dismissing them. In most instances the patients have (or the parents perceive there is) a problem. Sometimes, a parent's anxiety about a child's behavior can be allayed by reassurance. In other instances, a parent may insist that the child is ill, even though objective signs of organic illness are not present. Helping such patients obtain the type of help they need without alienating both the child and the parents may be difficult. Sometimes this can best be accomplished by explaining that you want simultaneously to assess both possible organic and possible behavioral causes of the problems.

9 Summary

We now have more than 30 years of solid, scientific research about Lyme disease, a relatively common, vector-borne illness in parts of the United States and of Europe. Although there is still widespread misunderstanding of and misinformation about the disease among the lay public, its clinical manifestations as well as how to diagnose and to treat it are now well understood. In the vast majority of cases simple treatment with a relatively short course of orally administered antimicrobials results in long-term cure with no adverse sequelae.

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Management of Myocarditis in Children: The Current Situation

Brigitte Stiller

1 Introduction

Acute myocarditis is an important cause of morbidity and mortality in children. It is defined as inflammation of the myocardium in association with non-ischaemic myocellular necrosis (Aretz 1987). Despite its clear-cut definition, the classification, diagnosis and treatment of myocarditis remain the subject of ongoing debate. The use of endomyocardial biopsy, which has become routine, has helped to better define the natural history of human myocarditis and to clarify clinicopathological correlations.

Clinical presentations of the disease range from non-specific systemic symptoms (fever, myalgia, palpitations or exertional dyspnoea) to fulminant haemodynamic collapse and sudden death. The extreme diversity of clinical manifestations has made the true incidence of myocarditis difficult to determine. Estimated prevalence ranges from 1 to 10 per 1,000,000 persons (Wakafuji and Okada 1986; Karjalainen and Heikkila 1999). Recent prospective post mortem data have implicated myocarditis in sudden cardiac death in young adults at rates of 8.6–12% (Doolan et al. 2004; Fabre and Sheppard 2006).

The Dallas pathological criteria, published in 1986, served as the first attempt to develop standardized diagnostic guidelines for the histopathological classification of myocarditis (Aretz 1987). Active myocarditis is characterized by an inflammatory cellular infiltrate with evidence of myocyte necrosis, whereas borderline myocarditis demonstrates an inflammatory cellular infiltrate without evidence of myocyte injury. This inflammatory infiltrate should be further defined as lymphocytic, eosinophilic or granulomatous. The amount of inflammation may be mild, moderate or severe, and its distribution may be focal, confluent or diffuse. A retrospective study of 112 consecutive patients with biopsy-confirmed myocarditis at the Massachusetts General Hospital demonstrated the following pathological distribution: lymphocytic 55%, borderline 22%, granulomatous 10%, giant cell 6%, and eosinophilic 6% (Magnani et al. 2006). Myocarditis progresses through stages with distinctively different mechanisms and manifestations. The etiopathogenesis of inflammatory myocarditis and cardiomyopathy is initiated by a viral (or rarely bacterial) infection or other noxes (Table 1). Viral proliferation triggers a host

Table 1 Major etiologies of myocarditis (modified from Magnani et al. 2006)

Viral
Adenovirus
Coxsackievirus
HCV
HIV
Bacterial
Mycobacterial
Streptococcal species
<i>Mycoplasma pneumoniae</i>
<i>Treponema pallidum</i>
Fungal
<i>Aspergillus</i>
<i>Candida</i>
Coccidioides
<i>Cryptococcus</i>
Histoplasma
Protozoal
<i>Trypanosoma cruzi</i>
Parasitic
Schistosomiasis
Larva migrans
Toxins
Anthracyclines
Cocaine
Interleukin-2
Hypersensitivity
Sulfonamides
Cephalosporins
Diuretics
Digoxin
Tricyclic antidepressants
Dobutamine
Immunological syndromes
Churg–Strauss
Inflammatory bowel disease
Giant cell myocarditis
Diabetes mellitus
Sarcoidosis
Systemic lupus erythematosus
Thyrotoxicosis
Takayasu’s arteritis
Wegener’s granulomatosis

immunological response, usually resulting in viral clearance. In a subset of individuals, however, a secondary phase evolves in which an immune reaction is initiated, involving both cellular and humoral effectors. This may lead to resolution of the infection or a chronic phase of inflammation, persistent infection or

autoreactive dilated cardiomyopathy (Maisch et al. 2002). Management decisions must take into account the particular phase of this disease continuum. For each of the phases, pathogenesis, diagnostics and treatment options differ considerably.

Therapeutic strategies in different centres vary from supportive therapy alone to the administration of steroids, immune globulin and other immunosuppressive medications (English et al. 2004). Clinical data regarding the efficacy of these strategies are lacking (Figs. 1 and 2).

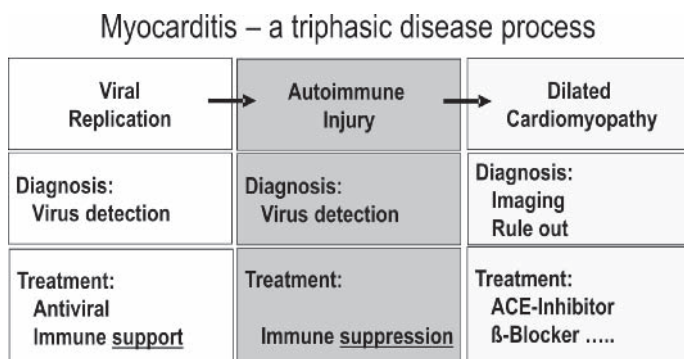


Fig. 1 Different phases of myocarditis

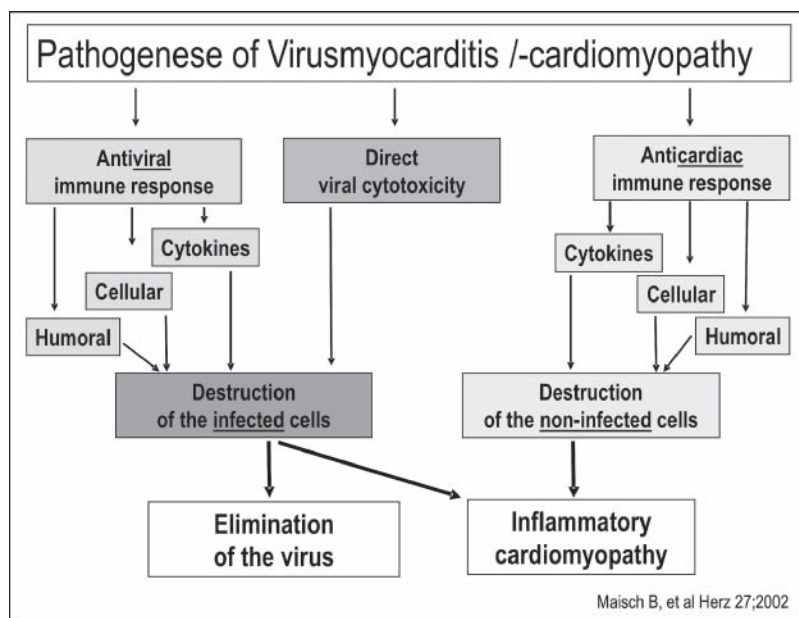


Fig. 2 The pathogenesis of virus myocarditis and cardiomyopathy

2 Viral Myocarditis: Three Different Phases

2.1 Phase 1: Viral Infection

Phase 1 is an induction phase during which the virus itself plays a major role. The enteroviral species, such as coxsackievirus B3 and B4 and adenoviruses, are detected either in serum or by direct molecular techniques such as polymerase chain reaction (PCR) or in situ hybridization. The coxsackie-adenoviral receptor (CAR) has been described by Bengelsson et al. (1997). CAR is a transmembrane protein with immunoglobulin-like domains that binds adenoviruses and coxsackieviruses and allows internalization of viral genomes. Both virus types have evolved independently to interact with this receptor that is itself important for normal heart development. CAR expression within the myocardium is highest during the embryonic stage and appears to be down-regulated after birth. However, expression within the heart is increased in certain diseases, including some forms of cardiomyopathy, and after myocardial infarction. It is possible that the function of CAR during myocardial development is recapitulated in cardiac diseases and that CAR acts to inhibit cardiomyocyte proliferation after myocardial injury (Chen et al. 2006). Coxsackievirus B (CVB) utilizes the complement-deflecting protein decay accelerating factor (DAF) as its co-receptor, whereas adenovirus uses integrin $\alpha_{v\beta3}$ and $\alpha_{v\beta5}$ as its co-receptors. DAF serves an important function as a co-receptor by significantly increasing the efficiency of coxsackievirus binding onto the DAF–CAR receptor complex to facilitate its internalization by CAR (Liu and Mason 2001).

The secondary immune response to viral infection probably plays a greater role in disease pathogenesis than the primary infection. After gaining a portal of entry into the host, through the intestine in the case of enteroviruses and through the respiratory tract for both the enteroviruses and adenoviruses, the virus is harboured in the immune cells of lymphoid organs, temporarily escapes immune clearance, and is secondarily transported to other target sites such as the heart.

