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The new face of enterohaemorrhagic Escherichia coli infections

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The unprecedented outbreak of Shiga toxin/verotoxin-producing *Escherichia coli* (STEC/VTEC) O104:H4 in Germany in May and June 2011 displayed several novel epidemiological, microbiological and clinical features. Infection with STEC/VTEC, also referred to as enterohaemorrhagic *E. coli* (EHEC), with or without haemorrhagic uraemic syndrome (HUS), which is usually a disease of pre-school children and equally distributed among the sexes, affected in the current outbreak mostly women over the age of 20 years (87%). In addition, several intriguing microbiological characteristics of the new epidemic strain have just been published [1,2].

With regard to the clinical characteristics, STEC/VTEC O104:H4 again differed remarkably from previously described STEC/VTEC infections. During a telephone conference on 9 June, organised by the European Centre for Disease Prevention and Control (ECDC) with clinical experts and nephrologists from 16 Member States of the European Union (EU) and several European and national professional societies, German colleagues shared their first clinical experiences from their patients. Severe infection with STEC/VTEC O104:H4 usually presented as a disease in three phases. On admission, about 80% of the patients suffered from bloody diarrhoea and 20% from watery diarrhoea. In 25% of the cases with bloody diarrhoea, signs of HUS (based on laboratory parameters of haemolysis, thrombocytopenia, and renal function tests) evolved after 3–5 days [3]. Completely unexpected, however, was the observation that severe neurological symptoms developed after about 3–10 days in roughly 50% of patients with HUS, even though clinical and laboratory markers of HUS were improving. These patients who had at first seemed to improve or respond to therapy, deteriorated again. Some patients even had to be re-hospitalised 3–4 days after they had been discharged. Neurologists were very concerned about the severity of neurological symptoms, ranging from mild disorientation and cognitive dissociation to stupor or severe, life-threatening seizures. Despite the impressive clinical presentation, routine neuroradiological examination revealed only mild alterations, if any. Worryingly, especially patients with seizures seemed to respond only weakly to standard antibody-based treatment regimes.

In this issue of *Eurosurveillance*, Cordesmeyer et al. [4] report about an unusual case of STEC/VTEC O104:H4 infection associated with colonic ischemia, and Kuijper et al. [5] describe a case of household transmission of STEC O104:H4 from a mother to her child. In both cases, neurological symptoms were present, with severe manifestation and as yet unclear neurological outcome in the child. From a public health perspective, these and other rather unusual clinical presentations and sequelae of STEC/VTEC O104:H4 infections are of importance when it comes to supporting and guiding the identification of STEC/VTEC cases, providing recommendations for the follow-up of patients, or adapting existing case definitions for the disease. In order to share and disseminate relevant clinical data among European clinicians and to foster the dialogue between clinicians and epidemiologists, a clinical support initiative was established by the ECDC as a reaction to the outbreak. Nominated clinical contact points, and up to two additional clinical STEC/VTEC experts per EU Member State were invited by the ECDC to join this initiative. It comprises a password-protected internet discussion forum for timely exchange of information, expertise and best practices. In addition, an audio podcast (available through the ECDC website) has been produced, in which a clinical expert from Germany describes his experiences with the presentation, treatment, and outcome of patients infected with STEC/VTEC O104:H4.

This clinical support initiative is one more component of the European response against this devastating outbreak and the possible future establishment and spread of the new STEC/VTEC O104:H4 strain in Europe. It will add to and support the ECDCs ongoing efforts in the field of scientific advice, outbreak response and surveillance.

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References

1. Scheutz F, Møller Nielsen E, Frimodt-Møller J, Boisen N, Morabito S, et al. Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing Escherichia coli O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. Euro Surveill. 2011;16(24):pii=19889. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19889
2. Bielaszewska M, Mellmann A, Zhang W, Köck R, Fruth A, Bauwens A, et al. The Characterisation of the Escherichia coli strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. Lancet Infect Dis. Early Online Publication, 23 June 2011. DOI: 10.1016/S1473-3099(11)70165-7
3. Frank C, Werber D, Cramer JP, Askar M, Faber M, an der Heiden M, et al. Epidemic Profile of Shiga-Toxin–Producing Escherichia coli O104:H4 Outbreak in Germany — Preliminary Report. N Engl J Med. 2011;22 Jun 22 2011 (10.1056/NEJMoa1106483).
4. Cordesmeyer S, Peitz U, Gödde N, Kasper HU, Hoffmann MW, Allemeyer E. Colonic ischaemia as a severe Shiga toxin/verotoxin producing Escherichia coli O104:H4 complication in a patient without haemolytic uraemic syndrome, Germany, June 2011. Euro Surveill. 2011;16(25):pii=19895. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19895
5. Kuijper EJ, Soonawala D, Vermont C, van Dissel JT. Household transmission of haemolytic uraemic syndrome associated with Escherichia coli O104:H4 in the Netherlands, May 2011. Euro Surveill. 2011;16(25):pii=19897. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19897

Colonic ischaemia as a severe Shiga toxin/verotoxin producing *Escherichia coli* O104:H4 complication in a patient without haemolytic uraemic syndrome, Germany, June 2011

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An increasing rate of infections with Shiga toxin/verotoxin-producing Escherichia coli (STEC/VTEC) O104:H4 has been observed in Germany since May 2011, with unusually high numbers of patients suffering from haemolytic uraemic syndrome (HUS). We report a STEC/VTEC O104:H4 case without HUS, presenting with colonic ischaemia demanding surgery. This atypical clinical presentation of STEC O104:H4 infection might indicate new severe complications associated with this uncommon strain, and highlights the importance of immediate interdisciplinary assessment of STEC/VTEC patients.

Clinical presentation and initial evaluation

A woman in her 80s presented to our Emergency Department on 1 June with increasing abdominal pain and a history of nausea, primarily associated with dysuria that had been lasting for five days. On 29 May, the day after the onset of those symptoms, non-bloody diarrhoea had followed. The initial physical examination showed a mildly distended abdomen with diffuse pain and left upper quadrant tenderness without rebound. Blood tests showed a massive elevation of white blood cell count (29,300/µl; normal range: 4,300–10,000/µl) as well as an elevation of both C-reactive protein (32.42 mg/dl; normal range: ‹0.5 mg/dl) and lactate (2.5 mmol/l; normal range: 0.5–2.4 mmol/l). Ultrasound and computed tomography (CT) scans of the abdomen revealed distinctive amounts of ascites and wall thickening of parts of the transverse and descending colon, but did not show any disturbance of the main arteries and veins. Considering pseudomembranous colitis as a differential diagnosis, we decided to perform a colonoscopy.

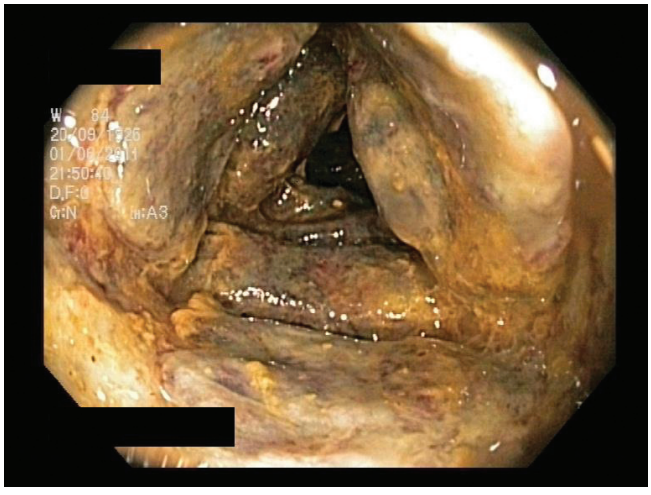
The endoscopy showed a normal rectum and sigmoid colon, macroscopically. The descending colon had a circularly swollen, partly pale, partly bluish mucosa, with no evidence of bleeding after biopsy, a combination of symptoms highly suspicious of ischaemia (Figure 1).

Surgical intervention

Following the confirmation of ischaemia by endoscopy of the descending colon on 1 June, surgery was performed immediately. Non-occlusive ischaemia of the descending colon with gangrenous bowel wall was detected during the operation, with patent macroperfusion of the medial colic artery and inferior mesenteric artery arcade, as well as the left colic artery up to the

Figure 1

Circularly swollen, partly pale, partly bluish mucosa of the descending colon, highly suspicious of ischaemia, endoscopy of STEC/VTEC O104:H4 patient, Germany, June 2011



STEC/VTEC: Shiga toxin/verotoxin-producing *Escherichia coli*.

gangrenous bowel wall. Therefore, a left hemicolectomy was performed, followed by thorough abdominal irrigation.

Pathology

Pathological examination of the removed part of the colon revealed wall thickening up to 1 cm and extensive necrosis throughout the entire intestinal wall with fibrinous-purulent exudation. The border area showed vital mucosa with erosive and phlegmonous inflammation, membranous-like fibrin exudation and crypt destruction, consistent with an ischaemic origin (Figures 2 and 3).

Postoperative course

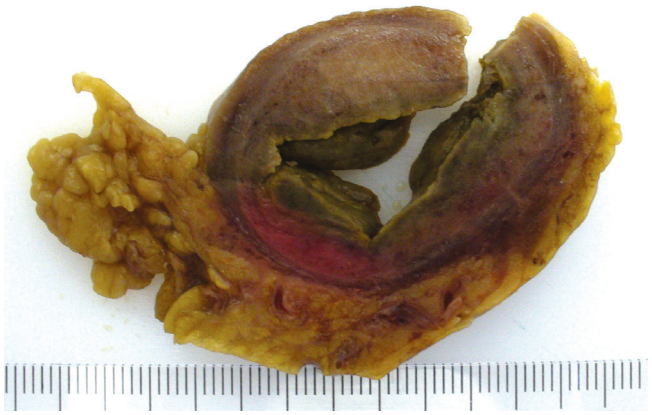
After the operation, which occurred late in the evening on 1 June, the patient was admitted to the intensive care unit. The next morning, as a precautionary measure, due to the ongoing Shiga toxin/verotoxin-producing Escherichia coli (STEC/VTEC) O104:H4 outbreak in Germany, the patient was placed in isolation and a stool sample was sent for further evaluation. The second day after surgery, seven days after the onset of the initial abdominal symptoms, neurological impairments were observed in terms of decelerated reactions of the patient, lack of orientation and intermittent response when addressed. Clinical examination was uneventful for abdominal, respiratory and haemodynamic findings. Laboratory studies were not suspicious for haemolytic uraemic syndrome (HUS): creatinine, elevated up to 1.7 mg/dl at admission (normal range: ‹1.2 mg/dl), had immediately revealed decreasing tendencies (0.8 mg/dl at day four) after intravenous fluid application, and was therefore most likely due to diarrhoea-induced hypovolemia. Lactate dehydrogenase (LDH) ranged between 139 U/l (normal range: 135-225 U/l) at admission and 318 U/l six days after, whereas platelets always presented within normal limits. The patient was monitored closely and improved neurologically during the next day. During the following 48 hours, however, noticeable neurological deficiencies with disturbance of vigilance, aphasia and apraxia were observed, as well as myoclonia of the extremities. Seizures did not occur. PCR analysis of the stool samples confirmed Shiga toxin 2-producing *E. coli* consistent with the strain responsible for the current outbreak, O104:H4, on 6 June. Monitoring the patient for an onset of HUS continued but has not eventuated as of 21 June.

