Introduction

Dengue virus (DENV) is the most important mosquito-borne viral disease, infecting approximately 50 million people in tropical and subtropical areas of the world each year. The annual average number of uncomplicated and severe dengue cases reported to the World Health Organization (WHO) has increased dramatically in recent years. Transmission of DENV typically follows a seasonal pattern punctuated every few years by a major epidemic. The factors leading to major epidemics are not completely understood. Dengue virus is a single-stranded RNA virus which is transmitted by Aedes spp. mosquitoes. Its spread is dependent on factors that promote the spread of its mosquito vector, in particular A. aegypti and A. albopictus. Since Aedes mosquitoes have proven to be exceptionally well adapted to human habitation, its global spread is difficult to control effectively. The geographical expansion of the Aedes mosquito, in particular A. albopictus, which is facilitated by increased mobilization and climate changes, may have contributed to the increased prevalence of dengue infections on a global scale. This is exemplified by the recent detection of DENV and Chikungunya virus infections in European residents.

Dengue is one of the most important causes of febrile illness in travellers returning from tropical regions where dengue is endemic or epidemic. The incursion of A. albopictus in some European countries resulted in emergence of Chikungunya virus in Italy and autochthonous transmission of both DENV and Chikungunya virus in France. Furthermore, changes in temperature and precipitation play important roles in the transmission cycle of DENV and may thus influence local and global incidence levels. To this end, the El Niño-Southern oscillation (ENSO), an ocean-atmosphere phenomenon of the Pacific ocean with a semiperiodic multiannual cycle, has been shown to be one of the important driving forces of temperatures and precipitation and consequently for dengue epidemics.

Clinical course, pathogenesis and treatment of dengue: An overview

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DENV is classified within the family Flaviviridae, genus Flavivirus and is maintained in sylvatic (nonhuman primate/forest-dwelling) and endemic (human/urban/peri-domestic) mosquito life cycles. Sylvatic DENV strains are evolutionarily and ecologically separated from endemic strains. Phylogenetic analyses revealed that each of the endemic DENV serotypes emerged independently from sylvatic ancestors. Based on neutralization assay data, four serotypes can be distinguished (DENV-1, DENV-2, DENV-3, DENV-4). Infection with any of the DENV serotypes may result in disease, or go asymptomatic in the majority of cases. Symptomatic infection ranges from a mild self-limiting, flu-like syndrome to a more severe disease characterized by coagulopathy, increased vascular fragility and permeability.

According to the new (2009) WHO classification system, the course of DENV infection can be divided into four distinct phases: incubation, febrile, critical, and recovery (Figure 1). During the critical phase, patients experience significant plasma leakage, resulting in a stage of profound shock, with a case fatality rate as high as 10% if patients do not receive prompt and appropriate treatment. Although the new WHO classification is likely to be an important and rational tool for clinicians in the management of patients, it does not provide
scientists with an appropriate framework for classifying disease based on the pathogenic mechanisms that are active during the course of infection. The pathogenesis of severe dengue is complicated and multifactorial, involving both viral and host factors. Although careful consideration and integration of knowledge, from the field of DENV, Ebola virus, Marburg virus, Yellow fever virus, Rift valley fever, and the New World arenaviruses, as well as from the field of bacterial sepsis, have led to a conceptual model for the pathogenesis of severe dengue, substantial gaps remain in our basic understanding of the molecular pathways leading to severe disease. Basically, studies of DENV pathogenesis are focused on understanding the mechanisms responsible for (1) increased manifestations of bleeding or tendencies of bleeding, and (2) vascular hyper-permeability as evidenced by massive plasma leakage.

Course of infection and clinical features
The course of DENV infection is schematically depicted in Figure 1. The incubation period following a mosquito bite is short, usually 3–5 days, but it may last up to 14 days in some cases. The febrile period is characterized by the abrupt onset of high fever, which usually lasts 2–7 days. This period is characterized by severe headache, retro-orbital pain, myalgia, arthralgia, gastrointestinal discomfort, and usually rash.