2.2 Phase 2: Autoimmune Injury

Phase 1 concludes with activation of the host immune system, which attenuates viral proliferation but may also enhance viral entry. Ideally, the immune system should down-regulate to a resting state once viral proliferation is brought under control. However, if host immune activation continues unabated despite elimination of the virus, autoimmune disease may result, initiating phase 2 of the disease. *T cells* may then target the host's own tissue through molecular mimicry. *Cytokine activation* and cross-reacting antibodies may further accelerate the process:

2.2.1 T Cells

T cells are triggered in the setting of viral infection of the myocardium through classic cell-mediated immunity. Viral peptide fragments are processed in the Golgi apparatus of the host cell and presented to the cell surface in a major histocompatibility complex-restricted manner. This immune activation is teleologically protective, as the T cells seek out virus-infected cells and destroy them by either cytokine production or perforin-mediated cell cytolysis. However, continuous, exuberant activation of the T cells is ultimately detrimental to the host, because both cytokine-mediated and direct T-cell-mediated myocyte damage reduces the number of contractile units. The cumulative effect causes impairment of contractile function, which leads to long-term remodelling and phase 3 of the disease, dilated cardiomyopathy.

2.2.2 Cytokines

Cytokines are major mediators of immune activation and of its maintenance. Matsumori et al. (1994) showed that patients with myocarditis have marked activation of cytokines including tumor necrosis factor- α (TNF- α), interleukin (IL)-1, and IL-6. The pattern of activation may indeed determine the type of T-cell reaction and the subsequent degree of autoimmune perpetuation (Penninger et al. 1997). Similar patterns of cytokine activation are seen in murine models of myocarditis, with many of these cytokines directly detectable in heart tissue. Cytokines contribute in a major way to disease phenotype. For example, mice deficient in the TNF p55 receptor (TNF-R1^{-/-}) have milder autoimmune myocarditis (Yamada et al. 1994). On the other hand, IL-12 has been shown to be protective by reducing viral replication, inflammation and myocyte necrosis and improving survival (Shioi et al. 1997). The delicate balance of all these mechanisms determines the degree of myocardial injury.

2.3 *Phase 3: Dilated Cardiomyopathy*

There is increasing evidence that viral myocarditis is the substrate for the development of many cases of dilated cardiomyopathy. Kühl and colleagues (2005) report a high prevalence of viral genomes and multiple viral infections in the myocardium of adult patients with dilated cardiomyopathy (DCM) and suggest that myocardial persistence of various viruses can play a role in the pathogenesis of DCM. In 245 DCM patients, amplification of the viral genome was possible in 67.4% and the most frequently found virus was ParvoB19 (51%).

A possible mechanism: coxsackieviral protease can cleave dystrophin, a major component of the myocardial cytoskeleton and the sarcoglycan complex in the myocardial myocytes. Badorff and Knowlton (2004) have shown that a coxsackieviral

protease can directly cleave dystrophin and modify the sarcoglycan complex in myocytes. This may be one of the mechanisms potentially explaining the significant ventricular dilation that may be seen soon after viral infection. This group has also shown that mice expressing the coxsackievirus genome, devoid of the capacity to replicate, develop cardiac dilatation, which could be explained by many mechanisms, including that of the viral protease mentioned above.

Cytokines may also contribute to the development of dilated cardiomyopathy. During the autoimmune phase, they activate the matrix metalloproteinases, such as gelatinase, collagenases, and elastases (Ono et al. 1998). The dilated cardiomyopathy seen in experimental models can be significantly attenuated by treatment to interfere with matrix degradation, for example with elastase inhibitors.

Viruses may also directly cause myocyte apoptosis. Ongoing viral persistence is associated with much worse outcome (early death or need of transplantation). Cytokines may activate death-domain or ceramide-mediated signalling pathways as part of the remodelling process (Liu and Sole 1999). In later stages of immune activation, cytokines play a leading role in adverse remodelling and progressive heart failure. This is clearly demonstrated by a study by Nakamura et al. (1999). They introduced a viral infection that initially produced only mild cardiac pathology. However, on the second introduction of the virus at a much later date, the heart rapidly became dilated. Cardiomyopathy developed despite the absence of viral proliferation but was correlated with elevated levels of cytokines such as TNF.

3 Diagnosis and Treatment of the Different Phases: Current Recommendations

The following recommendations draw on current knowledge but are unproved. The appropriate therapy for myocarditis requires specific knowledge of the phase of the disease at the time treatment is contemplated. This is important because each form of treatment has the potential to worsen rather than improve myocarditis if administered at the wrong time.

3.1 Phase 1: Diagnosis and Treatment in Viral Replication

3.1.1 Diagnosis

Viral infection may be suspected on the basis of history and presentation but can only be proved by direct or serological identification of virus. However, in this phase the presence of viral replication is difficult to prove quickly enough to allow application of appropriate antiviral therapies because of the rarity of obtaining a positive culture, the impracticality of obtaining serial viral titers, the unavailability of an established, rapid, non-invasive screening tool for the immediate detection of viral protein or

genetic material, and the need to know the specific infectious agent for optimal selection of antiviral therapy. This phase may transpire unnoticed, without symptomatic myocardial failure. When cardiac involvement is apparent during phase 1, in most patients the diagnosis of myocarditis is based on unspecific symptoms such as fever, lymphocytosis and symptoms of upper respiratory or gastrointestinal infection. The patient may also have chest pain and atrial or ventricular arrhythmia.

ECG findings in the acute phase of clinical myocarditis can include widened QRS complex, left bundle-branch block, ST-segment and T-wave changes, and heart block.

Echocardiography may reveal decreased systolic ventricular function and wall-motion abnormalities, but most often these are insufficient to differentiate myocarditis from other forms of cardiomyopathy. Ultrasonic tissue characterization may prove to be more useful. Transmission and reflection of ultrasound energy depends on tissue density, elasticity, and acoustic impedance. Changes in one or more of these factors lead to different ultrasonic backscatter and an altered image texture. Lieback et al. (1996) evaluated mean grey-scale values (indicative of average brightness) in 52 patients with biopsy-proven myocarditis; 12 patients had persistent myocarditis, 9 had healed myocarditis but no fibrosis, and 17 patients had healed myocarditis and fibrosis. Tissue characterization was highly effective in differentiating myocarditis from healthy control myocardium, with sensitivity and specificity values of 100% and 90%, respectively (Lieback et al. 1996). However, ultrasonic tissue characterization could not accurately differentiate between idiopathic dilated cardiomyopathy and active myocarditis. More recent techniques, particularly tissue Doppler imaging and myocardial velocity measurements, are better able to characterize tissue changes in acute myocarditis and to monitor changes in these parameters over time. Additional validation studies will be required to determine their clinical utility.

Contrast-enhanced MRI appears to be a promising technique for diagnosing myocardial inflammation and myocyte injury on the basis of small, observational clinical studies. Besides providing anatomic and morphological information, MRI can provide accurate tissue characterization by measuring T1 and T2 relaxation times and spin densities (Fenster et al. 2006). Because active myocarditis is typically associated with myocyte injury, including oedema and cellular swelling, assessment of relaxation times provides a sensitive measure for its detection. MRI may not only be useful in identifying patients who should undergo biopsy but can also facilitate a guided approach to the abnormal region of myocardium. It is hoped that this focused methodology will improve the sensitivity of endomyocardial biopsy for establishing a correct histological diagnosis. Serial MRI studies have also shown promise for tracking the natural history of the disease and may, in the future, allow non-invasive reassessment of the myocardial response to therapy.

Endomyocardial biopsy is generally not necessary in every case, but in patients with clinical evidence of myocarditis a virological diagnosis with myocardial biopsy offers a rapid, sensitive diagnostic method for myocardial viral infection. PCR used in conjunction with standard endomyocardial biopsy appears to enhance the likelihood of detecting viral genome in the myocardium.

3.1.2 Therapy

Appropriate treatment at this stage aims to eradicate the virus and attenuate viral injury. Treatment principles include the avoidance of potentially harmful immunosuppression and the performance of non-specific antiviral measures. Direct antiviral therapy should be used in the few cases in which an organism has been identified or in the context of a known viral epidemic. The reduction of viral entry, attachment, and proliferation diminishes the severity of myocarditis in experimental models. We look forward to the results of the European ESETCID trial (European Study of Epidemiology and Treatment of Cardiac Inflammatory Disease), which is addressing the roles of immune globulin and interferon in the different phases of the disease (Maisch et al. 2004).

3.2 Phase 2: Diagnosis and Treatment During Autoimmune Activation

3.2.1 Diagnosis

The autoimmune phase is diagnosed by endomyocardial biopsy. Serological markers may be abnormal as well. However, viral serology is often positive in this phase and high background and non-cardiac specificity detract from its significance.

3.2.2 Therapy

Theoretically, immune suppression is the appropriate therapy in this stage, unless significant viral replication persists but, in the absence of large, randomized, placebo-controlled trials of biopsy-proven viral myocarditis, the potential for benefit or harm from different immunomodulatory strategies remains uncertain. A variety of immunomodulatory therapies have been proposed, including immunosuppression, manipulation of cytokines, and anti-T-cell-receptor vaccines. Steroids, azathioprine, cyclosporine, and OKT-3 have been used as immunosuppressive agents in humans with myocarditis (Magnani and Dec 2006).