Discussion

The ongoing outbreak of infections with STEC/VTEC, also commonly referred to as enterohaemorrhagic *E. coli* (EHEC), in Germany is one of the largest worldwide [1]. Besides causing non-bloody and bloody diarrhoea, the STEC/VTEC subtypes may also lead to HUS, a severe complication that is characterised by thrombocytopenia, microangiopathic haemolytic anaemia, and decreased renal function. So far, *E. coli* O157:H7 had been described as the predominant serotype causing HUS in approximately 10% of all cases to date [2,3], whereas the strain responsible for the current outbreak, which has been identified as *E. coli* O104:H4, is an extremely rare strain, hardly described during the last decade [4]. With adults and predominantly women being infected, the age and sex distribution in the ongoing outbreak is unusual, but might be related to gender-specific differences in dietary habits: vegetables, which are generally more often consumed by women, are still suspected to have been contaminated and at the source of this outbreak. In addition, an unusually high number of patients have developed HUS: The latest data account for 814 patients with HUS from a total of 3,587 infected patients in Germany [1]. Neurological complications, which were seen, on average, in about 25% of HUS patients in former outbreaks [2], could also be more severe in this outbreak. Indeed,

Figure 2

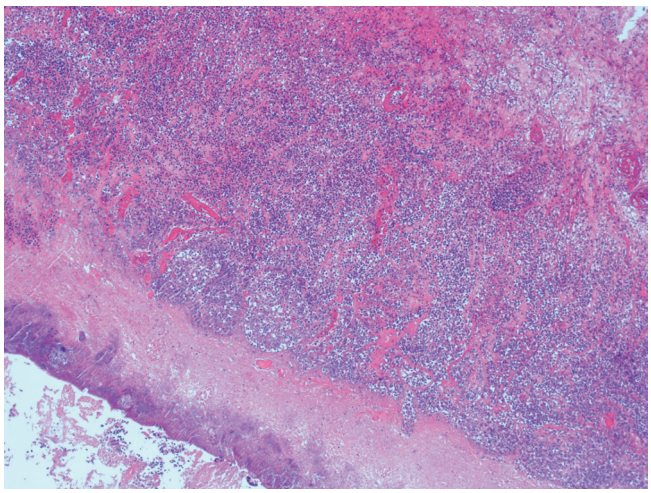
Left hemicolectomy specimen, revealing wall thickening up to 1 cm with induration and increased fragility, STEC/VTEC O104:H4 patient, Germany, June 2011



STEC/VTEC: Shiga toxin/verotoxin-producing *Escherichia coli*.

Figure 3

Extensive mucosal and submucosal necrosis affecting the muscular lining of the descending colon, with fibrinous-purulent exudation, left hemicolectomy specimen of STEC/VTEC O104:H4 patient, Germany, June 2011



STEC/VTEC: Shiga toxin/verotoxin-producing *Escherichia coli*.

the exchange among German clinicians, who set up a web-based platform to communicate clinical information in the context of the current outbreak, indicates higher numbers, but this has not been systematically evaluated so far. Taken together, the various aspects of the ongoing outbreak may suggest an increased virulent potential of the identified strain.

Besides HUS, STEC/VTEC-associated bowel ischaemia, as an additional severe complication, is rarely described in the literature. Very few reports of colonic necrosis and perforation due to Shiga toxin-induced intestinal damage exist, and in all these reports, this type of complication affected *E. coli* O157:H7-infected individuals. This complication was moreover mostly encountered in paediatric patients with concomitant HUS [3]. Only one case of ischaemic colitis in a non-HUS adult has been previously described [5].

Conclusion

Besides leading to its major complication HUS, infection with STEC/VTEC O104:H4 can also cause neurological complications and atypically present as bowel ischaemia, as shown in our patient. Since ischaemia-induced colonic wall thickening is difficult to differentiate from pseudomembranous colitis in CT imaging, endoscopy is essential and should be considered at an early diagnostic stage. Notably, our patient has not shown any signs of HUS to date, but obviously, even unexpected complications have to be considered as a differential diagnosis in STEC/VTEC O104:H4 infected patients, calling for interdisciplinary diagnostic investigations.

References

1. Informationen zum EHEC/HUS Ausbruchsgeschehen. [Information on the EHEC/HUS outbreak events]. Data from 20 Jun 2011, 3 pm. Berlin: Robert Koch Institut; 21 Jun 2011, 11 am. German. Available from: http://www.rki.de/cln_116/nn_205760/DE/Home/Info-HUS.html
2. Enterohaemorrhagic Escherichia coli. Factsheet N°125. Revised May 2005. World Health Organization. Geneva. Available from: http://www.who.int/mediacentre/factsheets/fs125/en/
3. Karch H, Tarr PI, Bielaszewska M. Enterohaemorrhagic Escherichia coli in human medicine. Int J Med Microbiol. 2005;295(6-7):405-18.
4. Scheutz F, Møller Nielsen E, Frimodt-Møller J, Boisen N, Morabito S, Tozzoli R, et al. Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing Escherichia coli O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. Euro Surveill. 2011;16(24):pii=19889. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19889
5. Kravitz GR, Smith K, Wagstrom L. Colonic necrosis and perforation secondary to Escherichia coli O157:H7 gastroenteritis in an adult patient without hemolytic uremic syndrome. Clin Infect Dis 2002;35(9):e103-5.

Household transmission of haemolytic uraemic syndrome associated with *Escherichia coli* O104:H4 in the Netherlands, May 2011

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Following the outbreak of haemolytic uraemic syndrome (HUS) and haemorrhagic colitis in Germany, two patients returning from a stay in Germany developed HUS due to *Escherichia coli* O104:H4 in the Netherlands. The index case developed symptoms eight days, and her child 15 days after their return. It is very likely that transmission resulted from secondary spread from mother to child. Recommendations should be made to prevent secondary transmission within households.

Introduction

Since early May 2011 one of the largest ever reported outbreaks of haemolytic uraemic syndrome (HUS) and bloody diarrhoea caused by Shiga toxin/verotoxin-producing *Escherichia coli* (STEC/VTEC), also referred to as enterohaemorrhagic *E. coli* (EHEC) has affected Germany [1]. The outbreak strain was identified as an enteroaggregative Shiga toxin-producing *E. coli* O104: H4 (EAggEC) [2]. Initial findings suggested raw vegetables and salads as vehicle of infections and recommendations were made to abstain from these products. Epidemiological investigations implicated an organic sprout farm in Lower Saxony near Hamburg as the potential source of the outbreak [3]. On 10 June 2011, German public health and food safety authorities issued a joint statement recommending people to abstain from consuming sprouts. A decrease in the number of new cases was seen after 6 June [4].

We describe here two cases of HUS and hemorrhagic colitis that occurred in the Netherlands and were associated with the outbreak in Germany.

Case descriptions

Patient A

On 24 May, a woman in her 30s was admitted to a hospital in the Netherlands with bloody diarrhoea and abdominal pain of two days. Common bacterial gastroenteritis was considered and no antibiotic treatment was initiated. Four days after admission, blood examination revealed severe HUS. The patient was transferred to the Leiden University Medical Centre (LUMC) for haemodialysis which eventually proved not to be necessary.

A tentative diagnosis of HUS due to Stx1-negative, Stx-2 positive, extended spectrum beta-lactamase (ESBL)-producing *E. coli* was confirmed by culture on sorbitol MacConkey agar of a stool sample collected two days after admission to the first hospital. Further identification of *E. coli* serotype O104 was performed at the Dutch National Institute for Public Health and the Environment (RIVM). A real-time PCR for Stx2 directly in stool sample was also positive [5]. Stool samples on admission to LUMC were already negative in culture and real-time PCR. Since 30 May 2011, the patient has gradually been recovering from HUS.

Patient A had travelled to Northern Germany for two days in May 2011, accompanied by six relatives, among them her 10 month-old child.

Patient B

The 10-month-old child of Patient A was examined first on 29 May 2011, one day after transfer of the mother to the LUMC. At the time, the child had mild diarrhoea free of blood, and blood examination did not reveal any abnormality. A stool sample was collected and tested positive for Stx2 in the real-time PCR. Culture revealed growth of ESBL-positive *E. coli* O104. On 30 May, the child developed fever and blood abnormalities compatible with HUS, and was admitted to hospital. The next day, it developed neurological symptoms, i.e. seizures, and was transferred to a specialised child dialysis centre, where experimental treatment with eculizumab was started. The patient received prolonged mechanical ventilation and inotropic therapy. Three weeks after transfer, the patient is still on dialysis and the neurological outcome is unsure.

Family members

Of the other five relatives of Patients A and B who had also travelled to Northern Germany, one developed mild diarrhoea 16 days after their return, but microbiological examinations were not performed since the diarrhoea resolved one day later and no HUS developed. The remaining relatives did not develop any symptoms and were not investigated for the presence of *E. coli* O104.

Microbiological and public health implications

HUS is a rare disease in the Netherlands, with approximately 20 patients per year [6]. During the outbreak in Germany, 854 cases of HUS and 2,848 non-HUS STEC/VTEC cases have been reported as of 22 June, of which 4.6% and 2.2%, respectively, were found in other Member States of the European Union [7]. In the Netherlands, four HUS (including Patients A and B described here) and five non-HUS laboratory-confirmed STEC/VTEC cases were detected. All acquired the infection during a recent visit to Germany.

Microbiological examination of stool samples from Patient A gave negative results in the real-time PCR and culture at the time of transfer to another hospital when HUS had fully developed, six days after disease onset, indicating the need to apply diagnostic tests early in the disease. Similarly, Patient B had positive faeces tests and excreted the organism before haemorrhagic colitis and HUS developed.

We hypothesise that Patient B probably acquired HUS by secondary transmission within the household, because the child developed illness only 15 days after return form Northern Germany. The child had spent much time with her mother during her stay at the first hospital, when the mother developed diarrhoea. Before admission of the child to LUMC, the mother had not been advised to take any specific hygienic measures. The child was not breastfed at the time. The age of the child makes it unlikely that it had eaten a food product such as sprouts.

Recently, a group of investigators reported that the strain causing the outbreak in Germany is in fact not a typical virulent Shiga toxin-producing *Escherichia coli* strain, but instead is a more rare, hybrid pathotype that harbours the phage encoded the Shiga toxin gene in an EAggEC background [2]. These findings are relevant for understanding the epidemiology since EAggEC is a common pathogen causing diarrhoea in travellers and persistent diarrhoea in infants and young children living in countries with poor sanitation. In contrast to STEC/VTEC strains, which that have an animal reservoir, mostly ruminants, EAggEC strains probably have a human reservoir only.

Secondary transmission frequently occurs in outbreaks caused by classical EHEC O157. A review of 90 confirmed outbreaks in the United Kingdom, Ireland, Scandinavia, Canada, the United States and Japan revealed that approximately 20% of all outbreak cases were the result of secondary spread [8]. Interestingly, the spread was significantly influenced by age and modes of transmission. A lower median age of the index patients was associated with a higher rate of secondary cases and household contacts aged one to four years were most likely to become infected. Immediate separation of a paediatric patient from its siblings when there is a clinical suspicion of STEC/VTEC O157 infection has been suggested as an important measure in the prevention of secondary cases [9]. Isolation of all symptomatic primary patients immediately after they receive a microbiological diagnosis of STEC/VTEC O157 infection could potentially decrease the number of secondary household cases by 50% [9]. In this family however, the primary case patient was an adult. Since young children usually have extensive close contacts with their parents; separation of young children from a parent with a suspected STEC/VTEC or *E. coli* O104 infection should be considered in order to prevent secondary transmission to the child.