During primary infection, fever coincides with the period of viraemia, whereas during secondary infection, viraemia may only last 2–3 days. The febrile phase may be accompanied by mild haemorrhagic manifestations like a positive tourniquet test, petechia, easy bruising and mucosal membrane bleeding.

The critical phase is short (1–2 days) and is preceded by defervescence in the majority of patients, where the temperature drops to 38°C or less. Concomitantly, an increase in capillary permeability may occur, resulting in significant plasma leakage as measured by increased haemoconcentration, or fluid effusion in chest or abdominal cavities. Shock develops as a result of massive plasma leakage, characterized by a rapid, weak pulse (≤20mmHg), or hypotension with cold, clammy skin. If left untreated, a stage of profound shock may follow, in which pulse and blood pressure become undetectable, resulting in death within 12–36 hours. The stage of prolonged shock may trigger or accelerate the development of metabolic acidosis and disseminated intravascular coagulation (DIC), which is often associated with multiple organ failure owing to perturbations in the microcirculation. Massive loss of blood, mainly restricted to the gastrointestinal tract, may occur due to blood being shunted away from the gastrointestinal tract leading to anoxia and cell death.

During the recovery phase, extravascular plasma is gradually reabsorbed and the haemodynamic balance is restored. If fluid replacement was extensive, patients may develop respiratory distress or congestive heart failure during the recovery phase. It is important to point out that, if patients received timely and appropriate treatment, the mortality rate is very low.

Laboratory diagnosis
The conclusive diagnosis of DENV infection can only be made in the laboratory by means of virus isolation, detection of viral antigens or RNA in serum or tissues, or detection of specific antibodies in serum. The sensitivity and positive predictive value of each method varies in epidemic and endemic regions. During primary infection, viraemia coincides with fever, and virus can usually be isolated on mosquito cell cultures.

However, virus isolation is more difficult during secondary infection (often in endemic regions) because of cross-neutralization of virus by pre-existing antibodies, and the window of success is short. Virus isolation is a time-consuming process and requires expertise and a complex infrastructure. A sensitive and much easier alternative
to virus isolation is detection of NS1 antigen. NS1 is produced in high amounts during viral replication and peaks 1–2 days after peak viraemia. However, presence of antibodies during a secondary infection may reduce sensitivity of the assay in endemic regions. Use of chaotropic agents may increase the sensitivity of this assay significantly.

Detection of DENV RNA during the febrile phase of a secondary infection using new generation PCR is the most sensitive assay for detection of virus. Several quantitative PCR methods have been described for DENV diagnosis. On the other hand, serology (IgM and/or IgG detection) still constitutes a powerful tool in everyday diagnosis of DENV infection. Detection of IgM antibodies during the febrile period is possible in 40–60% of primary infections, while in the remainder, IgM may only be detectable shortly before or shortly after defervescence.

**Pathogenesis**

Several theories have been formulated to explain the pathogenesis of severe dengue. It is clear that pathogenesis of dengue is a multifactorial process, resulting from the interaction between the host response of individuals with “high-risk genetic background” and (virulent) virus (Figure 2). Although the traits that predispose patients to development of severe disease are not completely elucidated, there is no doubt that the host genetics constitute a crucial determinant of disease outcome. For instance, polymorphisms in several genes have been associated with severe dengue. In general, viral tropism determines the nature of disease. Monocytes and macrophages represent the main target cells for DENV infection. Furthermore, DENV has been frequently found in the liver and endothelial cells of the spleen and lungs. However, viral replication does not result in discernable damage that could explain severe dengue, as revealed by histopathological examinations of autopsy samples. Occasionally, hepatic injury and damage to the vascular system have been reported. To date, the prevailing hypotheses suggest that severe disease results from an unbalanced host response to infection, analogous to the situation in bacterial sepsis.