Immunoglobulin, which may have immunomodulatory effects independent of its potential direct antiviral effects, has also been used and recommended (Maisch et al. 2004) in the literature, because of assumed improvement of myocardial function. Despite the potential therapeutic efficacy suggested by previous uncontrolled studies, treatment of adult patients with recent-onset cardiomyopathy with immune globulin in a placebo-controlled trial did not affect improvements in LVEF or functional capacity during follow-up. Although the mean improvement in EF at 1 year in the treated group was similar to that seen in the previous pilot study, this increase was essentially equalled in patients who were given placebo (McNamara et al. 2001). This high rate of spontaneous recovery with conventional therapy was

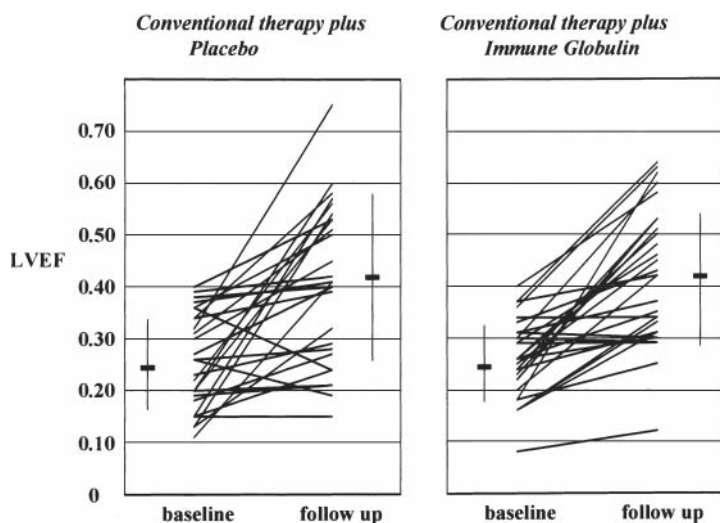


Fig. 3 LVEF over time by treatment: LVEF by radionuclide scan at baseline and after 12 months in patients randomized to placebo and IVIG. Overall, LVEF improved significantly over time (12-month LVEF significantly higher than baseline, $P < 0.001$). However, no differences by treatment group were evident ($P = \text{NS}$ for comparisons by treatment). (From McNamara et al. 2001.)

greater than anticipated but most likely underlies the apparent improvement reported in previous uncontrolled reports (see Fig. 3).

On the other hand this adult study by McNamara et al. cannot address the use of immune globulin for the treatment in children. In children this illness is more frequently associated with a positive endomyocardial biopsy, febrile illness, and a short duration of symptoms, lasting days or weeks rather than months. Children appear more likely than adults to present in the earlier inflammatory stage of the illness, and animal studies of the effects of immune globulin on virally induced cardiomyopathy show that the drug is most effective when given during the early viremic phase. Thus, failure of the study by McNamara et al. to prove efficacy for IVIG in the adult population does not rule out the potential effectiveness of immune globulin in children with a clinically similar but potentially pathologically distinct disorder. Gullestad et al. (2001) also studied the efficacy of intravenous immune globulin in a randomized trial of 40 adult patients with chronic (mean duration, 3.5 ± 0.5 years) rather than acute dilated cardiomyopathy. Biopsy was not performed, and therefore the percentage of patients with myocarditis remains unknown. IgG therapy was associated with a marked increase in serum anti-inflammatory markers (e.g., IL-10, soluble TNF), which correlated with significant improvement in LVEF (from $26 \pm 2\%$ to $31 \pm 3\%$; $P < 0.01$) at 6 months. Such changes were not observed in the control group.

The long-term benefit of this treatment strategy remains unknown. However, these studies do suggest that a prospective randomized study may be warranted in children to truly evaluate the potential efficacy of immune globulin in the paediatric population.

Parrillo et al. (1989) conducted the first immunosuppressive trial of patients presenting with unexplained dilated cardiomyopathy. The disease was classified as reactive or non-reactive on the basis of histopathology (fibroblastic or lymphocytic infiltrate), immunoglobulin deposition on endomyocardial biopsy, a positive gallium scan, or an elevated erythrocyte sedimentation rate. Patients with the reactive form treated with prednisone (60mg daily) had a statistically greater likelihood of achieving a predefined end-point of an increase in LVEF $\geq 5\%$ at 3 months. This improvement was not sustained at 6 or 9 months because the reactive control group showed comparable spontaneous improvement in myocardial function. It should be noted that the prednisone dose was decreased to 60mg on alternate days in the reactive group after 3 months, and this change may have confounded later end-point analyses.

The Myocarditis Treatment Trial (Mason et al. 1995) randomized 111 patients with biopsy-verified myocarditis to receive placebo or an immunosuppressive regimen of prednisone and either cyclosporine or azathioprine. Analysis compared the placebo group with the combined immunosuppressive cohorts. No difference in mortality was evident between treatment groups; furthermore, the degree of improvement in LVEF at 28 weeks was identical (controls, 24–36%; immunosuppression, 24–36%). Multivariate analysis identified higher initial LVEF, less intensive conventional therapy, and shorter duration of symptoms as independent predictors of subsequent improvement. These two controlled trials suggest that immunosuppression should not be prescribed for the routine treatment of viral myocarditis. Immunosuppression can benefit patients with myocarditis due to systemic autoimmune diseases, particularly lupus erythematosus, scleroderma, and polymyositis. Patients with idiopathic giant cell myocarditis have also been shown to benefit from aggressive immunosuppressive protocols.

Immunoabsorption: The elimination of anti-cardiac antibodies, which have been associated with idiopathic DCM, is a concept currently under discussion, which is supported by published registry data and a few very small controlled investigations but not by a randomized double-blind trial with clinical end-points of relevance. In some studies immunoglobulins have been substituted, so that a potential additional immunomodulatory effect has to be taken into account.

An even more challenging and still more attractive hypothesis is that cardiac inflammation caused by specific circulating β -adrenoceptor antibodies can be eradicated with the elimination of the β -receptor antibody, thus curing dilated cardiomyopathy. But this approach also lacks randomized trials and both of these pathophysiologically attractive concepts have to await further validation by double-blind, randomized clinical end-point trials.

Table 2 Differential diagnosis of myocarditis by age

Newborn and infant
Sepsis
Hypoxia
Hypoglycemia
Structural heart disease
Idiopathic dilated cardiomyopathy
Barth syndrome
Endocardial fibroelastosis
Anomalous origin of left coronary artery from the pulmonary artery
Cerebral arteriovenous malformation
Child
Idiopathic dilated cardiomyopathy
X-linked dilated cardiomyopathy
Autosomal-dominant dilated cardiomyopathy
Anomalous origin of left coronary artery from the pulmonary artery
Endocardial fibroelastosis
Chronic tachyarrhythmia
Pericarditis

Source: Towbin (2001)

3.3 Phase 3: Diagnosis and Treatment During Dilative Cardiomyopathy

3.3.1 Diagnosis

This stage of the disease is usually recognized by imaging and other diagnostic procedures that exclude other causes of dilatation. Differential diagnosis of myocarditis by age is shown in Table 2.

3.3.2 Therapy

Treatment centres on reversal of the continued remodelling process by promotion of myocyte survival, attenuation of continued neurohormone and cytokine activation, and reduction of haemodynamic stress. The main aim is ventricular unloading and prevention of mechanical and hormonal stresses that worsen dilatation.

4 Clinicopathological Subtypes of Myocarditis

Lieberman et al. (1991) have proposed a clinicopathological classification of viral myocarditis that divides patients into four distinct subtypes based on their presenting symptoms, clinical courses, outcomes, and histological findings (Fig. 4):

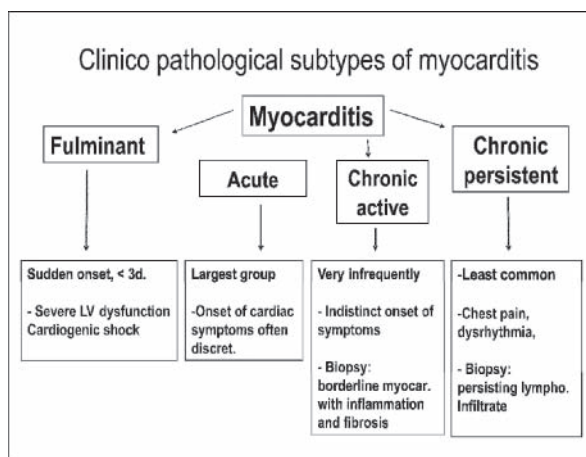


Fig. 4 Clinicopathological subtypes of myocarditis

4.1 Fulminant Myocarditis

Fulminant myocarditis is characterized by a distinct, sudden onset of cardiac failure (generally in <3 days), severe left ventricular dysfunction and cardiogenic shock. Endomyocardial biopsy reveals extensive inflammation and myocyte necrosis. Fulminant myocarditis, manifested by severe haemodynamic compromise requiring high-dose vasopressor support or mechanical circulatory support, was identified in 15 of 147 patients (10.2%) in the largest prospective study to use this classification system (McCarthy et al. 2000). Fulminant myocarditis was an independent predictor of survival after adjustments were made for age, histopathological findings, and haemodynamic variables. Patients with fulminant myocarditis were significantly less likely to die or require heart transplantation during follow-up than were patients with acute myocarditis (Fig. 5). The excellent long-term prognosis of children with this condition warrants aggressive haemodynamic support, as described at the end of this chapter.