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References

1. Frank C, Faber MS, Askar M, Bernard H, Fruth A, Gilsdorf A, et al. Large and ongoing outbreak of haemolytic uraemic syndrome, Germany, May 2011. Euro Surveill. 2011;16(21):pii=19878. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19878
2. Scheutz F, Møller Nielsen E, Frimodt-Møller J, Boisen N, Morabito S, Tozzoli R, et al. Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing Escherichia coli O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. Euro Surveill. 2011;16(24):pii=19889. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19889
3. Joint statement issued by the Federal Institute for Risk Assessment (BfR), Federal Office of Consumer Protection and Food safety (BVL), Robert Koch Institute (RKI). Press release. Berlin: BfR, Braunschweig: BVL, Berlin: RKI; 10 June 2011. Available from: http://ecdc.europa.eu/en/press/news/Documents/1106_Joint_Press_Release_German_authorities_issue_a_joint_statement.pdf
4. Informationen zum EHEC/HUS-Ausbruchsgeschehen [Information on the EHEC/HUS outbreak events]. Data from 21 Jun 2011, 10 pm. Berlin: Robert Koch Institut; 22 Jun 2011, 11 am. German. Available from: http://www.rki.de/cln_160/nn_205760/DE/Home/Info-HUS.html
5. Schuurman T, Roovers A, van der Zwaluw WK, van Zwet AA, Sabbe LJ, Kooistra-Smid AM, et al. Evaluation of 5'-nuclease and hybridization probe assays for the detection of shiga toxin-producing Escherichia coli in human stools. J Microbiol Methods. 2007;70(3):406-15.
6. Havelaar AH, Van Duynhoven YT, Nauta MJ, Bouwknegt M, Heuvelink AE, De Wit GA, et al. Disease burden in The Netherlands due to infections with Shiga toxin-producing Escherichia coli O157. Epidemiol Infect. 2004;132(3):467-84.

7. European Centre for Disease Prevention and Control (ECDC). Outbreak of Shiga toxin-producing E. coli in Germany (22 June 2011, 11:00). Stockholm: ECDC; 22 Jun 2011. Available from: http://www.ecdc.europa.eu/en/activities/sciadvice/Lists/ECDC%20Reviews/ECDC_DispForm.aspx?List=512ff74f-77d4-4ad8-b6d6-bf0f23083f30&ID=1119&RootFolder=%2Fen%2Factivities%2Fsciadvice%2FLists%2FECDC%20Reviews

8. Snedeker KG, Shaw DJ, Locking ME, Prescott R. Primary and secondary cases in Escherichia coli O157 outbreaks: a statistical analysis. BMC Infect Dis. 2009;9:144.

9. Werber D, Mason BW, Evans MR, Salmon RL. Preventing Household Transmission of Shiga Toxin-Producing Escherichia coli O157 Infection: Promptly Separating Siblings Might Be the Key. Clin Infect Dis. 2008;46(8):1189-96.

Surveillance of West Nile Virus Disease, Tel Aviv District, Israel, 2005 to 2010

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We present the findings of a six-year surveillance period (2005–2010) of human West Nile virus (WNV) infection in Tel Aviv district, Israel. Initial notifications of positively identified patients received from the Central Virology Laboratory were followed by epidemiological investigations of the local district health office. During 2005–2010, 104 patients, 79 with WNV neuroinvasive and 25 with WNV non-neuroinvasive disease were reported. The median age of the patients with a neuroinvasive disease was 74 years (range: 15 to 95 years) and 53 of such patients had encephalitis, 14 had acute flaccid paralysis, and 12 had meningitis. The case-fatality rate in these patients was 8%. The average annual incidence of neuroinvasive disease during 2005–2010 was 1.08 per 100,000 population. The incidence declined by 86% steadily between 2005 and 2009 (p for trend=0.005), but increased by more than six-fold in 2010. Elderly (≥65 years) men, comprising 25 patients of whom 24 were chronically-ill, had the highest incidence of WNV encephalitis (p<0.001). These findings are concordant with previous data, at the national level, published in Israel and the United States. Notably, the percentage of previously healthy patients, who developed a neuroinvasive disease was the highest (37%, p=0.001) in the surveillance period in 2010.

Introduction

West Nile virus (WNV) is a mosquito-borne arbovirus of the family *Flaviviridae*. Numerous avian species serve as the amplifying hosts. These include, migratory birds, such as the white storks which travel across Israel each autumn, as well as urban species, such as the common house sparrows. Transmission to humans mainly occurs by mosquito vectors, principally by mosquitoes of the genus *Culex*, during their active period, usually between mid-summer and early autumn. WNV has rapidly expanded in both the eastern and western hemispheres in the past two decades [1,2].

Human West Nile fever, caused by laboratory-confirmed WNV infection, was reported in Israel for the first time in the early 1950s, with several outbreaks in that decade, and an additional outbreak in 1980 [3]. The largest outbreak (439 serologically-confirmed cases with 29 deaths) of human WNV infection in Israel occurred in 2000, with a 73% rate of neuroinvasive disease in hospitalised patients [3,4]. Following this outbreak, a national surveillance system was established for humans and mosquitoes [5] and since 2001 WNV infection is notifiable in Israel. The surveillance system detects new cases of human WNV infections and characterises viral genotypes. The system is based on initial notifications of probable or confirmed cases of WNV infection by the Central Virology Laboratory (CVL) and on the subsequent epidemiological investigations of these patients by the local district health offices.

This report summarises a six-year period (2005–2010) of human WNV surveillance in the Tel Aviv district. This most densely populated urban district of Israel included 7,425 residents per square kilometre at the end of 2009, according to the Israel Central Bureau of Statistics [6].

Methods

Epidemiological investigation

The Tel Aviv District Health Office routinely receives notifications from the CVL about probable or confirmed cases of WNV infection in patients who are residents of the district. The local health office initiates an epidemiological investigation by collecting available information on the patients and by using a standard national-based questionnaire. For hospitalised patients, information is gathered from the patients' hospital records, and by conducting an interview with the patient or with a close family member, in order to obtain information regarding risk factors for WNV infection such as occupation, residence or travel near water bodies, recent exposure to mosquitoes, migratory birds and domestic fowl. For non-hospitalised patients, another source of information, in addition to the patient's interview, is usually the family physician.

The gathered demographic, clinical, laboratory and epidemiological data are reported to the Division of Epidemiology, Ministry of Health, Jerusalem.

Laboratory analysis

During the study period, the CVL used a combination of serology assays comprising in-house synchronised IgM-capture (until August 2007), indirect IgG, and indirect IgG avidity ELISAs [4], and from September 2007, the IgM InBios commercial kit (West Nile Detect IgM Capture ELISA, Seattle, Washington, USA) [7]. Results were interpreted according to the manufacturer's instructions. IgM and IgG tests were conducted simultaneously. The avidity IgG test was performed on single samples with IgM and IgG positive results. Unresolved cases were further tested by virus neutralisation assay [8].

Case definition

The following official national laboratory criteria of WNV infection are used by the CVL to define probable and confirmed cases.

• A confirmed case is defined as having at least one of the following criteria: (i) Serological conversion or at least two-fold increase in ELISA test results of IgM antibodies and/or IgG antibodies levels [4,7] in blood or in cerebrospinal fluid (CSF), in paired samples taken at least seven days apart; (ii) IgM antibodies level higher than IgG antibodies level and also IgG avidity lower than 30% in one blood sample; (iii) At least ten-fold higher optical density (OD) of IgM antibodies in CSF in one sample, compared to a reference cut-off level; (iv) Positive result of West Nile viral RNA in CSF, body fluids or body tissues, by real-time RT-PCR assays detecting WNV lineages 1 and 2 [8-10].

• A probable case is defined as detection of WNV IgM antibodies, without detection of IgG antibodies, in one blood or CSF sample or the lack of increase in IgM antibodies, without the presence of IgG antibodies, between paired samples of blood collected one to seven days apart.

The data in this report were summarised and analysed from the district health office's files of individual patients who had an onset of laboratory-confirmed or probable WNV infection, between 1 January 2005 and 31 December 2010. No clinical criteria were used, in addition to the laboratory criteria, for the case definition.

Clinical criteria

The term 'neuroinvasive disease' as used in our analysis refers to WNV-associated diagnoses of encephalitis, meningoencephalitis, meningitis and acute flaccid paralysis (AFP). The clinical definitions of encephalitis, meningitis and AFP, were adopted from previously described definitions [11]. Patients who have had a combined clinical picture of encephalitis and meningitis, hence, meningoencephalitis, were classified as having encephalitis, as in previous reports [12,13]. Also, any presentation of AFP combined with other illness (i.e. encephalitis) was classified as AFP only [12].

We assumed that in non-neuroinvasive patients, there would be a high level of cohort incompleteness due to lack of serological testing and therefore, lack of WNV diagnosis. These patients largely present with a mild nonspecific illness, which is often not followed by seeking medical care [14]. Thus, this report mainly analyses the cohort of WNV neuroinvasive disease patients who usually require diagnosis and treatment within a hospital setting, and therefore, this cohort is more likely to be complete.

Patients were also classified as to whether they had a pre-existing chronic medical condition, which was further classified as to whether it was an immunocompromised state. Any chronic medical condition, excluding psychiatric conditions, was included.

We abstracted these data, post hoc, at the information summary stage of the study from written medical diagnoses in patients' hospital records, where available.

Statistical analysis

For the purpose of p value calculations, we used the exact two-tailed Mann-Whitney U test in case of mean age differences, and not the Student's t-test, because of the small sample size. For the same reason, we used two-tailed Fisher's exact test in case of categorical data and Spearman's rank correlation coefficient for the trend of incidence. Also, the chi-square test with Yates' correction was used for testing differences in the average annual incidence between certain subgroups. A two-tailed p value inferior to 0.05 was considered significant. Statistical analyses were performed using SPSS version 15.0 software (Chicago, Illinois, USA).

The annual average population estimates for the years 2005–2009, used as denominators for incidence calculations, were taken from the Israel Central Bureau of Statistics. The annual average population estimate for 2010 was based on an estimated annual population growth rate of 1.725%, which was derived from the average population growth rate during 2005–2009.

Results

Between 1 January 2005 and 31 December 2010, 104 confirmed or probable cases of WNV infection were reported in the Tel Aviv district. Of these 104 cases, 94 (90%) had an onset of illness in the period from July to the end of October (Figure 1). Eighteen of the 23 (78%) neuroinvasive and non-neuroinvasive cases in 2010, and 18 of the 25 (72%) cases in 2005, occurred in July and August, compared to the occurrence of only 16 of the 56 (29%) cases in the years 2006–2009 combined during the same period.

Seventy-nine patients have had a neuroinvasive disease (Table), all of whom were hospitalised: 53 (67%) had encephalitis, 12 (15%) had meningitis and 14 (18%)

had AFP, of whom two patients had Guillain–Barré syndrome. The median age of all patients with a neuroinvasive disease was 74 years (range: 15 to 95 years) and 51 (65%) of these patients were aged 65 years or older. Fourty (51%) of these patients were males.

Of 79 patients with neuroinvasive disease, 25 (32%) were elderly men (≥65 years), of whom 24 were chronically-ill. Similarly, 26 (33%) elderly women including 25 chronically-ill were part of the 79 patients presenting with neuroinvasive disease.

Patients with encephalitis had a significantly higher mean age than patients with meningitis and AFP (74 years; 95% confidence interval (CI): 70–78 years vs 59 years; 95% CI: 51–67 years, p=0.02). The mean age of patients with meningitis was significantly lower than the mean age of patients with encephalitis and AFP (47 years; 95% CI: 35–60 years vs 73 years; 95% CI: 69–76 years, p<0.001).

Of all 79 patients with a neuroinvasive disease, 69 (87%) had a pre-existing chronic medical condition.

