Journal of Clinical Virology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Seroepidemiology and phylogenetic characterisation of measles virus in Ireland, 2004–2013

Bernadette O' Riordan*, Michael J. Carr, Jeff Connell, Linda Dunford, William W. Hall, Jaythoon Hassan

National Virus Reference Laboratory, School of Medicine and Medical Science, University College Dublin, Belfield, Dublin 4, Ireland

ARTICLE INFO

Article history:
Received 7 November 2013
Received in revised form 9 May 2014
Accepted 17 May 2014

Keywords:
Measles
Ireland
Seroepidemiology
Phylogenetics

ABSTRACT

Background: Ireland is classified as an area of high measles incidence. A World Health Organisation-European Region strategic plan exists for measles elimination by 2015.
Objectives: To retrospectively investigate measles outbreaks using all patient samples (sera and oral fluid) received for measles laboratory diagnosis and characterise the genetic diversity of circulating measles genotypes in Ireland.
Study Design: 704 cases of acute measles infection as determined by the presence of measles specific IgM in sera and oral fluids were confirmed at the National Virus Reference Laboratory. Measles positive samples ($n = 116$) were examined by genotyping, sequence analysis and phylogenetic characterisation.
Results: Three measles outbreaks occurred over the study period: 2004, 2009/2010 and 2011. Measles IgM positivity ranged from 22–29% in outbreak years to 5–10% in the intervening years. Age profile analysis revealed that whereas individuals >10 years accounted for only 8% of cases in the 2004 outbreak, this increased to 33% and 29% in the 2009/2010 and 2011 outbreaks, respectively. The <1 year cohort accounted for 18–20% of cases in all outbreaks. Phylogenetic analysis demonstrated both indigenous transmission and also importation events. Clade D viruses were exclusively found circulating in Ireland, with autochthonous transmission of diverse genotype D4 strains associated with large outbreaks across Europe. More recently, genotype D8 was identified and these were associated with importation events.
Conclusions: This study provides a comprehensive genetic analysis of circulating measles genotypes in Ireland and discriminated between indigenous and imported viral strains. Notably, an increase in laboratory-confirmed measles cases in the greater than 10 years of age group was seen over the study period. This information is valuable to inform vaccination strategies with a focus on those populations who remain susceptible to measles infection.

1. Background

Measles virus (MeV) is a member of the genus *Morbillivirus* within the family *Paramyxoviridae* and is a non-segmented, negative sense, single-stranded RNA virus [1]. Measles is typically a childhood infection of humans, spread via the respiratory route [2] and is one of the most contagious human pathogens with a reproductive number (R_0) of between 15 and 20 [3]. Symptoms of MeV infection include exanthema, coryza, cough, conjunctivitis and the pathognomonic Koplik's spots, however, severe complications such as pneumonia and encephalitis requiring hospitalisation can also arise [4]. One rare fatal complication of MeV infection, which manifests many years after primary infection, is subacute sclerosing panencephalitis (SSPE) caused by an infection of the central nervous system affecting 1 per 100 000 to 500 000 measles cases [5].

Laboratory confirmation of suspected MeV cases, complimented by the genotyping of circulating virus strains, remains crucial throughout the elimination phase, as clinical diagnosis is unreliable. World Health Organisation (WHO) guidelines for MeV genotyping requires that the minimum amount of data required for genotype assignment is the sequence of the 3′ hypervariable (HVR) 450 nucleotides that encode the variable C-terminal 150 amino acids of the nucleoprotein. Phylogenetic analysis of the MeV nucleoprotein (N) and haemagglutinin (H) gene have identified eight clades A-H, within which 23 genotypes are recognised [6]. This information allows molecular epidemiological analysis of circulating MeV genotypes to discriminate between

* Corresponding author. Tel.: +353 1 716 1319; fax: +353 1 716 1135.
E-mail address: bernadette.oriordan@ucd.ie (B. O' Riordan).

http://dx.doi.org/10.1016/j.jcv.2014.05.010
1386-6532/

endemic strains of MeV and importation events, which is of vital importance during the elimination phase. Despite the WHO strategic plan 2012–2020 and the availability of a highly effective vaccine, MeV remains a major cause of mortality and morbidity in children living in developing countries, with approximately 139 000 measles deaths globally recorded in 2010 [7]. The WHO European Region (WHO-ER) has set 2015 as the target date for the elimination of endemic MeV transmission in Europe. The European Centre for Disease Prevention and Control (ECDC) surveillance report for MeV and rubella monitoring in May 2013, reported 8127 cases of MeV from the 30 contributing countries in Europe and Croatia between April 2012 and March 2013. There were no MeV-related deaths during this time, however, there were six reported cases of acute MeV encephalitis. Ninety five percent of all MeV cases originated from France, Germany, Italy, Romania, Spain and the United Kingdom [8].