4.2 Acute Myocarditis

Acute myocarditis affects the largest group of patients. Interstitial aggregation of mononuclear cells, including lymphocytes, plasma cells, and eosinophils, is frequently seen in early myocarditis. Necrosis of the myocardium with loss of cross-striations of the muscle fibres can also be seen, particularly with coxsackievirus. The onset of cardiac symptoms is often indistinct, and patients seem to develop a more gradual deterioration in ventricular function. In myocardial biopsy active or

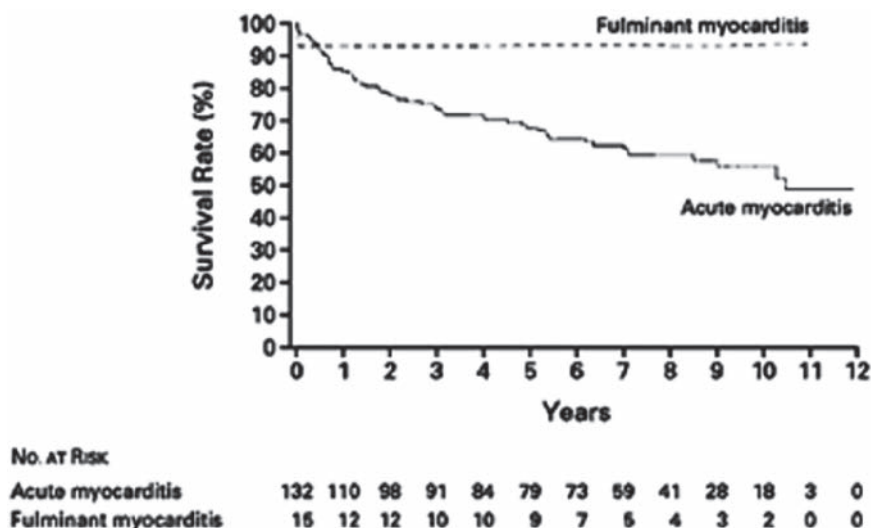


Fig. 5 Long-term outcome of fulminant myocarditis as compared with acute (non-fulminant) myocarditis (McCarthy et al. 2000) Patients with fulminant myocarditis were significantly less likely to die or require heart transplantation during follow-up than were patients with acute myocarditis

borderline myocarditis is seen, but inflammation may be absent in some specimens. Children may recover spontaneously or enter the autoimmune and cardiomyopathy phases described above.

4.3 Chronic Active Myocarditis

Chronic active myocarditis occurs very infrequently and is characterized by indistinct onset of congestive heart failure with left ventricular dysfunction. Endomyocardial biopsy reveals active or borderline myocarditis with persistence of inflammation combined with extensive fibrosis. There is a slow progressive deterioration in ventricular function.

4.4 Chronic Persistent Myocarditis

Chronic persistent myocarditis is the final and least common subtype. Patients present with atypical chest pain and dysrhythmia, but ventricular function is generally normal. Endomyocardial biopsy shows a lymphocytic infiltrate that persists in subsequent biopsies.

5 Therapeutic Strategies for Paediatric Heart Failure and Haemodynamic Instability

Children with myocarditis and only mild to moderate cardiac failure should be treated with conventional pharmacological agents, including *diuretics* and *ACE inhibitors*. Digoxin worsens viral myocarditis and thus its use in high doses should be avoided in patients suffering from viral myocarditis (Matsumori et al. 1999). *Anticoagulation* with aspirin, warfarin or heparin may be used to prevent mural thrombus formation when the left ventricle is markedly dilated and contractile function is severely depressed. After stabilization, a β -adrenergic antagonist, such as *carvedilol*, may be introduced cautiously and titrated up over 6–8 weeks. β -blockade in general and carvedilol in particular reduces mortality and improves left ventricular function in chronic heart failure (Packer et al. 1996; Laer et al. 2002). However, β -blockers are contra-indicated in children with acute severe circulatory failure when inotropes are required. Circulating catecholamines influence the production of cytokines through β -receptor stimulation. Carvedilol has been shown to reduce myocardial injury and mortality in murine myocarditis, possibly by increasing the production of IL-12 and interferon (Nishio et al. 2003). Ventricular ectopy is common and should be treated aggressively with amiodarone because of the high frequency of sudden death in patients with myocarditis (Doolan et al. 2004; Fabre and Sheppard 2006). *Extracardiac adjuncts* include mechanical ventilation, optimized haemoglobin and blood gas levels, spironolactone, diuretics and, in the case of renal failure, peritoneal dialysis or haemofiltration which should be instituted early on. If cardiac output is low a phosphodiesterase inhibitor such as milrinone should be considered (Hoffman et al. 2003; Duggal et al. 2005). *Milrinone* has the advantage that it does not increase oxygen consumption and improves diastolic function. The possibilities of pharmacological escalation in the case of further cardiogenic shock include the use of inotropic agents combined with afterload reduction to elevate not so much the blood pressure, but the cardiac output.

If pharmacological treatment fails and children remain in cardiogenic shock, mechanical circulatory support (MCS) is indicated. MCS has been applied with a broad variety of systems designed to unload the heart and provide adequate perfusion of the organs. It includes modifications of the original “heart-lung machine” circuits such as extracorporeal membrane oxygenation (ECMO) and extracorporeal centrifugal pumps. Application of these systems is limited to several days or a few weeks at the most and the patient must remain in the intensive care unit. True ventricular assist devices (VADs) are either extracorporeal pulsatile pneumatic or implantable electrical systems with a variety of designs and functional principles that allow patients to be mobile and lead an almost normal life except for the need for an external power source, which today is usually portable. Such VADs have gained wide acceptance for temporary and long-term assistance and have even supported adults as a permanent solution for more than 5 years. Several types of VADs have been used in children and adolescents with body surface area $>1.2\text{m}^2$, i.e. over 5 years of age. Miniaturized

VADs for smaller children and infants were introduced in 1992 (Berlin Heart Excor, Berlin Heart AG, Germany) and in 1994 (Medos HIA, Medos Medizintechnik AG, Stolberg, Germany) and these systems are still the only ones applicable in this age group at present. Both devices are extracorporeal and pneumatically driven; they come in a variety of pump sizes and with cannulae to be connected to the left ventricle (LVAD), the right ventricle (RVAD) or both ventricles (BVAD).

For children, no defined criteria for when to start with extracorporeal life support exist and we often have to deal with children suffering from unexpected acute deterioration of haemodynamic function, especially in cases of fulminant myocarditis.

The following parameters point to the severity of low cardiac output syndrome:

- Progressive or persistent poor peripheral perfusion
- Increased ventricular filling pressures
- Cardiac index below 2.0
- Mixed venous saturation <40%
- Persistent metabolic acidosis
- Oliguria (<1 mL/kg/min)
- FiO₂ requirements increasing
- Poor cardiac function (echocardiography).

In these extremely endangered children, we first connect the regular cardiopulmonary bypass by median sternotomy to gain time for safe implantation of the cannulae for longer-term support with the Berlin Heart. After sternal and skin closure the children are treated on our ICU. These severely ill infants with multi-organ failure after resuscitation often require the whole spectrum of ICU therapy, including peritoneal dialysis. Normally these problems disappear due to good cardiac/pump output within the first week on VAD. The blood pressure depends on pump rate and peripheral vascular resistance. Norepinephrine is sometimes necessary during the first few days but afterwards, with recovery of the organs and awakening of the patient, we try to reduce the afterload with ACE inhibitors and give β -blockers. This regime ensures good protection of the myocardium and potential recovery of the myocytes, especially in children with acute myocarditis.

Compared to ECMO (Cook 2004; Lin et al. 2005) or VADs in the form of centrifugal pumps (Duncan et al. 2001) the Berlin Heart offers a much longer gain in time in which to restore organ function and eliminate oedema and allows extubation, mobilization and improvement of nutritional status (Stiller et al. 1999, 2003, 2004, 2005; Hetzer et al. 2006).

Children with acute viral myocarditis are those who do best with the Berlin Heart. These patients were healthy until the onset of fulminant myocarditis, and prolonged circulatory support with a pulsatile pneumatic device is an effective method for bridging until cardiac recovery. If there is no improvement in myocardial function on the VAD, there is still the possibility of transplantation. Duncan et al. (2001) reported ECMO or centrifugal pump treatment of 15 children with fulminant myocarditis. They achieved excellent results with 80% survival, but survival without transplantation was only 47%. In their study the median support time was

only 6 days, and five children underwent early heart transplantation. Mechanical support for a longer time with a VAD such as the Berlin Heart Excor might have led to complete myocardial recovery, which we have seen after 10–21 days (Stiller et al. 1999), making pump explantation possible.

