SUMMARY

Aim: The aim of the study was to map the incidence of mumps in the Czech Republic in terms of clinical symptoms, epidemiological links, and characteristics of circulating genotypes.

Methods: Patients with suspected mumps examined in the Infectious Diseases Clinic of the Na Bulovce Hospital in 2013 were enrolled in the study. Buccal swab specimens were tested by means of nucleic acid detection (RT-qPCR) and when positive, they were cultured in tissue culture. Sequencing was carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit and Genetic Analyzer 3500. The SeqScape software was used for the analysis of sequencing data and filtering out low quality reads. The phylogenetic analysis and genotyping were performed using the Mega 6 software. To generate the phylogenetic tree, all sequences were aligned by the MAFFT tool and the alignment obtained was edited using the BioEdit software. In all patients, selected biochemical markers (C-reactive protein, white blood cell count and serum amylase) were measured. The EPIDAT system used for reporting infectious diseases, record keeping, and data analysis in the Czech Republic was the source of statistical data.

Results: Eighty-nine patients with suspected mumps were examined in the Na Bulovce Hospital and 65 of them were laboratory confirmed with mumps: 40 males (61.5%) and 25 females (38.5%). The mean age of the study cohort was 25.9 years (median age of 23 years, age range from 10 to 73 years) and 14 patients were under 18 years of age. Thirty-four (52.3%) patients were vaccinated in childhood, 28 (43.1%) were unvaccinated, and for three persons, vaccination data were not available. A severe course of the disease was reported in 15 (23.1%) patients. Fourteen of them needed hospitalization because of orchitis (9 males) and meningitis (5 patients). One patient with orchitis was treated on an outpatient basis. The need for hospitalization tended to be lower in the unvaccinated patients (14.7% vs. 35.7%, p = 0.076). In 2013, 1,553 cases of mumps were reported to the EPIDAT system. Of these, 640 were laboratory confirmed. The most often reported complications were orchitis (90 cases, i.e. 10.3%) and meningitis (21 cases, i.e. 1.4%). Orchitis was diagnosed in 30.3% of the unvaccinated and in 6.4% of the vaccinated males. Meningitis occurred in 3.1% of the unvaccinated and in 1.0% of the vaccinated patients.

Conclusion: Despite the emergence of mumps among the vaccinated population, the present study has confirmed a positive effect of the vaccine, particularly on the incidence of complications and inflammatory markers. All 30 sequenced mumps virus strains were assigned to group G. A secondary vaccine failure due to waning immunity seems to be a plausible explanation for the rise in mumps cases.

Key words: mumps, immunisation, vaccine failure, complications, genotyping

Address for correspondence: M. Havlíčková, National Institute of Public Health, Centre for Epidemiology and Microbiology, Šrobárova 48, 100 42 Prague 10, Czech Republic. E-mail: martina.havlickova@szu.cz

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INTRODUCTION

Mumps used to be a classical childhood illness before the vaccination programme was launched and mumps epidemics occurred regularly at intervals of two to five years, with the annual incidence ranging from 100 to 1,000 cases per 100,000 population. Although the post-infection antibody response was detected in more than 90% of the population, some cases of reinfection emerged in individuals who recovered from naturally acquired infection. After the incorporation of the mumps vaccine in the national childhood immunisation programme in 1987, the incidence of mumps in the Czech Republic (CR) declined substantially to hundreds of cases. The increase of mumps incidence to epidemic level was reported again in 1995–1996 (11,680 cases), 2002–2003 (1,501 cases), and 2005–2007 (with a peak of 5,172 cases in 2006). The unvaccinated population or single dose recipients had a severe course of the disease even five times as often as the vaccinated cohort. The last epidemic with nearly 4,000 reported cases occurred in 2012 (1). Epidemics of variable extent, despite the national vaccination programme in place, were reported by many other countries (2).

