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Laboratory-based investigation of suspected mumps cases submitted to the German National Reference Centre for Measles, Mumps, and Rubella, 2008 to 2013

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ABSTRACT

From 2008 to 2013, sample sets from 534 patients displaying clinical symptoms of mumps were submitted to the German Reference Centre for Measles, Mumps and Rubella. Mumps virus infection was confirmed in 216 cases (40%) by PCR and/or serology. Confirmed cases were more frequently seen in male than in female patients (128 vs. 81); the age group predominantly affected was 15 to 29 years old (65%, median age: 26.4 years). The majority of the confirmed cases had a remote history of vaccination with one or two doses of a mumps-containing vaccine (69%). Our results indicate that mumps virus caused two outbreaks in Bavaria in 2008 and 2010/2011 and a third one in Lower Saxony in 2011. Mumps virus genotype G was preponderantly detected from 2008 to 2013.

For 107 of the 216 patients with a confirmed mumps infection, we correlated the results from PCR and serology. PCR detected cases during the first week after onset of symptoms (74% positive results). PCR worked best with throat swabs and oral fluids (61% and 60% positive results, respectively). IgM was more reliable with a longer time after onset of symptoms (67%), but indirect IgM serology was of insufficient sensitivity for vaccinated mumps cases (30%); the IgM μ-capture assay detected more cases in this group.

Mumps virus is able to initiate an infection in vaccinated patients (secondary vaccine failure, SVF) although it is unclear to what extent. Since SVF does occur in highly vaccinated populations and IgM will not increase to detectable levels in all SVF patients, we strongly recommend using PCR plus serology tests to avoid false-negative diagnoses in vaccinated individuals with clinical signs of mumps.

1. Introduction

Mumps is an infectious disease induced by a virus of the family *Paramyxoviridae*. Typical symptoms are fever and a painful mono- or bilateral parotitis, but up to 30% of the infected patients are asymptomatic. Due to the rather unspecific symptoms, mumps can be mistaken for other viral or bacterial agents affecting the parotis gland. Confirmation by laboratory tests is therefore recommended, whenever mumps is suspected. Complications such as aseptic meningitis, orchitis and oophoritis are common in adults. Several live attenuated mumps vaccines have been in use worldwide for more than 50 years. A two dose uptake of the measles/mumps/rubella (MMR) vaccine is adapted in most industrialized countries but has not yet been globally implemented. In the pre-vaccine era, infection with mumps virus (MuV) was observed mostly in children, rising vaccination coverage shifted the incidence of mumps towards adolescents and young adults. Natural infection with MuV is thought to confer lifelong immunity, although reinfection has been described (Nojd et al., 2001).

Abbreviations: SVF, secondary vaccine failure; MuV, mumps virus; U, urine; TS, throat swab; OF, oral fluid; CPE, cytopathogenic effect; CMC, carboxymethylcellulose; n.r., not reported.

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Immunity after vaccination results in a lower IgG antibody titre compared to natural infection. Outbreaks among vaccinated individuals were observed, for example, in the Netherlands in 2004 among a student population of which 97% had received at least one dose of a mumps-containing vaccine (Brockhoff et al., 2010). A recent outbreak started in a U.S. Orthodox Jewish community and led to more than 3500 documented cases (Barskey et al., 2012), another in a British school in 2013 (Aasheim et al., 2014). Among the patients, 90% had received at least one dose of the MMR vaccine and transmission from vaccinated individuals with a secondary infection to close contacts was observed (Fanoy et al., 2011). These observations are puzzling since epidemiological studies demonstrated a vaccine efficacy of >91% (Takla et al., 2014; Deeks et al., 2011).

The outbreaks affecting highly vaccinated populations were caused by MuV of the genotype G. Genotyping analyses a hypervariable region of the MuV genome that encodes the SH gene, a modulator of virulence (Orvell et al., 2002; Muhlemann, 2004; Fontana et al., 2008). Worldwide, 12 genotypes (A to N) have been recognized; most of them are distributed regionally. Genotypes C, D, G, H, J and K are found primarily in the Western Hemisphere while genotypes B, F, I and L are predominantly found in the Eastern Hemisphere (Jin et al., 2014; Santos et al., 2008; WHO, 2010).

1.1. Mumps epidemiology in Germany

Mumps vaccination is recommended by the German Standing Committee on Vaccination (STIKO) as a two dose MMR schedule for children until the end of the second year of life. Vaccination coverage is assessed nationwide in 5 to 6 year old children prior to school entry. The last survey in 2012 showed 96.4% coverage for the first dose and 92.2% for the second dose of mumps-containing vaccine (RKI, 2014a). Different immunization policies were pursued in the formerly divided Germany. The STIKO recommended uptake of a mumps-containing vaccine since 1976 in the Western part of the country, while a mumps vaccine was not in use in the former German Democratic Republic. A nationwide recommendation was given in 1991 and since then Jeryl Lynn derived vaccine RIT 4385 is in use. The vaccination coverage for mumps in the eastern federal states is 97.3% coverage for the first does and 93.4% coverage for the second dose while the coverage in the western federal states was 96.3% and 92.1%, respectively (RKI, 2014a). A reporting system for mumps existed only in the Eastern federal states of the former German Democratic Republic but not in the Western federal states. From 2008 to 2013, the notification system in the Eastern federal states picked up between 30 and 90 mumps cases per year. In contrast to other European countries, knowledge about MuV circulation in Germany is rather scant, although single outbreaks affecting schools, universities and sports clubs were reported in the Western federal states from 2006 to 2011 (Otto et al., 2010; Takla et al., 2013; Koch and Takla, 2013). In addition, analysis of mumps-related diagnoses based on International Classification of Diseases (ICD-10) codes claimed through statutory health insurances between 2007 and 2011 revealed a severe underreporting of mumps and mumps-orchitis in Germany (Takla et al., 2013). A nationwide notification system was implemented in March 2013. In 2014, 834 cases were reported, mostly from North Rhine-Westphalia (237) and Bavaria (128).