The *Berlin Heart Excor* ventricular assist device (VAD) (Berlin Heart AG, Berlin, Germany) is a pulsatile system that has proved successful in children of all ages, from the newborn to the adolescent. The paediatric version of the Berlin Heart Excor VAD is mounted with trileaflet polyurethane valves and comes in pump sizes of 10, 25, 30, 50 and 60 mL (Hetzer et al. 2006). The smallest pumps are suitable for neonates and infants with body weight of up to 9 kg and the 25 and 30 mL pumps for children up to 25 kg body weight. Special silicon cannulae connect the blood pumps to the body (Fig. 6). These cannulae are anastomosed to the right atrium and pulmonary artery for right ventricular support, and to the apex of the left ventricle or, more rarely, to the left atrium and the ascending aorta for left ventricular support. During the past 5 years we have used apical cannulation whenever possible because the unloading of the left ventricle is then much more

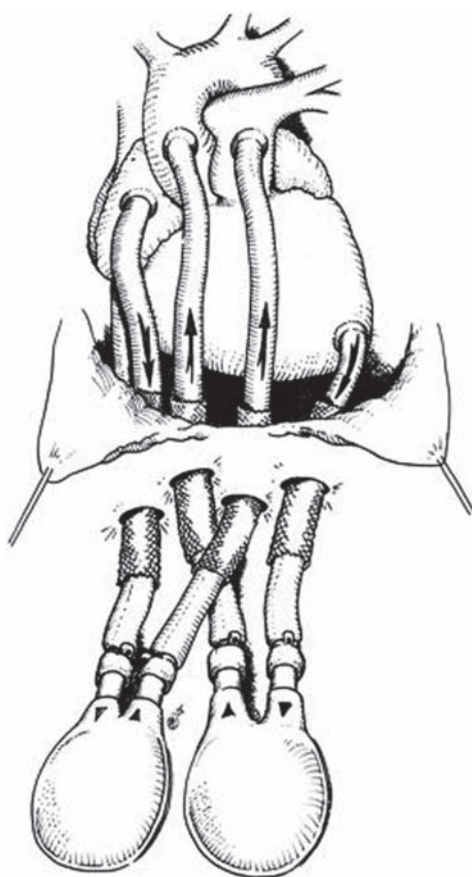


Fig. 6 Standard configuration of Berlin Heart Excor biventricular support. Preferably the apex of the left ventricle is cannulated, as this unloads the left ventricle more completely and in many cases makes the addition of a right VAD unnecessary

efficient than with left atrial cannulation and thus it is more often sufficient to apply a left-ventricular device rather than bi-ventricular support.

The largest numbers of children on Excor worldwide have been treated at our hospital, the German Heart Institute Berlin. We have experience with 73 children and more than 2,600 cumulative patient days in children aged from 2 days up to 17 years (mean age 7 years), with a body weight of 3–81 kg (mean 25 kg). All had severe circulatory failure resistant to pharmacological therapy. The children were suffering from cardiomyopathy, fulminant myocarditis, end-stage congenital cardiac defects or acute heart failure following congenital heart surgery. Mean Excor support time was 36 days (range 1–420 days). Forty-four patients (62%) survived up to transplantation or after weaning (Hetzer et al. 2006).

Within the past decade we have gradually developed a new concept with changes in cannulation and anticoagulation and a paradigm shift in ICU management. As a result, the rate of survival to discharge home in the past 5 years, during which 32 consecutive children were supported with Berlin Heart Excor, has increased to 78%. Seven children were successfully weaned from the device, 18 underwent heart transplantation, and only 7 died on the system). Of the 22 children treated for either cardiomyopathy or fulminant myocarditis within this group, 91% survived and were discharged home.

6 Conclusions

The treatment of myocarditis in 2006 remains largely supportive. Immunosuppression has not been shown to be effective as routine treatment for acute lymphocytic myocarditis. Early trials of antiviral agents, such as interferons, suggest a potential therapeutic role but they require further investigation. Currently, the standard procedure in acute cardiomyopathy remains haemodynamic and cardiovascular support, including the use of ventricular assist devices, and transplantation when necessary. Pharmacological treatment should consist of a heart failure regimen demonstrated to improve the haemodynamics and clinical symptoms. Although the high rate of spontaneous improvement in acute myocarditis and cardiomyopathy gives grounds for some optimism, patients who progress to chronic dilated cardiomyopathy have 5-year survival rates of <50%. Ongoing clinical trials should help to clarify whether immune-modulating strategies can improve this prognosis.

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Therapy of Herpes Virus Infections in Children

Richard J. Whitley

1 Introduction

Rapid advances have been achieved in the therapy of herpes virus infections of children over the past 25 years. Following the demonstration that vidarabine was an efficacious treatment for neonatal herpes simplex virus (HSV) infections, herpes simplex encephalitis, and varicella zoster virus (VZV) infections of children, significant advances were achieved with the development of second generation anti-viral drugs. The second generation anti-viral drug, namely acyclovir, and, subsequently, valacyclovir and famciclovir, now provide both efficacious as well as safe anti-viral therapy for a variety of herpes virus infections. More recently, the demonstration that ganciclovir proved efficacious for the treatment of congenital cytomegalovirus (CMV) infections provides impetus for improving therapeutics for the most common congenital infection of humans. The therapeutic advances achieved in the management of neonatal HSV infection, herpes simplex encephalitis, VZV infections of the normal and immunocompromised host, and congenital CMV infection will be summarized in this review.

2 Neonatal Herpes Simplex Virus Infection

Neonatal HSV infection is acquired at one of three times: intrauterine, peripartum, and postnatal. The majority of infants are infected during the peripartum period by exposure to infected maternal genital secretions at delivery. In the United States the incidence of neonatal herpes varies between one in 1,500 to one in 3,000 live births. This incidence is significantly higher than that recognized in other countries of the world, particularly the United Kingdom. Those infants born to women who experience a first episode genital infection during the third trimester of gestation are at significantly greater risk of developing neonatal herpes than are those whose mothers have recurrent genital herpes (Nahmias et al. 1971; Brown et al. 1987, 1991, 2003; Corey and Wald 1999). This increased risk is caused both by lower concentrations of transplacental HSV antibodies and the significantly greater

quantity of HSV that is shed in the genital tract for longer periods of time. Other factors that influence the acquisition of HSV infection during the peripartum period include the application of a scalp electrode and prolonged rupture of membranes (Whitley et al. In Press).

Neonatal HSV infections are classified according to the extent of disease. First, the most severe form of disease is disseminated multi-organ involvement that includes infection of visceral organs, including any combination of lung, liver, adrenal glands, skin, eye, and brain. Overall, approximately 25% of children present with this form of neonatal herpes. The second classification of disease is that which involves the central nervous system (CNS), namely encephalitis, with or without concomitant skin involvement. About 30% of children present with encephalitis. The last form of disease is that which is localized to the skin, eye or mouth, accounting for 45% of babies with neonatal HSV infection.

Disease classification is associated with unique demographic characteristics and is also predictive of both morbidity and mortality (Whitley et al. 1980, 1991a, 1991b; Kimberlin et al. 2001a, 2001b). Neonatal HSV infections involve both sexes equally and all races. Those children with disseminated disease are more likely to be born prematurely than newborns with either of the other two forms of disease. Babies with disseminated disease or skin, eye and mouth involvement tend to present to health care providers between 10–12 days of life. In contrast, children with central nervous system disease present for medical care between 16–19 days of life. As would be expected, disseminated disease is associated with the highest mortality while infection of the central nervous system is associated with the highest morbidity. Skin, eye and mouth involvement is the most benign form of infection but can progress to central nervous system disease if not carefully managed.

The diagnosis of neonatal HSV infection has been revolutionized by the application of polymerase chain reaction (PCR) to biologic specimens, especially the cerebrospinal fluid. The application of PCR to cerebrospinal fluid (Rowley et al. 1990; Kimura et al. 1991; Anderson et al. 1993; Troendle-Atkins et al. 1993; Schlesinger and Storch 1994; Kimberlin et al. 1996; Malm and Forsgren 1999) and blood (Barbi et al. 1998; Diamond et al. 1999; Malm and Forsgren 1999; Kimura et al. 2002; Lewensohn-Fuchs et al. 2003) provides a rapid assay for the detection of HSV DNA. When performed by a competent laboratory, the sensitivity and specificity of the assay exceeds isolation of virus in cell culture. For application to the cerebrospinal fluid, the sensitivity and specificity for the detection of HSV DNA in patients with herpes encephalitis is 98% and 95%, respectively. However, PCR results must be correlated with the clinical condition of the child since there is variability in inter-laboratory performance. Thus, if the PCR test is negative but the patient clinically has HSV infection, antiviral therapy should be continued. The isolation of HSV in cell culture continues to be an essential component of the diagnostic evaluation of the suspected infected patients. The isolation of HSV in cell culture allows for subsequent typing of HSV (HSV-1 versus HSV-2). Currently, approximately 25% of cases of neonatal HSV infection are caused by HSV-1 and the remainder are caused by HSV-2. Importantly, HSV-1 infection of the central nervous system is associated with a significantly better outcome than HSV-2.