It is conceivable that DENV could drive such a biased response in a strain-dependent way. In this respect, the ability of DENV to orchestrate an unbalanced host response may be defined as virulence. Tropism, replication rate, and immune-interfering properties of DENVs may constitute important virulence factors. For example, several studies suggested that different strains of DENV may exhibit differential tropism for endothelial cells from different tissues, suppress production of antiviral cytokines in vitro (e.g., interferon-α), or enhance the production of certain vasoconstrictive cytokines (e.g., nitric oxide). The role of the virus in causing the hyperpermeability syndrome characteristic of severe disease has been questioned, based on the observation that in only 30–40% of patients with severe disease can DENV be detected in plasma. Therefore, it is believed that the host response plays a critical role in the outcome of DENV infection.

Host response is the collective action of the immune, coagulation, hormonal and nervous systems. The systemic inflammatory response characteristic of DENV infection is a complex process, involving cytokines, chemokines, molecules belonging to the danger signalling pathway (such as defensins), acute-phase proteins, proteases of the complement and coagulation system, endothelial cells, and the liver.

Less is known about the role of the hormonal and nervous system in the pathogenesis of severe dengue. Conceptually, the host response to any infection may result in three states: (1) excessive pro-inflammatory, (2) excessive anti-inflammatory, or (3) mixed balanced responses. Following infection with DENV, resident dendritic cells and macrophages produce pro-inflammatory mediators, which lead to recruitment of neutrophils to the inflamed tissue. Enzymatic cleavage of chemokines by matrix metalloproteinase (MMPs)-8 and 9 further contributes to attraction of neutrophils. Neutrophils play an important role in the recruitment of inflammatory monocytes to the area of infection by production of the cathelicidin LL-37. Pro-inflammatory mediators are produced in an endeavour to eliminate the virus and promote tissue healing.

Once the inflammatory stimulus has been removed, neutrophils, inflammatory monocytes and macrophages collaborate to stop further recruitment of cells and produce an anti-inflammatory response meant to balance the detrimental effects of high concentrations of pro-inflammatory cytokines. Phagocytosis of microbes and debris from the inflamed site by neutrophils induce apoptosis of these cells, and the clearance of apoptotic cells by macrophages stimulates production of anti-inflammatory cytokines. In addition, lipid mediators, such as resolvins produced by neutrophils, play a key role in resolution of acute inflammation. Failure to balance the pro-inflammatory response leads to tissue damage, disseminated intravascular coagulation and ultimately multiple organ failure. On the other hand, an excessive anti-inflammatory response may lead to immune suppression and an increased risk for secondary infection. Therefore, it is believed that a balanced response is needed to restore homeostasis.

The majority of studies have compared the cytokine profiles in groups of patients classified according to the 1997-WHO system. Consequently, for most studies, it is not clear whether a certain cytokine profile is the cause or consequence of severe disease. In general, high levels of pro-inflammatory mediators, including IL-1β, IL-6, IL-8, IL-10, TNF-α, IL-1β, IL-13, and TGF-β1 have been measured in patients with severe disease. A drawback of most of these studies is that use of immunoassays mainly detects free circulating cytokine. Furthermore, interpretation of cytokine concentrations in relation to disease severity is complicated because levels of cytokine receptors, regulators of the respective pro-inflammatory cytokines, are not simultaneously measured. Therefore, analysis of mRNA transcript profiling provides a better way to study the complete immune response to DENV infection. Although most studies did not analyse transcription profiles in serial samples, the available evidence suggests that a mixed response (pro-inflammatory and anti-inflammatory) is seen in patients with severe dengue.