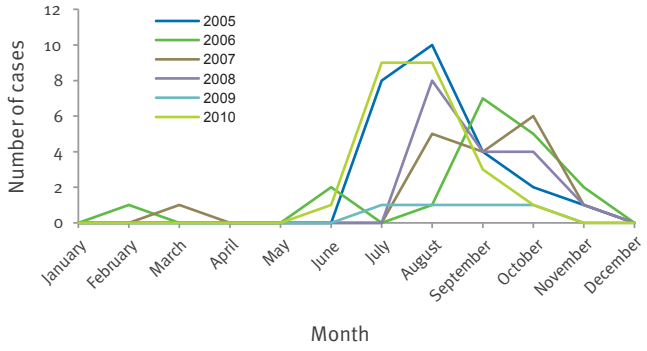
The group of patients with meningitis had a significantly lower percentage of chronic illness when compared to the group of patients with encephalitis (8/12, 67%; 95% CI: 35%-90% vs 49/53, 92%; 95% CI: 82%-98%, respectively, p=0.03). The percentage of patients with a pre-existing chronic medical condition was significantly lower in 2010, when compared to the period between 2005 and 2009 (12/19, 63%; 95% CI: 38%–84% vs 57/60, 95%; 95% CI: 86%–99%, p=0.001).

The case-fatality rate (CFR) of patients with a neuroinvasive disease was six of 79 (8%). The patients who died were older than 74 years of age, five of six were females, five of six had a pre-existing chronic medical condition, and four of six had a diagnosis of WNV encephalitis.

During the surveillance period, the annual percentage of patients with encephalitis, meningitis or AFP ranged between 55% and 90%, 0% and 25%, and, 10% and 33%, respectively (Figure 2).

Figure 1

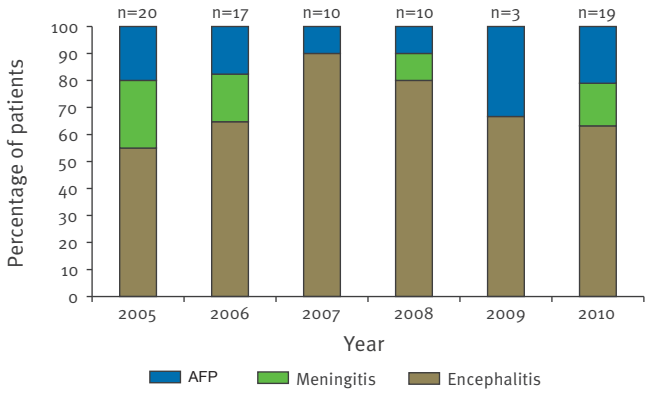
Human West Nile virus infection confirmed and probable cases, by month of illness onset, 2005–2010, Tel Aviv district, Israel (n=104)



Human West Nile virus infection confirmed and probable cases include 79 cases of neuroinvasive disease and 25 cases of non-neuroinvasive disease.

Figure 2

Percentage of patients with West Nile virus neuroinvasive disease, by year and clinical diagnosis, Tel Aviv district, Israel, 2005–2010 (n=79)



AFP: acute flaccid paralysis.

Table

Characteristics of cases of human West Nile virus neuroinvasive disease by clinical diagnosis, Tel Aviv district, Israel, 2005–2010 (n=79)

Characteristic	Encephalitis	Meningitis	AFP	**Total**
	(n=53)	(n=12)	(n=14)	**(n=79)**
Median age, years (range)	75 (38-95)	45 (15-79)	77 (41-95)	**74 (15-95)**
Male sex (%)	26 (49)	6 (50)	8 (57)	**40 (51)**
Deaths (%)	4 (8)	1 (8)	1 (7)	**6 (8)**
Immunocompromised (%)	5 (9)	0 (0)	1 (7)	**6 (8)**
Any chronic medical condition[a] (%)	49 (92)	8 (67)	12 (86)	**69 (87)**

AFP: acute flaccid paralysis.

[a] Most commonly included was a history of one or more illnesses such as essential hypertension, hyperlipidemia, and/or diabetes mellitus.

The average annual incidence of neuroinvasive disease between 2005 and 2010 was 1.08 per 100,000 population (Figure 3). The incidence of neuroinvasive disease declined significantly, between 2005 and 2009, from 1.69 to 0.24 per 100,000 population (an 86% decrease, rs=-0.97, p for trend=0.005), but increased more than six-fold in 2010, to 1.47 per 100,000 population, compared to the previous year. The change in trend, during 2005–2010, of the WNV-associated central nervous system diagnoses (encephalitis and meningitis) was similar to the trend of the WNV-associated peripheral nervous system illness (AFP).

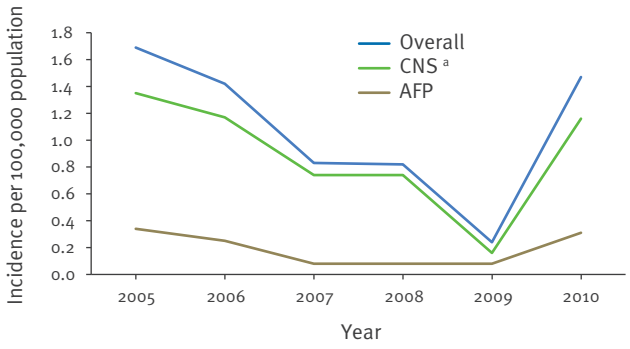
The average annual incidence of neuroinvasive disease, by age, between 2005 and 2010 (Figure 4) demonstrated a peak in incidence among patients aged 75 years or older (7.03 per 100,000 population), while no patients aged less than 15 years were reported. The peak of the average annual incidence of encephalitis and of AFP was in patients aged 75 years or older (5.18 and 1.48 per 100,000 population, respectively) (Figure 5).

The average annual incidence of neuroinvasive disease had a similar pattern among men and women (Figure 6) with the exception that the incidence in male patients aged 65–74 years, was almost three-fold higher than in female patients of the same age group (4.11 per 100,000 population vs 1.41 per 100,000 population, respectively). The latter difference could not be confirmed with statistical significance, but this could be due to the small number of cases in each of the male and female subgroups.

During the surveillance period, elderly men (≥65 years) had the highest average annual incidence of WNV encephalitis in the cohort, and it was significantly higher than the rest of the patients with encephalitis, aged between 35 and 64 years (4.54 per 100,000 population vs 1.19 per 100,000 population, p<0.001).

Figure 3

Annual incidence of human West Nile virus neuroinvasive disease by year and clinical diagnosis, Tel Aviv district, Israel, 2005–2010

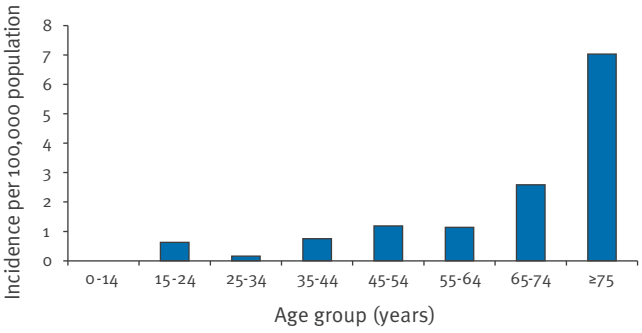


AFP: acute flaccid paralysis; CNS: central nervous system group of diagnoses.

[a] The CNS group of diagnoses includes encephalitis and meningitis.

Figure 4

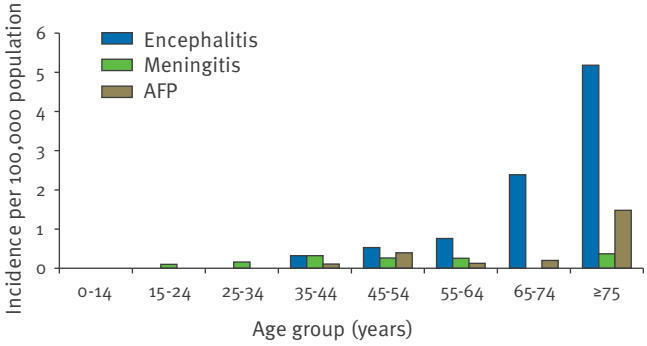
Average annual incidence of human West Nile virus neuroinvasive disease by age group, Tel Aviv district, Israel, 2005–2010



Average annual incidence is calculated using Israel Central Bureau of Statistics average population estimates of Tel Aviv district for 2008.

Figure 5

Average annual incidence of human West Nile virus neuroinvasive disease by age group and clinical diagnosis, Tel Aviv district, Israel, 2005–2010

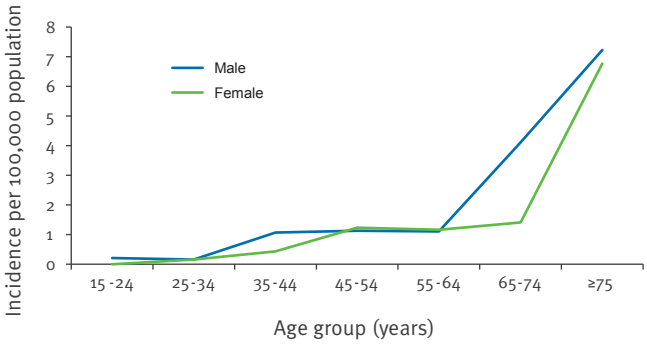


AFP: acute flaccid paralysis.

Average annual incidence is calculated using Israel Central Bureau of Statistics average population estimates of Tel Aviv district for 2008.

Figure 6

Average annual incidence of human West Nile virus neuroinvasive disease by age group and sex, Tel Aviv district, Israel, 2005–2010



Average annual incidence is calculated using Israel Central Bureau of Statistics average population estimates of Tel Aviv district for 2008.

Similarly, male patients aged 75 years or older had the highest average annual incidence of encephalitis or AFP in the cohort during the surveillance period, which was significantly higher than the rest of the patients, aged between 35 and 74 years, with encephalitis (5.42 per 100,000 population vs 1.46 per 100,000 population, p<0.001) or AFP (1.81 per 100,000 population and 0.33 per 100,000 population, p=0.004). In contrast, female patients aged 75 years or older had the highest average annual incidence of meningitis, but it was not significantly higher than the rest of the patients with meningitis, aged between 15 and 74 years (0.62 per 100,000 population vs 0.17 per 100,000 population, p=0.3).

Discussion

The data that were summarised and analysed in this study reflect a six-year period of WNV surveillance in Tel Aviv district. After several years of successive declines in WNV neuroinvasive disease, its incidence increased sharply in 2010.

We observed, as previously reported by Lindsey et al. [12], a higher incidence of encephalitis in elderly male patients during our surveillance period. These patients were also chronically ill, which may serve as an independent risk factor for WNV neuroinvasive disease [15]. However, five of the six fatalities in the observation period were female patients.

Two-thirds of the patients with a neuroinvasive disease had encephalitis (53/79, 67%), followed by patients with AFP (14/79, 18%). Of those latter 14 patients with AFP, four were diagnosed in 2010 alone. WNV-associated AFP was hardly observed during the large outbreak of 2000 in Israel: 98% of 233 hospitalised patients were diagnosed as having West Nile fever, encephalitis or meningitis but not AFP, which was reported as myelitis by Chowers et al. [13].

In our series, patients with meningitis were significantly younger than patients with other forms of neuroinvasive disease. This age discrepancy was described previously by Lindsey et al. [12].

Our reported CFR for the patients with neuroinvasive disease (6/79, 8%), who were all hospitalised, was similar to the overall CFR in Israel during the outbreak in 2000 (29/439, 7%) [4]. The CFR in our study was however half the CFR of hospitalised patients (33/233, 14%), for whom data were obtained in 2000 [13]. Our reported CFR was also similar to that in the United States (US), which was 9% of the patients with a neuroinvasive disease during 1999–2008, most of whom were hospitalised [12].