Polymerase chain reaction can also be used to study disease pathogenesis and predict outcome. The application of PCR to detect evidence of HSV DNA at the completion of antiviral therapy appears to be a predictor of morbidity. Infants classified as having central nervous system involvement who were PCR positive for HSV DNA at the completion of antiviral therapy are more likely to either die or have severe neurologic sequelae (Kimberlin et al. 1996). This recognition indicates that an end-of-therapy cerebrospinal fluid examination should be performed in order to determine clearance of HSV DNA. If HSV DNA is detected, therapy should be extended for a longer period of time (see below).

3 Antiviral Therapy of Neonatal HSV Infection

3.1 Mortality

Prior to the development of antiviral therapy, 85% of babies with disseminated HSV disease died by 1 year of age as did 50% of patients with central nervous system infection (Whitley et al. 1980). Evaluation of two different doses of vidarabine and a lower dose of acyclovir (30 mg/kg/day for 10 days) documented that both of these antiviral drugs reduced the mortality to comparable degrees (Whitley et al. 1980, 1983, 1991). The mortality rates at 1 year for disseminated disease decreased to 54% and for central nervous system (CNS) disease to 14%. Despite the lack of superiority of acyclovir, this medication quickly replaced vidarabine because of its improved safety profile and ease of administration.

With the administration of a high dose of acyclovir (60 mg/kg/d for 21 days), the mortality at 1 year of life was further reduced to 29% for disseminated disease and 4% for central nervous system infection (Figs. 1 and 2, respectively) (Kimberlin et al. 2001b, Kimberlin et al. 2001a). An altered level of consciousness and consumptive coagulopathy are associated with a higher mortality for babies with disseminated disease, as are prematurity and seizures in newborns with central nervous system disease (Kimberlin et al. 2001b).

3.2 Morbidity

3.2.1 Disseminated and CNS Neonatal HSV Disease

Improvement in morbidity with antiviral therapies has not been as dramatic as that for mortality. The proportion of survivors with disseminated neonatal HSV disease who have normal neurologic development has increased from 50% in the pre-antiviral era to 83% today (Whitley et al. 1980; Kimberlin et al. 2001a). In the case of central nervous system disease, while survival has improved dramatically, no improvement

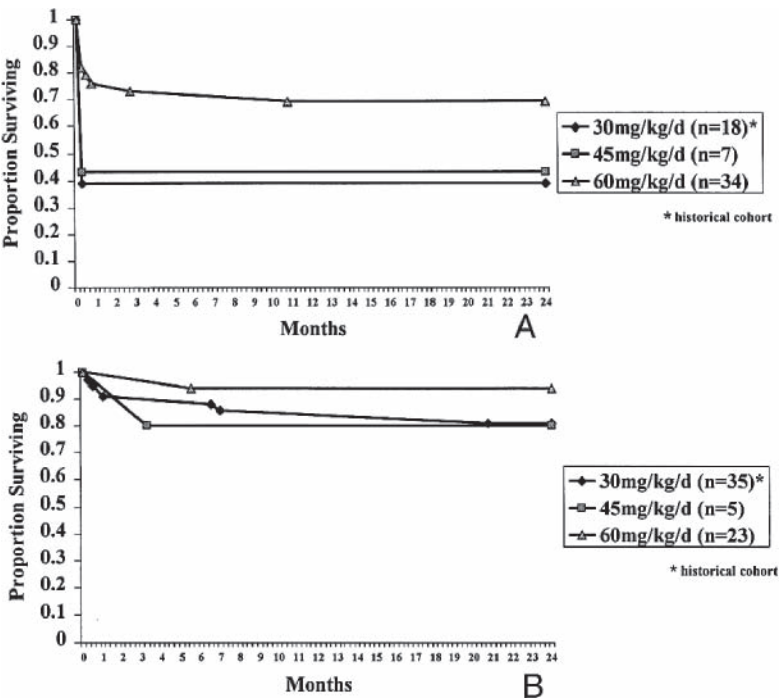


Fig. 1 Mortality in patients with A) disseminated disease and B) CNS disease (with permissions from the American Academy of Pediatrics).

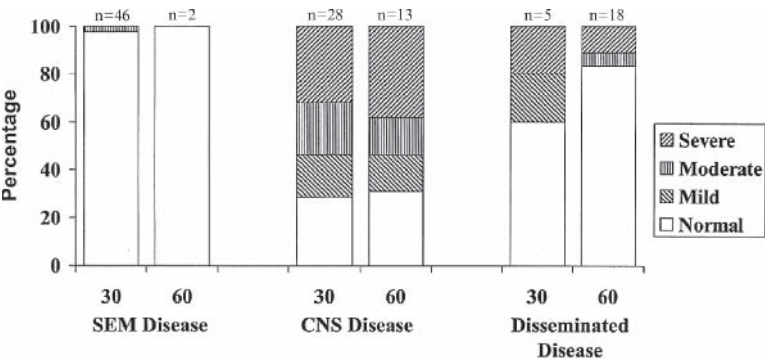


Fig. 2 Morbidity and mortality among patients after 12 months of age by viral type, 1981–1997 (with permissions from the American Academy of Pediatrics).

in morbidity has been documented, as 33% of babies in the pre-antiviral era and 31% of those today develop normally following treatment (Fig. 3) (Whitley et al. 1980; Kimberlin et al. 2001a). Although these outcome data indicate an area for which improvement is unquestionably needed, it is important to reiterate that as more neonates survive neonatal HSV encephalitis, the total numbers of patients who

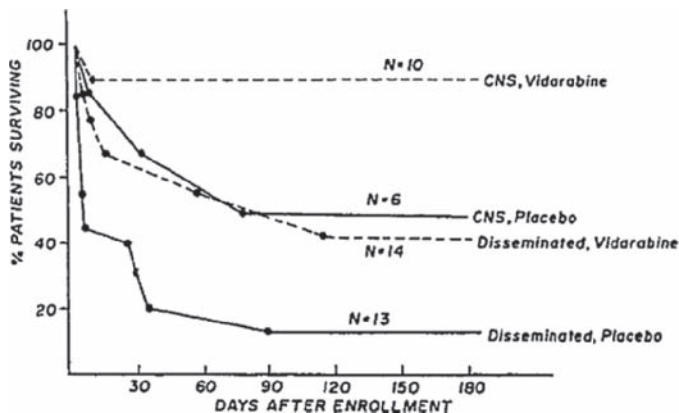


Fig. 3 Outcome of herpes simplex virus infection in neonates according to type of disease and therapy. Points represent last death(s) (with permissions from the American Academy of Pediatrics).

subsequently develop normally is higher. Seizures that occur at or before the initiation of antiviral therapy are associated with increased risk of morbidity both in patients with central nervous system disease and in patients with disseminated infection (Kimberlin et al. 2001b).

Infection of the central nervous system caused by HSV-2 is associated with greater morbidity than HSV-1 infection, as noted above.

3.2.2 Skin Eye and Mouth Disease

Unlike disseminated or central nervous system infection, morbidity following skin, eye or mouth disease has dramatically improved with antiviral therapy. Before the development of vidarabine or acyclovir, 38% of babies with SEM experienced developmental impairment at 12 months of age (Whitley et al. 1980) in large part because disease progressed to involve the central nervous system. Today, fewer than 2% of acyclovir recipients have developmental delays after recovering from skin, eye and mouth disease. The propensity to develop neurologic impairment with this form of localized disease can be directly attributed to progression to involve the central nervous system, in spite of antiviral therapy. With the development of PCR technology, many babies had evidence of HSV DNA in the cerebrospinal fluid at the time of presentation even though all biochemical parameters and neurologic assessments were totally normal, including normal head computed tomographic scans and ophthalmologic examination. Thus, these children had asymptomatic infection of the brain, placing them in a disease classification of increased risk for neurologic impairment.

3.2.3 Summary

The improvement in morbidity and mortality achieved with the administration of high dose acyclovir (60 mg/kg/day intravenously in three divided doses) is the currently recommended treatment. Drug is administered for 14 days for skin, eye and mouth disease and 21 days for either central nervous system disease or disseminated infection (Kimberlin et al. 2001; American Academy of Pediatrics 2003). As noted previously, it is prudent to perform an end-of-therapy PCR evaluation of the cerebrospinal fluid in order to document that it is negative.

The primary toxicity associated with the administration of high dose intravenous acyclovir is neutropenia, as approximately one-fifth of patients develop an absolute neutrophil count of 1,000/microL or less (Kimberlin et al. 2001a). Although the neutropenia resolves either during continuation of intravenous therapy or after its cessation, it is prudent to monitor neutrophil counts throughout the course of acyclovir therapy.

4 Herpes Simplex Encephalitis

The pathogenesis of herpes simplex encephalitis (HSE) in older children (greater than 3 months of age), adolescents, and adults is only partially understood, but likely is similar for all age groups. Herpes simplex encephalitis can be the consequence of either primary or recurrent HSV infection. Approximately one-third of HSE cases are the consequence of primary infection and, logically, these occur in children and adolescents (Nahmias et al. 1982). The remaining two-thirds of cases occur in the presence of pre-existing antibodies. The route of access of HSV to the central nervous system following primary infection is a subject of debate. Classic studies define pathways for access of HSV to the brain in animals and include both olfactory and trigeminal nerves (Johnson, Olson and Buescher 1968); however which of these nerve tracks uniformly leads to HSV infection of the brain of humans is not clear.