Interestingly, the data suggest an important role for neutrophils in the pathogenesis of severe disease. T cells, known to play an important role in down-regulating the pro-inflammatory innate response, have been shown to be suppressed in patients with severe disease. Corroboration of these data has been hampered by the lack of a DENV strain which replicates efficiently in mice, which has also impeded in-depth studies of the role of different cytokines and chemokines in pathogenesis of severe dengue.
The use of mouse-adapted DENV strains provided strong evidence that the lipid mediator PAF (platelet activating factor) and the chemokine receptors CCR1, CCR2, and CCR4 play a role in development of severe dengue. Interestingly, these disease-associated mediators were not essential for control of virus replication in the mouse model. DENV infection also induces production and release of reactive nitrogen and oxygen species (RNS/ROS), which have been shown to play a role in development of severe disease. These both contribute to lipid oxidation, resulting in PAF-like lipids, which are believed to be involved in the initial phase of the inflammatory response. Future studies are needed to determine the quantitative nature of the pro-inflammatory and anti-inflammatory response in patients with severe disease in relation to the pathophysiological events. In particular, the causes underlying the selective plasma leakage seen in patients with severe disease is an important issue to be addressed. In addition, a better understanding of the interaction between different phagocytes in initiation, progression and resolution of inflammation in patients with uncomplicated dengue is necessary for identifying disease-associated mediators.

**Treatment**

There are no specific antiviral treatments or licensed vaccines against dengue. The high economic burden that DENV infections pose to affected areas makes the development of an effective vaccine an international health priority. Several vaccine candidates, without increasing the risk for enhancing disease severity. In addition, incomplete understanding of the role of cellular immunity regulation by immune complexes.

**Concluding Remarks**

DENV infection has become an increasing global health concern with over two billion people being at risk of infection. The effects of changing demographics, urbanization, climate change and travel have made DENV infections one of the most important emerging vector-borne diseases. To date, there is no specific treatment for dengue, and numerous efforts are ongoing to develop safe and effective vaccines and antivirals. Deciphering host responses involved in severe dengue will be an important contribution to efforts to develop new and effective intervention strategies.

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**References**

Q Fever in the Netherlands, a review.

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Coxiella burnetii, an obligate intracellular bacterium found around the world, is the causal agent of the rare zoonotic illness, Q Fever. It has a complex life cycle with spore-like forms, similar to anthrax, that remain biologically active in harsh environments. The bacteria are only infectious when transmitted from animals to humans, while human-to-human infections have not been conclusively proven. The bacteria are highly resistant to many conventional disinfectants, notably retaining infectivity after 24 hour contact with 5% formalin, 5% Lysol® or 0.5% hypochlorite. Coxiella burnetii is also highly resistant to UV radiation and can spread widely in the atmosphere. In an outbreak in the United Kingdom, believed to be from a single source, 147 cases were identified and spread over an area 6.7 kilometers east/west and 18.3 kilometers north/south.

The name Q (for query) fever comes from the original description of a febrile illness of unknown cause among abattoir workers in Brisbane, Australia in the 1930s. Around the same time on the other side of the world, at the Rocky Mountain Laboratories in Hamilton, Montana in the United States of America, researchers were studying Rickettsia rickettsii, the causative agent of Rocky Mountain Spotted Fever, and came across a similar illness but with a different clinical presentation in guinea pigs. The name, Q fever, has remained even after the pathogen was identified and named as Coxiella burnetii, after the two researchers in Australia and the United States responsible for performing the majority of the research, Dr. H. R. Cox and Dr. F. M. Burnet, respectively.

Recent Cases in the Netherlands

In the Netherlands, the presence of C. burnetii is well established, but until recently, there were on average not more than 12 cases per year. In 2007, the number of symptomatic cases jumped to 178, with most being reported from a single municipal health service in the southern province of Noord Brabant. A small cluster (n=55) was observed around a single village, but the source of the outbreak was not identified. In 2008, 1000 patients were reported to the municipal health services, and in 2009, there were more than 2300 patients. A clearly defined, epidemiological peak can be seen in Figure 1 and coincides, when the 2 week incubation period is included, almost perfectly with the lambing period of the year. From 2007, the number of cases continued to rise and showed similar annual peaks (Figure 2).