Remarkably, the highest percentage of WNV neuroinvasive disease occurring in previously healthy patients was observed in 2010, when compared to the period between 2005 and 2009 (7/19, 37% vs 3/60, 5%, p=0.001).

In our series, meningitis affected patients who were significantly younger on average than patients with other forms of neuroinvasive disease. The year 2010 did not appear to present an exception, although the total number of cases was small (39 years on average (n=3) for patients with meningitis vs 47 years in all years of the surveillance period (n=12)). Because of their younger age, patients with WNV meningitis would be less likely to have underlying medical conditions. Therefore, we examined whether the overall increase of previously healthy patients presenting with WNV neuroinvasive disease in 2010 could have been due to an increase in the number of patients with WNV meningitis in that year; however, of the patients with a neuroinvasive disease in 2010, only three of 19 had meningitis, which was well within the range of annually reported cases of WNV meningitis in the district, between 2005 and 2009 (0/10 in 2007 and 0/3 in 2009 up to 5/20 in 2005).

We also verified if the number of previously healthy patients presenting with a neuroinvasive disease had increased in other years than 2010, such as in 2005, in which a peak in the proportion of meningitis was observed (5/20). There was, however, no lower proportion of chronically-ill patients (17/20) in that year.

Additionally, to our knowledge, there was no change in the surveillance practice over the study time period that could account for the highest percentage of previously healthy patients presenting with WNV neuroinvasive disease observed in 2010.

In the years following the major national outbreak of WNV infection in 2000, various strains of WNV lineage 1 predominated in Israel either in isolated foci or in the entire country [16]. Representative Israeli WNV genomic sequences of viruses isolated from mosquitoes and humans between 2000 and 2009 were deposited in the GenBank/EMBL/DDBJ database under accession numbers GU246634–GU246714 and HM152773–HM152780, respectively.

The emergence of new viral genotypes simultaneously with higher number of human cases was observed in Israel in 2000, 2005, and 2007 [16]. Particularly, in 2005, an increased nationwide WNV activity, with 102 laboratory-confirmed human patients [16] of whom 20% were from the Tel Aviv district, which was a part of the epicentre, was reported [17]. Molecular analysis of human and mosquito isolates revealed a genotype most similar to the one that was isolated in the equine WNV outbreak in Morocco in 2003 [18], and which had not been isolated previously from humans or mosquitoes in Israel [17].

Notably, 2005 and 2010 shared some epidemiological similarities, as we observed in the district: both years had a similar incidence of WNV neuroinvasive disease, higher than the other years of surveillance, including a higher incidence of AFP, which is a long-term

complication of considerable morbidity and mortality [19,20]. In addition, the majority of WNV infection cases in both years had occurred early, already in July and August, compared to later months of occurrence, in the other years.

One possible explanation to the increased incidence of morbidity observed in 2010, which was also accompanied by a higher fraction than before of previously healthy patients could be the recent emergence of another WNV strain or variant, as in 2005. A genotype characterisation of the 2010 WNV strain is however not available to date.

Another explanation for the 2010 epidemiological characteristics could be the early arrival of an extremely hot summer, already in May. This might have contributed to the early peak of WNV season in 2010, in the district. The period of May–July 2010 was warmer than the perennial average of the years 1981–2000 [21-23]. In addition, August 2010 was the warmest measured month in Israel [24,25].

The summer of 2010 was extremely hot also in areas, which usually have a temperate climate, such as northern Greece and Romania. A human WNV outbreak emerged in northern Greece for the first time, early in July 2010 [26]. An additional noticeable outbreak, which also began in early July 2010, was reported in Romania, where a neuroinvasive lineage 2 WNV strain was detected for the first time [27]. Whether there is a possible association between recent climatic extremes in the region, WNV activity, and its mosquito vectors should be extensively studied.

Conclusions

We report on the epidemiological and clinical characteristics of human WNV infection in the Tel Aviv district between 2005 and 2010. As such, it may be limited in time, place and person. Nevertheless, our main body of findings, such as the patients' characteristics, was concordant with previous data, at the national level, published in Israel and the US.

Any successful WNV surveillance system should integrate and maintain both epidemiological and laboratory capabilities for prolonged periods of time, particularly in endemic and densely populated areas, such as the Tel Aviv district.

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References

1. Petersen LR, Marfin AA, Gubler DJ. West Nile virus. JAMA. 2003;290(4):524-8.
2. Malkinson M, Banet C, Weisman Y, Pokamunski S, King R, Drouet M, et al. Introduction of West Nile virus in the Middle East by migrating white storks. Emerg Infect Dis. 2002;8(4):392-7.
3. Weinberger M, Pitlik SD, Gandacu D, Lang R, Nassar F, Ben David D, et al. West Nile fever outbreak, Israel, 2000: epidemiologic aspects. Emerg Infect Dis. 2001;7(4):686-91.
4. Bin H, Grossman Z, Pokamunski S, Malkinson M, Weiss L, Duvdevani P, et al. West Nile fever in Israel 1999-2000: from geese to humans. Ann N Y Acad Sci. 2001;951:127-42.
5. Orshan L, Bin H, Schnur H, Kaufman A, Valinsky A, Shulman L, et al. Mosquito vectors of West Nile Fever in Israel. J Med Entomol. 2008;45(5):939-47.
6. Israel Central Bureau of Statistics. Statistical Abstract of Israel 2010-No.61. 2010. [Accessed 29 December 2010]. Available from: http://www.cbs.gov.il/reader/shnatonenew_site.htm.
7. Welch RJ, Anderson BL, Litwin CM. Evaluation of a new commercial enzyme immunoassay for the detection of IgM antibodies to West Nile virus using a ratio method to eliminate nonspecific reactivity. J Clin Lab Anal. 2008;22(5):362-6.
8. Hindiyeh M, Shulman LM, Mendelson E, Weiss L, Grossman Z, Bin H. Isolation and characterization of West Nile virus from the blood of viremic patients during the 2000 outbreak in Israel. Emerg Infect Dis. 2001;7(4):748-50.
9. Lanciotti RS, Kerst AJ, Nasci RS, Godsey MS, Mitchell CJ, Savage HM, et al. Rapid detection of west nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. J Clin Microbiol. 2000;38(11):4066-71.
10. Linke S, Ellerbrok H, Niedrig M, Nitsche A, Pauli G. Detection of West Nile virus lineages 1 and 2 by real-time PCR. J Virol Methods. 2007;146(1-2):355-8
11. Sejvar JJ, Haddad MB, Tierney BC, Campbell GL, Marfin AA, Van Gerpen JA, et al. Neurologic manifestations and outcome of West Nile virus infection. JAMA. 2003;290(4):511-5.
12. Lindsey NP, Staples JE, Lehman JA, Fischer M; Centers for Disease Control and Prevention (CDC). Surveillance for human West Nile virus disease - United States, 1999-2008. MMWR Surveill Summ. 2010;59(2):1-17.
13. Chowers MY, Lang R, Nassar F, Ben-David D, Giladi M, Rubinshtein E, et al. Clinical characteristics of the West Nile fever outbreak, Israel, 2000. Emerg Infect Dis. 2001;7(4):675-8.
14. Zou S, Foster GA, Dodd RY, Petersen LR, Stramer SL. West Nile fever characteristics among viremic persons identified through blood donor screening. J Infect Dis. 2010;202(9):1354-61.
15. Jean CM, Honarmand S, Louie JK, Glaser CA. Risk factors for West Nile virusneuroinvasive disease, California, 2005. Emerg Infect Dis. 2007;13(12):1918-20.
16. Bin H, Shulman L, Hindiyeh M, Weiss L, Schlezinger S, Orshan L, et al. Clinical and molecular aspects of West Nile virus infections in Israel from 2000 to 2008. The 9th Tel Aviv University Research Fair; 2010 Mar 25; Tel Aviv, Israel. Available from: http://medicine.tau.ac.il/fair-abstracts/uploads/Z35.doc.
17. Bin H, Shulman L, Weiss L, Schlezinger S, Kaufman Z, Orshan L, et al. WNF resurgence in Israel in 2005 was associated with introduction of new WNV genotypes and high rate of mosquito infections. The Second Jerusalem Symposium on Arthropod-Borne Viral Diseases; 2006 Jun 25-30; Mitzpe Haymim, Israel.
18. Schuffenecker I, Peyrefitte CN, el Harrak M, Murri S, Leblond A, Zeller HG. West Nile virus in Morocco, 2003. Emerg Infect Dis. 2005;11(2):306-9.
19. Saad M, Youssef S, Kirschke D, Shubair M, Haddadin D, Myers J, et al. Acute flaccid paralysis: the spectrum of a newly recognized complication of West Nile virus infection. J Infect. 2005;51(2):120-7.
20. Sejvar JJ, Bode AV, Marfin AA, Campbell GL, Pape J, Biggerstaff BJ, et al. West Nile Virus-associated flaccid paralysis outcome. Emerg Infect Dis. 2006;12(3):514-6.
21. Israel Meteorological Service. Monthly summary of weather - May 2010. Israel Meteorological Service. [Accessed 1 September 2010]. Hebrew. Available from: http://www.ims.gov.il/NR/rdonlyres/A61ACC44-DE6D-4F5F-90F6-7CF3CC978CC2/0/may10.pdf.
22. Israel Meteorological Service. Monthly summary of weather - June 2010. Israel Meteorological Service. [Accessed 1 September 2010]. Hebrew. Available from: http://www.ims.gov.il/NR/rdonlyres/E6150B31-B8DA-462D-9EFC-28F45B4FBB94/0/june10.pdf.

23. Israel Meteorological Service. Monthly summary of weather - July 2010. Israel Meteorological Service. [Accessed 1 September 2010]. Hebrew. Available from: http://www.ims.gov.il/NR/rdonlyres/A732F141-D50D-4A95-A4A3-70D62EC423C7/0/july2010.pdf.

24. Israel Meteorological Service. Monthly summary of weather - August 2010. Israel Meteorological Service. [Accessed 1 September 2010]. Hebrew. Available from: http://www.ims.gov.il/NR/rdonlyres/59992847-D836-49DF-B8D7-2D62F7892555/0/aug10.pdf.

25. Zafrir R. It's official: Last month marked Israel's hottest August. Haaretz. 1 September 2010. Available from: http://www.haaretz.com/news/national/it-s-official-last-month-marked-israel-s-hottest-august-1.311546.

26. Papa A, Danis K, Baka A, Bakas A, Dougas G, Lytras T, et al. Ongoing outbreak of West Nile virus infections in humans in Greece, July - August 2010. Euro Surveill. 2010;15(34):pii=19644. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19644.

27. Sirbu A, Ceianu CS, Panculescu-Gatej RI, Vázquez A, Tenorio A, Rebreanu R, et al. Outbreak of West Nile virus infection in humans, Romania, July to October 2010. Euro Surveill. 2011;16(2):pii=19762. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19762.

Seroprevalence to cytomegalovirus in the Portuguese population, 2002-2003

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The prevalence of cytomegalovirus (CMV) infections ranges between 50% and 85% in adults in the United States, and its epidemiology varies in different regions of the world and between socioeconomic and age groups. In Portugal, no study has been carried out to date to determine the prevalence of CMV in the general population. Under the second National Serological Survey conducted in continental Portugal in 2001–2002, we estimated the prevalence of individuals with antibodies to CMV using indirect immunofluorescence to detect virus-specific IgG. The population sample included 2,143 individuals of both sexes and different ages from all 18 districts in Portugal. The national seroprevalence of CMV was determined as 77%. We analysed the proportion of CMV IgG by sex, age group and district of residence. This was the first nationally representative study of seroprevalence of CMV in Portugal. The results of the study indicate that CMV infection is highly prevalent in the population and occurs mainly in the first years of life.