The measles-mumps-rubella (MMR) vaccination was introduced as a routine childhood vaccination in the Republic of Ireland in 1988 [9]. Since 2008, the national vaccination policy recommends that children receive MMR_1 at 12 months of age and MMR_2 between the ages of 4 and 5 [10]. As a result of the MMR childhood vaccination, the numbers of measles cases reduced considerably [11], however outbreaks of MeV continue to occur [10]. The WHO recommends a vaccination target of 95% to facilitate population protection of susceptible individuals arising from herd immunity. However, no national immunisation database currently exists in Ireland and therefore childhood vaccination records are maintained on a number of separate systems and with parents/guardians [12]. The estimated national MMR_1 uptake rate was 92% for Quarter 1, 2013 which is below the recommended 95% target as stated by the WHO. No comprehensive data exists for MMR_2 immunisation levels [13]. The Republic of Ireland is classified as an area of high MeV incidence with an incidence of 2.29 measles cases/100 000 population recorded in 2012 and a MeV incidence rate of 1.30 MeV cases/100 000 population for the first 6 months of 2013, which exceeds the WHO-ER target of ≤1 measles case/100 000 [14].

1.1. Objectives

The principal aims of the present study were to retrospectively investigate the seroepidemiology of measles outbreaks in Ireland and characterise the genetic diversity of circulating MeV genotypes in laboratory-confirmed MeV cases identified in Ireland between January 2004 and June 2013.

2. Study design

2.1. Seroepidemiological review, 2004–2013

A retrospective review of acute MeV infection was performed, using the results generated at the National Virus Reference Laboratory (NVRL) between 1st January 2004 and 30th June 2013. The assay results of the Measles IgM capture EIA (Microimmune, Middlesex, UK) were categorised as positive or negative according to the criteria in the kit insert, and for analysis all equivocal results were classified as negative.

Patients were identified based on forename, surname and date of birth and any duplicates were removed before analysis. Patients were categorised into five age cohorts: ≤1 year of age, 1 to 2 years, 2 to 5 years, 6 to 10 years, 11 to 20 years and ≥21 years of age.

2.2. Specimen collection

As previously described by Reid et al. [15], oral fluid specimens were collected using the OraCol collection device (Malvern Medical Developments, Worcester, United Kingdom) and oral fluid extracted using 1.5 ml of freshly prepared Phosphate Buffered Saline (PBS) containing 0.5% Tween 20. All samples were then subsequently stored at −20 °C.

2.3. Nucleic acid extraction and measles virus real-time PCR

Total nucleic acids were extracted from 200 μl of oral fluid using the total nucleic acid isolation kit and the automated Magnapure extractor (Roche, Sussex, UK) [16], Real-time reverse transcription polymerase chain reaction (PCR) amplification of the haemagglutinin (H) gene was performed using the TaqMan ABI 7500 FAST platform (Applied Biosystems, Warrington, UK).

2.4. Measles virus genotyping, sequence analysis and phylogenetic characterisation

MeV phylogenetic analysis was performed by a one-step RT-PCR amplification of a 590-bp fragment encompassing the MeV N gene 3′ HVR with the Qiagen RT-PCR KIT (Qiagen, Crawley, UK) using oligonucleotide primers: 5′-TGGAGCTATGCCATGGGAGT-3′ and 5′-TAACAATGATGGAGGGTAGG-3′ [17]. The measles virus N gene cDNA were sequenced bidirectionally using the ABI 3500 Dx sequencing platform.

In total, Irish measles virus N gene fragments ($n = 116$) were compared with representative reference sequences available in Genbank. Lasergene version 8 (DNASTAR, Madison, WI, USA) was used for contiguous sequence assembly [18], and the sequences were aligned with ClustalW [19] implemented in Bioedit version 7.05 [20]. A rooted maximum likelihood phylogenetic tree was constructed in PAUP* version 4.0 beta10 using a sub-model of GTR with a gamma distribution [21]. Statistical support for the topology of the trees was provided by 1000 bootstrap replicates using a neighbour joining algorithm.

2.5. Statistical analysis

Chi-square analysis was performed to compare the incidence of MeV infection in males and females.

3. Results

3.1. Serological analysis (January 2004–June 2013)

Monthly analysis of MeV IgM positive results identified three MeV outbreaks in Ireland over the study period, occurring in 2004 (May 2004 to August 2004; $n = 127$), 2009/2010 (November 2009 to April 2010; $n = 230$) and 2011 (June 2011 to November 2011; $n = 114$) (Fig. 1). During the outbreak years, the MeV IgM positivity rate, ranged from 22 to 29%, whereas during the intervening years ranged from 5 to 10% (Fig. 2). The MeV IgM positivity rate for the first 6 months of 2013 was 11%.

3.2. Gender analysis

Gender analysis revealed that the total number of samples received from females ($n = 2251$) and males ($n = 2063$) were comparable. Those samples collected from individuals where gender was not identified were excluded from this analysis ($n = 84$). Reviewing the MeV IgM positive cases (males; $n = 365/2063$, females; $n = 339/2251$) during the study period revealed overall that there was a male gender bias ($P = 0.02$) (Table 1a). However, a review of the gender of MeV IgM positive cases associated with the three identified outbreaks revealed no significant gender bias in the 2004 outbreak ($P = 0.60$) and 2009/2010 outbreak ($P = 0.09$) but revealed a statistical significant association in the 2011

Please cite this article in press as: O' Riordan B, et al. Seroepidemiology and phylogenetic characterisation of measles virus in Ireland, 2004–2013. J Clin Virol (2014), http://dx.doi.org/10.1016/j.jcv.2014.05.010