The outbreak in the Netherlands is unlike previously documented outbreaks. When first documented in Australia, the infected individuals all worked in slaughterhouses. As animals were slaughtered, the workers became infected, representing a high risk group based upon profession. Other than high risk groups, there are few well documented outbreaks, which are often attributed to super-spreading events, single events responsible for the infection of a group of people. Examples of super-spreading events are documented in the United Kingdom, in Switzerland and in Germany.

In the Netherlands, the cyclic nature of the outbreak suggests other factors, dry periods in spring and high density of farms and people, are responsible for the spread of C. burnetii.

Organism Replication and Hosts

Coxiella burnetii is an intracellular organism that requires a host in which to replicate. The largest source of hosts are domesticated goats, sheep and cattle living close together, but it is also found in many other animal species, including ticks, and in free-living amoeba which inhabit a variety of air, soil and water environments. The relationship between abattoir workers and their direct contact with animals was hypothesized but only proven after successful culture from a patient. The lifecycle of the organism typically involves the ingestion by phagocytic cells following their inhalation in aerosols. Once in the macrophages or monocytes, the lowered pH in the vacuoles activates the spore-like bacterium, which then undergoes intracellular replication in the vacuoles and survives by resisting lytic enzymes.

In animals, C. burnetii infections are largely asymptomatic but can cause abortions. In the super-infected postpartum material, there are massive numbers of bacteria; up to 1 billion per gram. On Q fever affected goat farms in the Netherlands, there have been abortion storms, where more than 50% of pregnant goats aborted. Goat farming in the Netherlands has, in the past two decades, undergone a large shift from mostly hobbyists to professional goat farms, with an increase from a negligible number of 3300 goats in 1984 to upwards of 350,000 goats in 2008. Other countries have noted a direct relationship between livestock numbers and number of cases. Given the large increase in the concentration of goats in the most densely populated country in Europe, it is understandable that such a large scale outbreak could occur.
Human Disease

Coxiella burnetii is highly infectious in humans, the infectious dose is believed to be fewer than 10 bacteria\(^1\). Most infections remain asymptomatic. The clinical presentation of symptomatic C. burnetii infection is Q fever, a flu-like febrile illness with an average incubation period of 14 days and average duration of symptoms of 14–21 days. The infection is often self-resolving and typically does not require hospitalization. Given the lack of specific characteristics, diagnosis of the acute illness is often delayed unless physicians are aware of the presence of C. burnetii infections in the region. Initial laboratory diagnosis of acute Q fever (AQF) is difficult, as antibodies are only detectable two weeks after initial symptoms. However, Schneeberger et al.\(^{12}\) have shown recently that real-time PCR is indispensable in early diagnosis of symptomatic Q fever, in the period before antibodies can be detected.

A chronic form of Q fever (CQF) can develop as well, often months or years after an acute infection, and presents itself mainly as a culture-negative endocarditis. Patients that develop CQF often have underlying heart valvulopathies or vascular diseases. Other complications of CQF are infections of vascular aneurisms and prosthetic valves. Approximately 2% of AQF patients will develop CQF, but diagnosis is difficult and relies upon follow-up serology and early identification of valve damage\(^1\). In a French reference laboratory, C. burnetii infections made up 48% of the culture-negative infectious endocarditis (CNIE) between 1983 and 2001 and were the single largest cause of CNIE in samples\(^1\).

The culturing of C. burnetii is difficult, given that the exact intracellular processes have not been thoroughly researched, and only recently have steps been made to successfully grow it in a cell free medium\(^15\). An easier way to study the bacterium is with only recently have steps been made to successfully grow it in a cell free medium\(^15\). An easier way to study the bacterium is with cell free medium. For laboratory associated infections in 1976\(^16\). The CDC requires a negative infectious endocarditis (CNIE) between 1983 and 2001 and were the single largest cause of CNIE in samples\(^10\).