Introduction

Cytomegalovirus (CMV) is a common virus with no known seasonal predominance and with a prevalence that ranges between 50% and 85% of adults in the United States [1-3]. The epidemiology of CMV varies in different regions of the world and in different socioeconomic and age groups [3-5].

CMV establishes a latent state following primary infection, reactivating when there are changes in immune status [6,7]. CMV infections are most often asymptomatic, but when symptomatic, can cause a syndrome similar to clinical and haematological infectious mononucleosis. The virus is excreted through body fluids, and the most common modes of transmission are via the oropharyngeal and genital tract, although transmission can also occur through breast milk, organ transplant or blood transfusions [8-11].

CMV primary infection occurs mostly in childhood and adolescence, but primary infections are also observed in adults [3,11-14]. The infection is important in certain risk groups such as immunocompromised individuals and pregnant women. In immunocompromised individuals, CMV infection is the leading cause of morbidity and mortality, especially in connection with transplants, haemodialysis, cancer, immunosuppressive medication and infection with human immunodeficiency virus (HIV) [8]. Transplacental transmission can occur and primary infection in the first 16 weeks of pregnancy is associated with higher rate of damage in fetal development [15-20]. In primary maternal infection, the probability of transmission of CMV to the fetus is approximately 30% to 40%. In women with CMV reactivation during pregnancy the probability of fetal CMV transmission decreases to approximately 0.5% to 1.4% [21-24]. In developed countries, CMV is a major cause of congenital infection, with an incidence of 0.4% to 2.2% of total live births per year, and is responsible for neonatal morbidity and mortality [19,23,25-27]. The congenital CMV infection is asymptomatic in the neonatal period (the first 28 days of life) in approximately 85% to 90% of infants, but nearly 5% to 15% of these infants show late sequelae during the first years of life, typically hearing deficits and visual impairments [22,28-30]. Approximately 10% to 15% of infants with congenital CMV infection are symptomatic at birth, with manifestations including growth retardation, jaundice, purpura, hepatosplenomegaly, microcephaly, intracerebral calcifications, and retinitis. The risk of long-term neurodevelopmental disabilities is high in these children and include microcephaly, hearing loss, motor deficits, cerebral palsy, mental retardation, seizures, ocular abnormalities and learning disabilities [23,31-34].

In a recent review of priorities for vaccine development, the Institute of Medicine of the National Academy of Sciences in the United States concluded that in terms of healthcare costs and years of life and disability saved, a vaccine against CMV infection should be a priority [35,36].

In Portugal, the prevalence of CMV in the population and the incidence of CMV congenital infection have

not previously been determined. However, in 2003, preliminary results of a prospective study indicated that the proportion of CMV congenital infections was 0.7% in a consecutive population of newborns in the area of Lisbon, as determined using the gold standard technique, shell vial culture of urine [37]. According to a survey by Paixão et al. [38] that screened for CMV congenital infection by PCR of blood samples obtained from Guthrie cards, the proportion of congenital infection by CMV was 1.1% in Portugal and 0.7% in the region of Lisbon in the period from August 2003 to September 2004.

The second National Serological Survey conducted in continental Portugal in 2001–2002 was carried out with two objectives: firstly, to determine the prevalence of individuals with antibodies to vaccine-preventable diseases, as a means of evaluating the national immunisation programme, and secondly, to determine the prevalence of individuals with antibodies to infections deemed important in terms of public health. As part of the second National Serological Survey, we estimated the proportion of individuals with CMV antibodies in Portugal in 2002-2003 in order to assess the prevalence of CMV in the population [39].

Methods

Sampling

The main sample frame that was used to study the immunity to diseases included in the national immunisation programme was calculated to be nationally representative and to adequately cover all age groups, at a sample size of 3,304 individuals of both sexes, homogeneously distributed in eight age groups: 2–4, 5–9, 10–14, 15–19, 20–29, 30–44, 45–64, and ≥65 years. Each age group included individuals from each of the 18 districts of mainland Portugal, to a number proportionally representative for the population of each district. Between 2002 and 2003, the national immunisation programme recruited 3,525 participants at 38 private and public serum collections points, distributed in all 18 districts of mainland Portugal. Individuals were invited to participate until the district and age grids prepared were completed. For the participation of individuals, a fact sheet was prepared with the objectives and benefits of the study and informed consent was obtained either from the participants themselves or from their legal representatives [39].

For our specific study on the prevalence of CMV, we used a sub-sample of 2,143 individuals. Serum samples were taken from the same batch collected during 2002–2003 and analysed in 2003–2004 for the presence of CMV-specific antibodies.

Serological analyses

IgG antibodies specific for CMV were detected by indirect immunofluorescence, using commercial reagents (Merifluor CMV IgG, US) according to the manufacturer's instructions. Samples were added to a layer of human fibroblasts fixed on glass slides on which approximately 10% of cells are infected with CMV strain AD169, and the formation of antigen-antibody complex is viewed using a fluorescent dye. Uninfected fibroblasts on the same slide were used as an internal control of the specificity of the test. The tests were validated with negative and positive control sera. The use of reference sera ensured the reproducibility of results between batches. According to the manufacturer, the test has 97% sensitivity, 100% specificity, 100% predictive value of a positive test and 99% predictive value of a negative test.

Statistical analysis

Statistical analysis consisted in the determination of absolute and relative frequencies (percentages). Binomial confidence intervals were calculated using the exact method that uses the relationship between the F and Binomial distributions attributed to Bliss and Brownlee as described by Zar [40].

Table

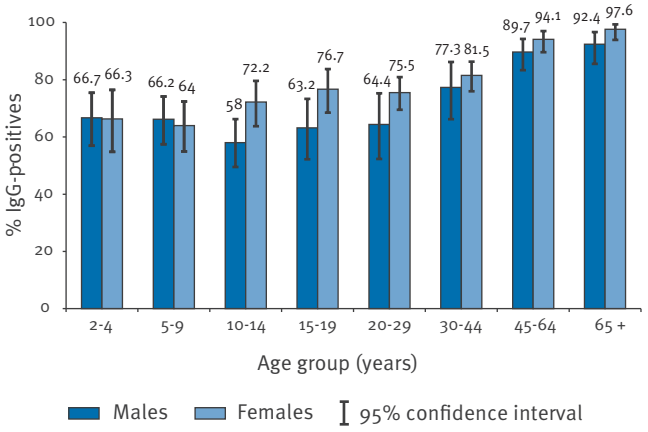
Individuals IgG-positive for cytomegalovirus, by sex, Portugal, 2002-2003 (n=2,143)

Sex	Study participants n	CMV-positive n (%)	95% confidence interval
Male	860	622 (72.3)	69.2–75.3
Female	1,283	1,029 (80.2)	77.9–82.4
Total	**2,143**	**1,651 (77%)**	**75.2–78.8**

CMV: cytomegalovirus.

Figure 1

Percentage of individuals IgG-positive results for cytomegalovirus, by age and sex, Portugal, 2002-2003 (n=2,143)



IgG: Immunoglobulin G.

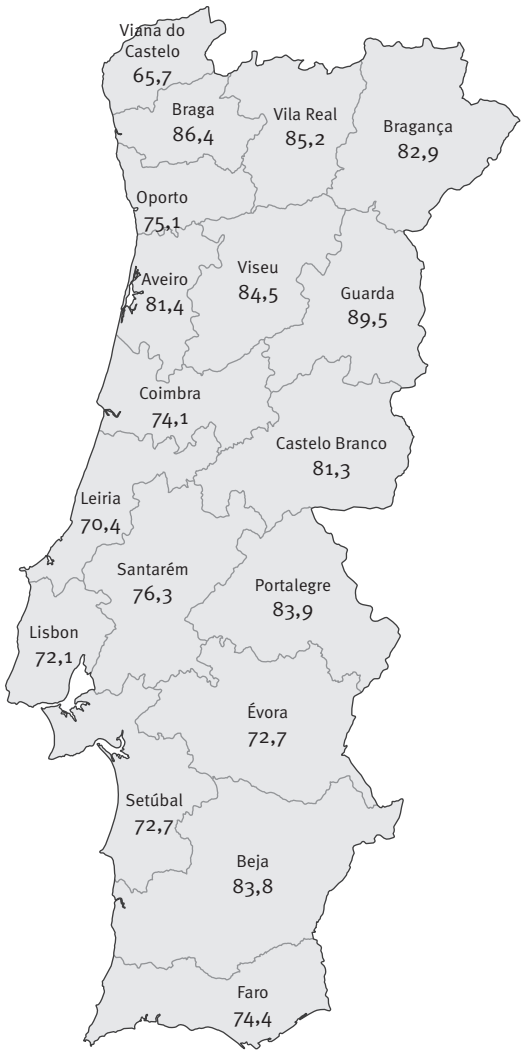
Results

In a total of 2,143 individuals, 1,651 had IgG antibodies to CMV (77%, 95% confidence interval (CI): 75.2–78.8) and 492 were seronegative (23%, 95%: CI 21.2–24.8). Among the male participants, 622 (72.3%) were CMV-positives and among the female participants 1,029 (80.2%) were CMV-positives (Table).

When analysing the distribution of CMV IgG-positive individuals by age, 66.5% of children in the youngest age group (2–4 years) were shown to have antibodies. The percentage of seropositive individuals was similar in age group of 5-9 year-olds and 10-14 year-olds with 65.1% and 64.9%, respectively, and then increased with age, reaching 71.3% in the age group of 15–19 year-olds, 80.5% in the 30–44 year-olds and 95.6% in the oldest group (≥65 years). The percentage of males and females with CMV IgG in each age group is shown in Figure 1.

Figure 2

Proportion of individuals IgG-positive for cytomegalovirus, by district of residence, Portugal, 2002-2003 (n=2,143)



IgG: Immunoglobulin G.

Analysis of the distribution of CMV IgG-positive individuals by district of residence showed that only one of the 18 districts of Portugal, Viana do Castelo, had a prevalence of less than 70% of seropositive individuals. The highest prevalence of CMV-positive residents was found in the districts of Guarda, Braga and Vila Real, with 89.5%, 86.4% and 85.2%, respectively (Figure 2).

Discussion

In Portugal, the second National Serological Survey has established, for the first time, the prevalence of individuals with CMV-specific IgG. The results of this study indicated that CMV infection was highly prevalent in the population (77%), similar to what has been described for other countries, and that it occurred predominantly in the first years of life [1,3,41-46].

Seroprevalence for CMV between the age of two and four years was high, with 66.5% of IgG-positive children in this age group (95% CI: 59.3–73.2). Children younger than two years were not included in the study and conclusions on the situation at that age can therefore not be drawn. However, breastfeeding is known to be a significant source of CMV transmission to children and plays an important role in the epidemiology of CMV infection as CMV is reactivated during lactation in nearly every seropositive mother [47-49]. The proportion of infants who acquire CMV during the first year of life is directly related to the prevalence of maternal infection and to the proportion of mothers who breastfeed. In countries where breastfeeding is widely practiced and most mothers are seropositive, for example in south and south-eastern Europe, regions of Asia, Africa and Latin America, more than 50% of infants acquire CMV within the first year of life [8,50]. The seroprevalence found in the age group between two and four years (66.5%) was probably the result not only of transmission via breastmilk but also of oral transmission from other children and from seropositive adults they are in close contact with when they start attending day care centers at that age [51,52]. This was similar to the seroprevalence of 53.8% found in several studies performed in children of that age in Brazil (region of São Paulo) [53]. It was higher than that in Italy (region of Parma, 28% at two years of age) and Finland (41% at eight years of age) and lower than in Venezuela (region of Valencia; 83.3% between two and four years of age) and Turkey (region of Antalya, 82.1% in children between one and six years of age) [41,54-56].