Herpes simplex virus infections of the central nervous system are among the most severe of all viral infections of the human brain. Currently HSE is estimated to occur in approximately 1 in 250,000 individuals annually (Longson 1984; Skoldenberg et al. 1984). In the United States, HSE is thought to account for as many as 10–20% of all viral infections of the brain, excluding West Nile Virus encephalitis. Herpes simplex encephalitis occurs throughout the year and in patients of all ages with approximately one-third of cases occurring in patients younger than 20 years of age but older than 3 months. Caucasians account for approximately 95% of all individuals with disease. Both sexes are affected equally.

The clinical presentation of HSE is that of a focal encephalitic process. Evidence of fever, altered mentation, and focal neurologic findings is characteristic of HSV infection of the brain. In addition, patients exhibit a progressive decrease in level of consciousness as the disease progresses, usually in spite of the initiation of antiviral therapy. Supporting neurodiagnostic studies include the use of

electroencephalogram (spike and slow wave activity that localizes to the temporal lobe), computed tomographic scan or magnetic resonance image scan by which demonstration of a lesion localized to the temporal lobe can be demonstrated.

Examination of the cerebrospinal fluid is essential in the establishment of a diagnosis of HSE. Biochemical parameters will demonstrate an elevated white blood count (lymphocytic predominance) and a cerebrospinal fluid protein that becomes progressively elevated as the disease progresses. The presence of cerebrospinal fluid red cells or, conversely, their absence is not diagnostic of HSE. Importantly, approximately 5–10% of patients will have a normal CSF formula on first evaluation.

Today, the application of PCR for the detection of HSV DNA in the cerebrospinal fluid has become the diagnostic method of choice (Rowley et al. 1990; Aurelius et al. 1991, 1993; Puchhammer-Stockl et al. 1993; Shoji et al. 1994; Sakrauski et al. 1994; Lakeman and Whitley 1995; Fodor et al. 1998; DeBiasi and Tyler 1999). The sensitivity and specificity of PCR detection of HSV DNA are 94% and 98%, respectively. As was noted previously, the presence or absence of HSV DNA in the cerebrospinal fluid must be correlated with the clinical condition of the patient. A negative PCR test for HSV DNA in the presence of progressive clinical deterioration with focal neurologic findings and without another etiology of infection should not result in the discontinuation of antiviral therapy.

4.1 Treatment

The initial therapeutic trials that established efficacy for the treatment of HSE utilized vidarabine for the management of biopsy proven disease (Whitley et al. 1977, 1981). However, as with neonatal HSV infection, acyclovir has replaced this medication in the physician's armamentarium. Acyclovir is superior to vidarabine for the treatment of HSE (Whitley et al. 1986). Acyclovir decreases the mortality rate to 19% 6 months after therapy. Importantly, 38% of patients irrespective of age returned to normal function. However, conversely most patients are left with significant neurologic sequelae. During the conduct of these studies, the variables of age, duration of disease, and level of consciousness at the outset of therapy proved to be the major determinants of outcome. For younger patients, and those with a more normal level of consciousness (lethargic as opposed to comatose), the probability of return to normal function was significantly greater than older patients, particularly those greater than 50 years of age. Ideally, therapy should be instituted before the onset of hemorrhagic necrosis of a dominant temporal lobe and a significant deterioration in consciousness.

Regarding morbidity, the vidarabine studies indicated that approximately 15–20% of patients overall developed normally following treatment. However, 22% had moderate or severe sequelae, and, ultimately, 65% died during follow up period. For acyclovir recipients approximately 40% of patients were normal or had minor impairment on long term follow-up, 10% had moderate sequelae and 50% were left with severe impairment or died. Relapse of HSE has been reported, although not well documented in patients receiving acyclovir (Wang et al. 1994; VanLandingham et al. 1988).

The current treatment of choice is acyclovir administered at a dose of 30 mg/kg/day for a period of 14–21 days. Medication should be administered at 8 h intervals. While ideally, one would like to increase the dose to match that which is administered to newborns, renal function of older individuals dictates that this is not usually feasible.

5 Varicella Zoster Virus

Varicella occurs worldwide but its epidemiology differs in temperate as compared to tropical climates. In tropical climates, varicella occurs at a much older age for reasons that are not entirely clear. Historically, the incidence of varicella paralleled the birth rate in the United States, namely 4 million cases annually. However, since the licensure of the varicella vaccine, the incidence of disease has plummeted. Varicella has a striking seasonal pattern, with the peak incidence most commonly reported in the winter and early spring. The natural history, pathogenesis and prevention of varicella by vaccination have attracted a great deal of attention in the pediatric infectious diseases community over the past decade. The impact of the varicella vaccine has been documented by a decreased incidence of chicken pox in selected populations, decreased hospitalizations and a lower mortality. As a consequence, the focus on treatment of chicken pox has been reserved for high risk individuals, namely the immunocompromised host and children with underlying diseases that leave them at a greater propensity for progressive disease. Thus, this section will focus primarily on the treatment of varicella in young children and adolescents.

5.1 *Treatment of Varicella in the Immunocompetent Host*

Varicella, even in the immune competent host, can be associated with significant morbidity. Medical management involves preventing avoidable complications especially secondary bacterial infection of the skin. These infections can be averted by meticulous care of the skin and by measures to decrease pruritus. Pruritus can be significantly lessened with topical dressings of calamine lotion or the oral administration of benadryl or atorax at appropriate dosages.

Oral acyclovir therapy accelerates cutaneous healing in the immune competent host but only when therapy is instituted within the first 24 h after disease onset. For children between 2 and 12 years of age, the total and mean number of lesions are significantly decreased as defined in a large, placebo-controlled trial (Dunkle et al. 1991). Similar benefit was evident in adolescents and adults. The dosage of oral acyclovir is 20 mg/kg (up to 800 mg) either 4 (adolescents) or 5 (adults) times daily for 5–7 days.

The outcome of treatment of chicken pox in the immune competent child with oral acyclovir can be summarized by the following observations: 1. The maximum number of lesions is significantly decreased in acyclovir recipients, by approximately 75, based upon counting a maximum of 500 lesions. 2. The time to cessation of new

lesion formation and the time to appearance of maximum number of lesions were significantly shorter for acyclovir than placebo recipients. For the cessation of lesion formation, the mean was 2.7 days for the acyclovir-treated group and 3.2 days for placebo recipients. 3. The acyclovir-treated children healed more rapidly than those receiving placebo, as measured by days to 50% reduction in the maximum number of lesions (2.9 versus 4.1 days for acyclovir and placebo recipients, respectively). 4. Systemic symptoms, including fever and a mean constitutional illness score, was also significantly reduced for acyclovir recipients. 5. Acyclovir was well-tolerated in all populations (Balfour et al. 1983; Dunkle et al. 1991; Wallace, Bowler and Murray 1992).

Because of the marginal clinical benefit for young children, as noted above, the American Academy of Pediatrics Committee on Infectious Diseases suggests that routine acyclovir therapy is optional for the immune competent child. Populations that could be considered for treatment include: adolescents, secondary or tertiary household exposures, and children receiving corticosteroid therapy for asthma, or who have cardiopulmonary disease (cystic fibrosis, congenital heart disease), diabetes, or chronic or severe skin disorders (atopic dermatitis, etc).

5.2 Treatment of Varicella in the Immunocompromised Host

The data from three successful antiviral trials in the immunocompromised child indicate that vidarabine, leukocyte interferon, and acyclovir are all useful in the management of chicken pox in these populations. However, neither vidarabine nor leukocyte interferon is available in the twenty-first Century for therapy. As a consequence, acyclovir is the treatment of choice for the treatment of varicella in the immunocompromised host. In placebo-controlled trials, treatment with intravenous acyclovir improved outcome of varicella as evidenced by reduction of varicella-zoster virus pneumonia from 45% to less than 5% (Prober et al. 1982; Nyerges et al. 1988). No significant toxicity was reported in either trial. In spite of the lack of appropriately powered controlled studies, the safety of acyclovir and its efficacy for other varicella-zoster virus infections has led to its preferential use in this disease. The dose is 500 mg/m²/ivq 8 h for 5–7 days.

5.3 Valacyclovir and Famciclovir

No data are available that evaluate the value of the prodrugs valacyclovir (prodrug of acyclovir) and famciclovir (prodrug of penciclovir) for the treatment of chicken pox in either the immune competent or the immunocompromised child. However, because of improved pharmacokinetics, likely these medications will be equally as efficacious in the management of chicken pox. Notably, however, a pediatric formulation is not licensed for either medication. Some physicians institute intravenous

antiviral therapy with acyclovir until there is evidence of early resolution of disease (i.e. cessation of lesion formation and early scabbing) and, then, complete treatment with oral therapy, utilizing either acyclovir, valacyclovir, or famciclovir.