The outbreak of the human cases since 2007 has followed a typical geographical spread from a central location in the southern rural countryside of the Netherlands. Epidemiological research has shown that the risk of infection is highest within 5km of the source\(^19\). These human cases spread within the Netherlands from the southern countryside to all 12 provinces of the Netherlands in 2009. While there have been no documented cases in the Netherlands of human to human spread, there is a case study of a United States serviceman infecting his wife, possibly through sexual contact\(^20\).

### Interventions in the Netherlands

In 2009 three interventions, 1) mandatory hygiene measures including manure management; 2) the vaccination of goats and sheep; and 3) the culling of pregnant animals on infected farms, were employed to reduce the number of human Q fever infections in the Netherlands\(^21\). The number of cases in 2010 has dramatically dropped to fewer than 500 cases, less than one quarter of the cases in 2009\(^9\). However, the question of whether the interventions resolved the problem of Q fever or whether culling temporarily removed the source of C. burnetii from the environment remains. The vaccine currently used by the Dutch government and farmers seems to be effective in preventing abortions and in reducing the shedding of C. burnetii in milk, vaginal mucus and faeces\(^22,23\). However, there is consensus that vaccination of small ruminants must be sustained for at least several years. Outbreak vaccination, i.e. vaccinating herds that already are infected, is much less effective.
Containment regulations implemented in the Netherlands as a result of the recent outbreak are restricted to farms that test positive for *C. burnetii* in the mandatory tank-milk monitoring programme. Positive farms are forbidden to transfer animals to other milking farms, to have visitors on the location, and are required to retain manure for 3 months before being able to use it for fertilizer. By retaining the manure for an extended period of time, the heat of the decomposition of covered manure raises the temperature to a level sufficient to kill the bacteria. With the belief that *C. burnetii* is endemic in the Netherlands, the animals were able to be transferred to the slaughter houses for slaughter. Only at the end of 2009 was this reconsidered, as there was a new hypothesis that the presence of a new strain was the cause of the outbreak. Intense monitoring is in place to ensure farmers can quickly identify and remove infected animals. When infected farms test negative for a year, restrictions are lifted. The above mentioned regulations are implemented on farms that test positive for the first time, but the removal of animals and a lifelong breeding ban are not necessary as long as the farmers have vaccinated their goats according to a letter sent by the ministers of Health and Agriculture.

**Treatment of infection**

The treatment of patients is complicated by resistance of *C. burnetii* to a number of antibiotics. Many antibiotics commonly used for respiratory infections, such as β-lactam antibiotics and azotrimycines, are not effective because the bacteria are located in a low pH vacuole. The bacterium is able to avoid the immune system by avoiding degradation in these vacuoles. In combination with the fact that the diagnosis is often after the resolution of the AQF symptoms, the use of antibiotics is not necessary in all instances. In more severe infections, a dose of 100mg of doxycycline taken orally twice daily for 15–21 days is a frequently prescribed therapy. For chronic infections, patients are given doxycycline in combination with hydroxychloroquine for a minimum of 1.5 years and a maximum of 3 years. Hydroxychloroquine is included in the regime as an alkalinizing agent, to raise the pH and prevent the inhibition of doxycycline action at the low pH levels found in the vacuoles. In some instances, patients with valvulopathy or other heart disease are advised to undergo regular screening of heart dynamics by ultrasonography.

**Summary**

Much remains unclear about the transmission of *C. burnetii* from animals to humans, about the impact of veterinary control measures, about the best treatment for the individual patient, and about the best follow-up strategy of acute Q fever for the early detection and treatment of chronic Q fever. Future research should focus on these topics. *Coxiella burnetii* is still poorly understood, and until 2007, was regarded as an exotic or rare illness, but the large outbreak in the Netherlands has put it in the spotlight.

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