The antibody prevalence in children at school age (age groups 5–9 years and 10–14 years) was similar to that at pre-school age, but increased further to 71.3% (95% CI: 64.8–77.2) in the age group between 15 and 19 years, which corresponds to a greater sexual exposure, in addition to close non-sexual contact [3,8,13,57]. The antibody prevalence in this age group was identical to that in the 20–29 year-olds. Studies with similar age groups conducted in other countries, such as the United States, Japan, France, England, Poland and Russia, describe seroprevalences ranging between 51.5% and 78.0% [3,44,58-62].

The prevalence of individuals with CMV IgG gradually increased further in the three oldest age groups, with values of 80.5% (95% CI: 75.6%–84.8%), 92.2% (95% CI: 88.7%–94.9%) and 95.6% (95% CI: 92.4%–97.7%), suggesting that sexual transmission was an important route of transmission of the virus in the population [8,63,64]. Another recognised source of adult CMV infection are children. Children infected with CMV shed virus in saliva and urine for years, providing an opportunity for continued spread to other children and susceptible adults (close relatives and day care workers) [8,51,65–67].

IgG-positivity was equally common in both sexes in the age groups of 2–4 and 5–9 year-olds, while in the older age groups, females were more likely to be IgG-positive than males. The statistically significant difference of 8% between males and females in the prevalence of individuals with CMV IgG could be explained by the fact that women may have more contact with children. This horizontal mode of transmission presents a risk to mothers, pregnant women and those with occupations associated with exposure to children, such as teachers and day care providers [23,63,68,69].

Nevertheless, it should be noted that in our study, 24.5% and 18.5% of women of reproductive age (from 20 to 29 years and 30 to 44 years, respectively) were susceptible to CMV, which led us to conclude that there is a considerable risk for congenital infection due to maternal primary CMV infection, which leads to fetal infection in approximately 40% of cases [11,16].

Possible approaches to preventing congenital CMV infections include improved hygiene behaviour of seronegative pregnant women, administration of CMV hyperimmune globulin (HIG) to pregnant women with primary infection, and vaccines, once available, administered to girls or women before pregnancy [70].

Several studies have been done to determine whether changing protective behaviour prevents child-to-mother transmission of CMV during pregnancy [71-74]. The United States Centers for Disease Control and Prevention recommend that seronegative pregnant women assume that children are secreting CMV in their urine or saliva. They advise on simple hygiene such as frequent hand washing, wearing gloves for specific childcare tasks and avoiding intimate contact with their child such as sharing utensils, food or towels, and kissing on or near the mouth [75-77].

Despite advances in the diagnosis of maternal-fetal CMV infection and approaches to prevent congenital CMV, an effective prenatal therapy is unavailable. A prospective, non-randomised study of pregnant women who acquired CMV infection during pregnancy and who received passive immunisation with CMV HIG, showed that this therapy was associated with a significantly reduced risk of congenital CMV disease and infection and had no adverse effects [70,78,79]. Recent case reports supported safe administration of oral ganciclovir to mothers of CMV-infected fetuses, with no teratogenic side effects when given in the early stages of pregnancy [70,80,81]. The efficacy of ganciclovir still remains to be defined in controlled trials. Other early experience with treatment of intrauterine CMV infection using maternal oral administration of valaciclovir showed that it decreased the viral load in fetal blood significantly and could potentially also reduce the morbidity of prolonged intrauterine infection [82]. The absence of adverse effects or teratogenecity of valaciclovir is compatible with its clinical use, but a well-designed randomised controlled trial is needed.

Currently, there is no approved vaccine for CMV, but two vaccines are in phase II studies: one is a recombinant vaccine containing the major envelope glycoprotein B of the virus with the adjuvant MF59 (gB/MF59) that induces high levels of neutralising antibodies, is safe and immunogenic in adults and infants, preventing also maternal CMV infection [36,83]. The other vaccine is the live attenuated CMV Towne strain that stimulates neutralising antibodies comparable to those induced by wild type virus and protects renal transplant patients from severe CMV after transplantation [78,84].

The main interventions for the prevention of CMV infection should be aimed at women who wish to become pregnant, women who care for children and immunocompromised individuals. These individuals in whom exposure to CMV can be most detrimental will be the target groups for possible administration of a future vaccine.

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References

1. Centers for Disease Control and Prevention (CDC). Cytomegalovirus (CMV) and Congenital CMV infection. Atlanta: CDC. [Accessed 23 Jun 2011]. Available from: http://www.cdc.gov/cmv/overview.html
2. Burchett SK, Dalgic N. Viral infections. In: Cloherty JP, Eichenwald EC, Stark AR, editors. Manual of Neonatal Care. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2008. p. 244-74.
3. Staras SA, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ. Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. Clin Infect Dis. 2006;43(9):1143-51.
4. Fowler KB, Stagno S, Pass RF. Maternal age and congenital cytomegalovirus infection: screening of two newborn populations. J Infect Dis. 1993;168(3):552-6.

5. Chandler SH, Alexander ER, Holmes KK. Epidemiology of cytomegaloviral infection in a heterogeneous population of pregnant women. J Infect Dis. 1985;152(2):249-56.
6. Mocarski ES, Jr. Cytomegalovirus biology and replication. In: Roizman B, Whitley RJ, Lopez C, editors. The human herpesviruses. New York: Raven Press; 1993. p. 173-226.
7. Mocarski ES, Jr, Tan CS. Cytomegaloviruses and their replication. In: Knipe DM, Howley PM, editors. Fields' Virology. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 2629-73.
8. Pass, RF. Cytomegalovirus. In: Knipe DM, Howley PM, editors. Fields' Virology. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 2675-705.
9. Grangeot-Keros L, Audibert F, Vial-Courmont M. CMV et grossesse. Revue Française des Laboratoires 2002;345:41-6. French.
10. Ross SA, Boppana SB. Congenital cytomegalovirus infection: outcome and diagnosis. Semin Pediatr Infect Dis. 2005;16(1):44-9.
11. Stagno S, Britt W. Cytomegalovirus infections. In: Remington JS, Klein JO, Wilson CB, Baker CJ, editors. Infectious Diseases of the Fetus and Newborn Infant. 6th ed. Philadelphia: Elsevier Saunders; 2006. p. 739-81.
12. Spiteri LJ, Chamberlain R, Pollard R, Tobin JO. Antibody to cytomegalovirus in Malta. J Hyg (Lond) 1982;88(2):355-9.
13. Nageswaran A, Kinghorn GR. Sexually transmitted diseases in children: herpes simplex virus infection, cytomegalovirus infection, hepatitis B infection and molluscum contagiosum. Genitourin Med. 1993;69(4):303-11.
14. Kuijpers TW, Vossen MT, Gent MR, Davin JC, Ross MT, Van Lier RA, et al. Frequencies of circulating cytolitic, CD45RA+ CD27-, CD8+ T lymphocytes depend on infection with CMV. J Immunol. 2003;170(8):4342-8.
15. Lazzarotto T, Varani S, Gabrielli L, Spezzacatena P, Landini MP. New advances in the diagnosis of congenital cytomegalovirus infection. Intervirology. 1999;42(5-6):390-7.
16. Stagno S, Pass RF, Cloud G, Britt WJ, Henderson RE, Alford CA, et al. Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus, and clinical outcome. JAMA. 1986;256(14):1904-8.
17. Pass RF, Fowler KB, Boppana SB, Britt WJ, Stagno S. Congenital cytomegalovirus infection following first trimester maternal infection: symptoms at birth and outcome. J Clin Virol. 2006;35(2):216-20.
18. Liesnard C, Donner C, Brancart F, Gosselin F, Delforge ML, Rodesch F. Prenatal diagnosis of congenital cytomegalovirus infection: prospective study of 237 pregnancies at risk. Obstet Gynecol. 2000;95(6 Pt 1):881-8.
19. Maine G, Lazzarotto T, Landini MP. New developments in the diagnosis of maternal and congenital CMV infection. Expert Rev Mol Diagn. 2001;1(1):19-29.
20. Donner C, Liesnard C, Brancart F, Rodesch F. Accuracy of amniotic fluid testing before 21 weeks gestation in prenatal diagnosis of congenital cytomegalovirus infection. Prenat Diagn. 1994;14(11):1055-9.
21. Lazzarotto T, Varani S, Spezzacatena P, Gabrielli L, Pradelli P, Landini MP, et al. Maternal IgG avidity and IgM detected by blot as diagnostic tools to identify pregnant women at risk of transmitting cytomegalovirus. Viral Immunol. 2000;13(1):137-41.
22. Stagno S, Pass RF, Dworsky ME, Henderson RE, Moore EG, Alford CA, et al. Congenital cytomegalovirus infection: The relative importance of primary and recurrent maternal infection. N Engl J Med. 1982;306(16):945-9.
23. Schleiss MR. Congenital cytomegalovirus infection: Update on management strategies. Curr Treat Options Neurol. 2008;10(3):186-92.
24. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol. 2007;17(4):253-76.
25. Lazzarotto T, Guerra B, Spezzacatena P, Varani S, Gabrielli L, Landini MP, et al. Prenatal diagnosis of congenital cytomegalovirus infection. J Clin Microbiol. 1998;36(12):3540-4.
26. Ahlfors K, Ivarsson SA, Harris S. Report on a long-term study of maternal and congenital cytomegalovirus infection in Sweden. Review of prospective studies available in the literature. Scand J Infect Dis. 1999;31(5):443-57.
27. Marino T, Laartz B, Smith SE, Gompf SG, Allaboun K, Marinez JE, et al. Viral Infections and Pregnancy. Medscape Reference [Accessed 23 Jun 2011]. Available from: http://emedicine.medscape.com/article/235213-overview
28. Boppana SB, Pass RF, Britt WJ, Stagno S, Alford CA. Symptomatic congenital cytomegalovirus infection: neonatal morbidity and mortality. Pediatr Infect Dis J. 1992;11(2):93-9.
29. Conboy TJ, Pass RF, Stagno S, Britt WJ, Alford CA, Boll TJ, et al. Intellectual development in school-aged children with asymptomatic congenital cytomegalovirus infection. Pediatrics. 1986;77(6):801-6.
30. Bale JF, Miner L, Petheram SJ. Congenital Cytomegalovirus Infection. Curr Treat Options Neurol. 2002;4(3):225-30.
31. Noyola DE, Demmler GJ, Nelson CT, Griesser C, Williamson WD, Yow MD, et al. Early predictors of neurodevelopmental outcome in symptomatic congenital cytomegalovirus infection. J Pediatr. 2001;138(3):325-31.
32. Boppana SB, Fowler KB, Vaid Y, Hedlund G, Stagno S, Pass RF, et al. Neuroradiographic findings in the newborn period and long-term outcome in children with symptomatic congenital cytomegalovirus. Pediatrics. 1997;99(3):409-14.
33. Kylat RI, Kelly EN, Ford-Jones EL. Clinical findings and adverse outcome in neonates with symptomatic congenital cytomegalovirus (SCCMV) infection. Eur J Pediatr. 2006;165(11):773-8.
34. Sharon B, Schleiss MR. Congenital cytomegalovirus infection: an unrecognized epidemic. Infect Med. 2007;24(9):402-15.
35. Stratton K, Durch J, Lawrence R. Vaccines for the 21st century: A tool for decision making. Washington, DC: National Academy Press; 2001.
36. Pass RF. Development and evidence for efficacy of CMV glycoprotein B vaccine with MF59 adjuvant. J Clin Virol. 2009;4 Suppl 4:6:S73-6.
37. Lopo S, Palminha P, Pité M, Caçador T, Vinagre E, Pereira MA, et al. Infecção Congénita pelo vírus citomegálico - Resultados preliminares de estudo prospectivo. [Cytomegalovirus congenital infection – preliminary results of a prospective study]. Revista Portuguesa de Doenças Infecciosas 2004b;Aug-Dec:14-7. Portuguese.
38. Paixão P, Almeida S, Gouveia P, Vilarinho L, Vaz Osório R. Study of the prevalence of HCMV congenital infection in portuguese newborns using dried blood spots. Abst. 10th international CMV/Betaherpesvirus workshop; 2005 Apr 24-28; Williamsburg, United States.
39. Ministério da Saúde. Direcção-Geral da Saúde (Portugal). Avaliação do programa nacional de vacinação e melhoria do seu custo-efectividade: 2º inquérito serológico nacional: Portugal Continental 2001-2002. [Evaluation of the national vaccination programme and its cost-effectiveness. Second national serological survey: Portugal Continental 2001-2002]. Lisboa: DGS; 2004. Available from: http://www.dgs.pt/upload/membro.id/ficheiros/i009552.pdf. Portuguese.
40. Zar J. Biostatistical analysis. 3rd ed. Englewood Cliffs (NJ): Prentice-Hall International Editions; 1996.
41. Natali A, Valcavi P, Medici MC, Dieci E, Montali S, Chezzi C. Cytomegalovirus infection in an Italian population: antibody prevalence, virus excretion and maternal transmission. New Microbiol. 1997;20(2):123-33.
42. de Ory F, Sanz JC, Castaneda R, Ramirez R, Leon P, Pachon I. [Cytomegalovirus seroepidemiology in the community of Madrid]. Rev Esp Salud Publica 2001;75(1):55-62. Spanish.
43. Linhares MI, de Andrade GP, Tateno S, Eizuru Y, Minamishima Y. Prevalence of cytomegalovirus antibodies in Brazilian and Japanese populations in the north-east of Brazil. Microbiol Immunol. 1989;33(11):975-80.
44. Takeda N, Isonuma H, Sekiya S, Ebe T, Matsumoto T, Watanabe K. [Studies of anti-cytomegalovirus IgG antibody positive rate and cytomegalovirus mononucleosis in adults]. Kansenshogaku Zasshi 2001;75(9):775-9. Japanese.
45. Kothari A, Ramachandran VG, Gupta P, Singh B, Talwar V. Seroprevalence of cytomegalovirus among voluntary blood donors in Delhi, India. J Health Popul Nutr. 2002;20(4):348-51.
46. Sarov B, Naggan L, Rosenzveig R, Katz S, Haikin H, Sarov I. Prevalence of antibodies to human cytomegalovirus in urban, kibbutz and Bedouin children in southern Israel. J Med Virol. 1982;10(3):195-201.
47. Schleiss MR. Acquisition of human cytomegalovirus infection in infants via breast milk: natural immunization or cause of concern? Rev Med Virol. 2006;16(2):73-82.
48. Hamprecht K, Witzel S, Maschmann J, Dietz K, Baumeister A, Jahn G, et al. Rapid detection and quantification of cell free cytomegalovirus by high-speed centrifugation-based microculture assay: comparison to longitudinally analysed viral DNA load and pp67 late transcript during lactation. J Clin Virol. 2003;28(3):303-16.