5.4 Resistant Varicella Zoster Virus Infections

In children with human immunodeficiency virus infection, varicella can be chronic in nature with the appearance of verrucous-like lesions. In this population as well as in bone marrow transplant recipients, therapy has been associated with the development of resistance to all drugs that have been utilized. Resistance to acyclovir usually occurs because of a deficiency in varicella-zoster virus thymidine kinase (Kost et al. 1993; Kimberlin et al. 1995). These isolates remain susceptible to foscarnet. As a consequence, foscarnet can be used in the treatment of children who have apparent acyclovir resistant isolates.

6 Cytomegalovirus

Cytomegalovirus (CMV) infection is the most common perinatal viral infection in the developed world, occurring in approximately 1% of all newborns (Demmler 1990). Cytomegalovirus is an endemic virus infection that occurs without seasonal variation. Seroprevalence studies demonstrate that CMV infection occurs in all human populations. In most developed countries, approximately 50% of the adult population will have serological evidence of past infection. In developing regions of the world, the seroprevalence may be as high as 90–100% in older children and adults. The prevalence of CMV infection also is higher in populations from lower socioeconomic backgrounds in developed nations (Stagno et al. 1982; Pass 2001). In most individuals, CMV infection is not associated with clinical symptomatology. In a small percentage of normal hosts, CMV will cause a mononucleosis-like illness. However, the burden of disease is encountered in the newborn who suffers from congenital infection and the immunocompromised host. Once an individual is infected, virus is excreted in the urine, semen, saliva, oropharyngeal secretions, breast milk and tears. In babies with congenital infection, virus excretion is known to persist up until at least the second decade of life. Cytomegalovirus can be transmitted horizontally, particularly between sexual partners and in child care centers. Between 20–40% of toddlers in day care shed virus for years and these children function as an important reservoir for transmission of infection to other children, parents and day care workers (Hutto et al. 1985; Adler 1985; Murph et al. 1985). A second route of infection is vertical whereby CMV can be transmitted transplacentally, intrapartum, or through breast feeding. Transplacental transmission is the cause of congenital CMV infection. Perinatal and postnatal acquisition usually do not result in clinically symptomatic disease, unless the child is born prematurely and did not receive transplacental maternal antibodies.

Between 10% and 15% of congenitally infected children are symptomatic at birth. Clinical symptoms involve the reticuloendothelial and the central nervous systems. Evidence of disease includes hepatosplenomegaly, jaundice, microcephaly, and lethargy. These findings are associated with laboratory aberrations that include both elevated bilirubin and hepatic transaminases. Thrombocytopenia is a common finding in the symptomatic congenitally infected children (Boppana et al. 1992).

The outcome of symptomatic congenital infection varies. Approximately 5% of children die. Of the children who survive infancy, most will develop severe psychomotor and perceptual handicaps. Sensorineural hearing loss and mental retardation are exceedingly common (Boppana et al. 1992; Williamson et al. 1982; Conboy et al. 1987). Predictors of hearing loss in this population include intrauterine growth retardation, petechiae, hepatosplenomegaly, hepatitis, thrombocytopenia, and intracranial calcifications (Rivera et al. 2002).

Most children with congenital infection are asymptomatic at birth; however, 10% of these children will develop sensorineural hearing loss. Approximately half of the children who develop hearing loss will do so bilaterally. Hearing loss can be progressive or even have a delayed onset (Harris et al. 1984; Williamson et al. 1992; Fowler et al. 1997; Dahle et al. 2000). Essential to the development of appropriate antiviral therapeutics is the requirement for laboratory assays that are predictive of hearing impairment in these congenitally infected children.

6.1 *Diagnosis*

The accepted gold standard for the diagnosis of congenital CMV infection is the detection of virus or viral DNA in the urine or saliva within the first 2 weeks of life. Detection of CMV in saliva and urine is accomplished easily because these children shed large quantities of virus from multiple body fluids. The availability of monoclonal antibodies to stain cell culture can lead to a rapid diagnosis within 24–36 h (Gleaves et al. 1984; Alpert et al. 1985).

Amplification of viral DNA by PCR has become essential in the diagnosis of CMV infections as is the case with disease caused by HSV. Several studies have noted variability in the sensitivity and specificity of PCR detection of CMV DNA (Demmler et al. 1988; Warren et al. 1992). In contrast, in the immunocompromised host, the detection of CMV DNA by PCR is applied routinely, including quantitative detection of CMV DNA copy number. The demonstration of a reduction in CMV DNA copy number has been a useful adjunct in the demonstration of therapeutic response.

6.2 *Treatment*

Over the past 15 years, ganciclovir has been extensively utilized for the treatment of CMV infections in the immunocompromised host, particularly retinitis in patients

with HIV/AIDS and infection in organ transplant recipients. In contrast to acyclovir and penciclovir that are phosphorylated by HSV or VZV thymidine kinase, ganciclovir is phosphorylated by the CMV U_L97 gene, a protein kinase and phosphotransferase (Sullivan et al. 1992; Littler, Stuart and Chee 1992). Like acyclovir monophosphate and penciclovir monophosphate, ganciclovir monophosphate is then converted to the triphosphate derivative by cellular kinases whence it becomes a competitive inhibitor of CMV DNA polymerase. While this was the first molecule licensed for the treatment of CMV disease, particularly in individuals with HIV/AIDS and transplant recipients, significant toxicity was identified as myelosuppression (granulocytopenia, anemia, and thrombocytopenia). Because of the myelosuppression, dose reduction or discontinuation of therapy and, even, the administration of granulocyte colony-stimulating factors, is often necessary.

The experience using ganciclovir for the treatment of congenital CMV infection is much more limited. Phase I pharmacokinetic and pharmacodynamic studies indicated that ganciclovir therapy of congenital CMV was associated with decreased virus load and improved laboratory abnormalities (Trang et al. 1993; Zhou et al. 1996). Drug was administered at 12 mg/kg per day intravenously for a period of 42 days. Furthermore, data from the Phase II studies substantiated the potential utility of this compound in the management of congenital infection (Whitley et al. 1997), however, in its early development, the incidence and severity of myelosuppression paralleled that encountered in the immunocompromised host. Indeed, the Phase II study substantiated a significant reduction in the quantity of virus excreted in the urine; however, once medication was discontinued, there was a rebound. Importantly, hearing improvement or stabilization occurred in 16% of treated children.

More recently, a Phase III randomized, controlled study of parenteral ganciclovir in neonates with symptomatic congenital CMV that involved the central nervous system has been reported (Kimberlin et al. 2003). The primary endpoint for this clinical trial was improved brain stem evoked response (BSER) between baseline and 3 month follow-up or for those infants with normal hearing at enrollment, maintenance of normal hearing between baseline and 3 month follow-up. While 100 children were enrolled in this study, only 42 had both a baseline and 3 month follow-up BSER audiometric examination. Of the 25 ganciclovir recipients, 21 (84%) had improved hearing or maintained normal hearing between the two time intervals. Among those children who received no therapy 10 of the 17 controls (had improved or stable hearing) ($P = 0.006$). More importantly, of the ganciclovir recipients none had worsening in hearing between the two time intervals as compared to seven (41%) of the control recipients, $P < 0.001$.

While not a primary endpoint, these children were followed and assessed audiometrically longitudinally. Of the 43 patients who had a BSER at baseline and at 1 year, 5 of the 24 ganciclovir recipients (21%) had worsening of hearing, as compared to 13 of 19 (68%) control patients, $P < 0.01$.

The administration of ganciclovir to these children required the placement of a central or continuous infusion peripheral line. The maintenance of these lines was associated with complications in approximately 10% of treated patients (need for

replacement, thrombophlebitis, secondary infection). For those children who received ganciclovir, dosage reduction and interruption of therapy was required in 40% of children.

Nevertheless, taken together, these data strongly imply that children with symptomatic congenital CMV infection involving the central nervous system derive direct benefit of therapy as it relates to the primary disability outcome event, namely hearing impairment. Initial analyses of developmental milestones achieved by each of the two patient populations indicates that those babies who received ganciclovir were more likely to meet developmental milestones than the counterpart no treatment recipients, even when adjusting for hearing loss (Oliver et al. 2006).

Lessons learned from the current Phase III clinical trial would suggest that the prodrug of ganciclovir, namely valganciclovir, would offer an improved therapeutic modality for this disease. In so doing, the requisite placement of a continuous infusion line could be averted. Toward this end, pharmacokinetic and pharmacodynamic studies are in progress by the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group in order to determine the optimal dosage for prevention of hearing loss.

7 Summary

Significant progress has been made in the treatment of viral infections in children, particularly those caused by HSV, varicella-zoster virus and cytomegalovirus. While not discussed in this review, licensed therapies are available for influenza infection in children as well. However, and notably so, numerous viral diseases of children represent unmet medical needs and warrant dedicated drug discovery efforts, including hepatitis B and C, and respiratory syncytial virus, among others.

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Fig. 1 Erythema migrans



Fig. 2 Borrelial lymphocytoma of the ear lobe



Fig. 3 Acrodermatitis chronica atrophicans