The German Reference Centre Measles, Mumps, Rubella (NRC MMR) performs serology, virus genome detection by PCR, and molecular surveillance of circulating measles, mumps and rubella viruses. Our clients are encouraged to submit samples from suspected mumps cases that meet the clinical case definition which is defined as "swollen parotis gland(s) for two days or longer without another evident cause" together with a filled-in questionnaire stating the age, gender and vaccination status of the patient in addition to the dates of sampling and the onset of symptoms. This study summarizes the results of mumps laboratory investigations conducted at the NRC MMR from 2008 to 2013. The test results are correlated with the epidemiological details from the questionnaires.

2. Materials and methods

2.1. MuV cultivation

MuV was routinely cultivated on Vero/SLAM cells (received via the WHO Global Measles and Rubella Lab Network (GMRLN) from Dr Yusuke Yanagi (Kyushu University, Japan). Clinical samples were passed through a 0.45 μm filter and the resulting filtrate was applied to a medium-deprived cell culture in a 25 cm^2 flask. After 30 min incubation, 10 ml DMEM +2% FCS were added. Cells were incubated for a maximum of 8 days with a change of medium every 3 days. The successful cultivation of MuV was confirmed by sight and microscopic inspection for the development of cytopathogenic effects (CPE). Cells were inspected every day and passaged after one week. Moreover, the MuV genome was detected by PCR. The supernatant was harvested and titrated when the following criteria were fulfilled: (i) the MuV genome was detected by PCR and (ii) 80% of the cells showed CPE.

2.2. PCR and genotyping

RNA from clinical specimens was extracted with the QIAcube instrument using the Viral RNA extraction kit (Qiagen/Hilden). A nested protocol was used for PCR. Viral RNA was reversely transcribed and amplified with MuV-specific primers 5′-AGTGTACTAATCCAGGCTTG-3′ and 5′-ACCCACCATTGCATAGTATC-3′ targeting the N-gene of MuV in a OneStep amplification (QIAGEN OneStep RT-PCR Kit, Qiagen/Hilden; 50 °C 30 min; 95 °C 15 min; [94 °C 30 s; 52 °C 30 s; 72 °C 60 s] 30×; 72 °C 10 min). One μl of the 261 base pair (bp) product was amplified in the second round reaction with primers 5′-GTATGTCAGCGTACGACCAAC-3′ and 5′-GATAGCAACCCCTGCCGTCT-3′ (95 °C 5 min; [94 °C 30 s; 52 °C 30 s; 72 °C 60 s] 30×; 72 °C 10 min). The fragments were separated by agarose gel electrophoresis. A fragment length of 198 bp indicated detection of the MuV genome in the clinical specimen. This n-PCR approach had a detection limit of 100 copies of a plasmid encoding a cloned N-gene of MuV. Genotyping was performed in a similar approach using primers 5′-GTAGCAGCCTTAGTTTTGAGCAT-3′ and 5′-TTGATTCATTACTCCCACAGCAG-3′ for the first round and 5′-TATCATTTCGCTATTGTTTTGCTG-3′ and 5′-TGAAGAGTTTTGAGGGCTCCAT-3′ for the second round. The product was sequenced using the latter primers in a Big Dye based sequencing reaction. Phylogenetic analysis was performed as recommended by the WHO (Jin et al. 2014) using the 316 nucleotide (nt) fragment comprising the SH gene (Mega5 software (Tamura et al., 2011); Neighbor-Joining method (Saitou and Nei, 1987); bootstrap test, 1000 replicates; *p*-distance method.

2.3. ELISA

Serum samples were analyzed using the indirect anti-Mumpsvirus-AT IgG-ELISA and the anti-Mumpsvirus G5 IgM-ELISA (Euroimmun/Lübeck, Germany) using a Euroimmun Analyzer 4P. This test uses lysate from mumps virus infected cells as antigen. The cut-off of the IgG test is defined as ≥22 relative units (RU)/ml (positive) with ≥16–22 RU/ml as equivocal and <16 RU/ml as negative test results. The semiquantitative IgM test defines a ratio of ≥1.1 as positive (with ≥0.8 to <1 as equivocal and <0.8 as negative). Selected samples were analyzed with the μ-capture anti-mumps IgM test (Microimmune Ltd., UK). Capture tests use anti-human IgM