49. Hamprecht K, Maschmann J, Jahn G, Poets CF, Goelz R. Cytomegalovirus transmission to preterm infants during lactation. J Clin Virol. 2008;41(3):198-205.
50. Arvin AM, Fast P, Myers M, Plotkin S, Rabinovich R. Vaccine development to prevent cytomegalovirus disease: report from the National Vaccine Advisory Committee. Vaccines Clin Infect Dis. 2004;39(2):233-9.
51. Hutto SC, Ricks RE, Garvie M, Pass RF. Epidemiology of cytomegalovirus infection in young children: day care vs home care. Pediatr Infect Dis. 1985;4(2):149-52.
52. Mello A, Ferreira E, Vilas Boas L, Pannuti C. Cytomegalovirus infection in a day care center in the municipally of São Paulo. Rev Inst Med Trop São Paulo. 1996;38(3):165-9.
53. Almeida L, Azevedo R, Amaku M, Massad E. Cytomegalovirus seroepidemiology in an urban community of São Paulo, Brazil. Rev Saúde Pública. 2001;35(2):124-9.
54. Aarnisalo J, Ilonen J, Vainionpaa R, Volanen I, Kaitosaari T, Simell O. Development of antibodies against cytomegalovirus, varicella-zoster virus and herpes simplex virus in Finland during the first eight years of life: a prospective study. Scand J Infect Dis. 2003;35(10):750-3.
55. Chacón M, Naveda O, Castillo O, Flores M, Casanova L, Naveda M, et al. Prevalencia de anticuerpos anti-citomegalovirus y anti-virus epstein-barr en Valencia, estado Carabobo, Venezuela [Cytomegalovirus and Epstein-Barr vírus prevalence in Valencia, Venezuela]. Rev Soc Venez Microbiol. 2002;22(2):131-5. Spanish.
56. Ataman S, Colak D, Günseren F, Senol Y, Colak T, Gültekin M. et al. [Investigation of cytomegalovirus seroepidemiology in Antalya with a population-based cross-sectional study and review of related data in Turkey.] Mikrobiyol Bul. 2007;41(4):545-55. Turkish.
57. Pass RF. A key role for adolescents in the epidemiology of cytomegalovirus and genital herpes infections. Clin Infect Dis. 2004;39(10):1439-40.
58. Gratacap-Cavallier B, Bosson J, Morand P, Dutertre N, Chanzy B, Seigneurin J, et al. Cytomegalovirus seroprevalence in French pregnant women: parity and place of birth as major predictive factors. Eur J Epidemiol. 1998;14(2):147-52.
59. Tookey P, Ades A, Peckham C. Cytomegalovirus prevalence in pregnant women: the influence of parity. Arch Dis Child. 1992;67(7 Spec No):779-83.
60. Nishimura N, Kimura H, Yabuta Y, Tanaka N, Ito Y, Morishima T, et al. Prevalence of maternal cytomegalovirus (CMV) antibody and detection of CMV DNA in amniotic fluid. Microbiol Immunol. 1999;43(8):781-4.
61. Karas Z, Blok R, Zabel J, Zaba C. [Seroepidemiologic study of cytomegaly and rubella in pregnant women and prostitutes]. Przegl Epidemiol. 1992;46(4):303-7. Polish.
62. Odland J, Sergejeva I, Ivaneev M, Jensen I, Stray-Pedersen B. Seropositivity of cytomegalovirus, parvovirus and rubella in pregnant women and recurrent aborters in Leningrad County, Russia. Acta Obstet Gynecol Scand. 2001;80(11):1025-9.
63. Staras S, Flanders WD, Dollard SC, Pass RF, McGowan JE, Cannon MJ. Influence of sexual activity on cytomegalovirus seroprevalence in the United States, 1988-1994. Sex Transm Dis. 2008;35(5):42-9.
64. Drew WL. Condoms and the transmission of cytomegalovirus. Sex Transm Dis. 1998;25(9):481-2.
65. Hutto SC, Little EA, Ricks R, Lee JD, Pass RF. Isolation of cytomegalovirus from toys and hands in a day care center. J Infect Dis. 1986;154(3):527-30.
66. Pass RF, Hutto SC, Ricks R, Cloud GA. Increased rate of cytomegalovirus infection among parents of children attending day care centers. N Engl J Med. 1986;314(22):1414-8.
67. Fowler KB, Pass RF. Risk factors for congenital cytomegalovirus infection in the offspring of young women: exposure to young children and recent onset of sexual activity. Pediatrics. 2006;118(2):286-92.
68. Bale JF Jr, Zimmerman B, Dawson JD, Souza IE, Petheram SJ, Murph JR. Cytomegalovirus transmission in child care homes. Arch Pediatr Adolesc Med. 1999;153(1):75-9.
69. Staras SA, Flanders WD, Dollard SC, Pass RF, McGowan JE Jr, Cannon MJ. Cytomegalovirus seroprevalence and childhood sources of infection: a population-based study among pre-adolescents in the United States. J Clin Virol. 2008;43(3):266-71.
70. Adler SP, Nigro G, Pereira L. Recent advances in the prevention and treatment of congenital cytomegalovirus infections. Semin Perinatol. 2007;31(1):10-8.
71. Adler SP, Finney JW, Manganello AM, Best AM. Prevention of child-to-mother transmission of cytomegalovirus by changing behaviors: a randomized controlled trial. Pediatr Infect Dis J. 1996;15(3):240-6.
72. Adler SP, Finney JW, Manganello AM, Best AM. Prevention of child-to-mother transmission of cytomegalovirus among pregnant women. J Pediatr. 2004;145(4):485-91.
73. Picone O, Vauloup-Fellous C, Cordier A-G, Parent Du Châtelet I, Senat M-V, Grangeot-Keros L, et al. A 2-year study on cytomegalovirus infection during pregnancy in a French hospital. BJOG 2009;116:818-23.
74. Vauloup-Fellous C, Picone O, Cordier A-G, Parent Du Châtelet I, Senat M-V, Grangeot-Keros L, et al. Does hygiene counseling have an impact on the rate of CMV primary infection during pregnancy? Results of a 3-year prospective study in a French hospital. J Clin Virol. 2009;46 Suppl 4:S49-53.
75. Cannon MJ, Davis KF. Washing our hands of the congenital cytomegalovirus disease epidemic. BMC Public Health. 2005;5:70.
76. Centers for Disease Control and Prevention (CDC). Department of Health and Human Services (USA). About CMV – General information. 2006. [Accessed 24 Nov 2009]. Available from: http://www.cdc.gov/cmv/index.html
77. Ross DS, Dollard SC, Victor M, Sumartojo E, Cannon MJ. The epidemiology and prevention of congenital cytomegalovirus infection and disease: activities of the Centers for Disease Control and Prevention Workgroup. J Womens Health (Larchmt). 2006;15(3):224-9.
78. Adler SP. Human CMV vaccine trials: what if CMV caused a rash? J Clin Virol. 2008;41(3):231-6.
79. Nigro G, Adler SP, La Torre R, Best AI; Congenital Cytomegalovirus Collaborating Group. Passive immunization during pregnancy for congenital cytomegalovirus infection. N Engl J Med. 2005;353(13):1350-62.
80. Brady RC, Schleiss MR, Witte DP, Siddigi TA, Fame PT. Placental transfer of ganciclovir in a woman with acquired immunodeficiency syndrome and cytomegalovirus disease. Pediatr Infect Dis J. 2002;21(8):796-7.
81. Puliyanda DP, Silverman NS, Lehman D. Successful use of oral ganciclovir for the treatment of intrauterine cytomegalovirus infection in a renal allograft recipient. Transpl Infect Dis. 2005;7(2):71-4.
82. Jacquemard F, Yamamoto M, Costa J-M, Romand S, Jaqz-Aigrain E, Ville Y, et al. Maternal administration of valaciclovir in symptomatic intrauterine cytomegalovirus infection. BJOG. 2007;114(9):1113-21.
83. Pass RF, Zhang C, Evans A, Simpson T, Andrews W, Cloud G, et al. Vaccine prevention of maternal cytomegalovirus infection. N Engl J Med. 2009;360(12):1191-9.
84. Griffiths PD. CMV vaccine trial endpoints. J Clin Virol. 2009;46 Suppl 4:S64-7.