In the Czech Republic, combined measles, mumps, and rubella (MMR) vaccine is currently used in the frame of the national childhood immunisation programme in accordance with the leg-

Clinical Specimens

Buccal swab specimens collected from patients with suspected mumps were placed into test tubes containing 1.5 ml of viral medium and were stored and transported at refrigerator temperature. After one-minute agitation the dacron swab was removed from the test tube under sterile conditions and the specimen was analyzed without delay. Nucleic acid was isolated using the MagNaPure Compact, Nucleic Acid Isolation Kit (Roche) by Sabine Santibanez and Anne Wolbert – Robert Koch Institut, Nationales Referenz Zentrum für Masern, Mumps, Röteln) using the OneStep RT-PCR Kit (Qiagen) at a volume of 12.5 or 25 µl. The amplification quality was checked by electrophoresis on 1.5% agarose gel. Prior to sequencing analysis, amplicons were purified using the HighPure PCR Product Purification Kit (Roche).

Virus Isolation in Tissue Culture

Vero cells were used for virus isolation and the growth medium was Minimal Essential Medium (MEM – Gibco) with 2% of tissue culture bovine serum (Bioveta). After six days of culture, the second passage was made. Virus propagation in infected cells resulted in cytopathic effect (CPE), characterized by the presence of rounded cells with syncytia formation and subsequent cell destruction. The first signs of virus propagation were observed early during the first passage. The identification of virus isolates was carried out using RT-qPCR.

Pre-sequencing Amplification

Nucleic acids were isolated from RT-qPCR positive swabs and CPE positive tissue cultures using the MagNaPure Compact, Nucleic Acid Isolation Kit (Roche). A 316pb fragment of the SH gene (small hydrophobic protein) was amplified for genotyping by nested RT-PCR (in accordance with the protocol recommended by Sabine Santibanez and Anne Wolbert – Robert Koch Institut, Nationales Referenz Zentrum für Masern, Mumps, Röteln) using the OneStep RT-PCR Kit (Qiagen) at a volume of 12.5 or 25 µl. The amplification quality was checked by electrophoresis on 1.5% agarose gel. Prior to sequencing analysis, amplicons were purified using the HighPure PCR Product Purification Kit (Roche) and subsequently eluted into a volume of 50 µl. The purification was checked by electrophoresis on 2% agarose gel.

Sequencing

Sequencing was carried out using the BigDye Terminator v3.1 Cycle Sequencing Kit, (Life Technologies) and Genetic Analyzer 3500 (Life Technologies). The sequencing products
were purified on a column of Sephadex G-50 (Sigma-Aldrich). The SeqScape software (Life Technologies) was used for the analysis of sequencing data and filtering out low quality reads. The phylogenetic analysis and genotyping were performed using the Mega 6 software (6), with each sequence being compared to the corresponding GeneBank reference sequences. To generate the phylogenetic tree, all sequences were aligned by the MAFFT tool (7) and the alignment obtained was edited using the BioEdit software (8).

EPIDAT

The EPIDAT system used for reporting infectious diseases, record keeping, and data analysis in the Czech Republic was the source of statistical data.

Statistical Methods

Categorial data are presented as absolute frequencies and percentages. The Fisher factorial test was used for the comparison between groups. Continuous data were analyzed by the t-test. A 0.05 level of significance was used to interpret the tested hypotheses.

RESULTS

Clinical Data

Eighty-nine patients were enrolled in the study and mumps were laboratory confirmed in 65 of them: 40 males (61.5%) and 25 females (38.5%). The mean age of the study cohort was 25.9 years (median age of 23 years, age range from 10–73 years) and 14 patients were under 18 years of age. Thirty-four (52.3%) patients were vaccinated in childhood, 28 (43.1%) were unvaccinated, and for 3 persons (4.6%), vaccination data were not available. Demographic data are summarized in Table 1.

A severe course of the disease was reported in 15 (23.1%) patients. Fourteen of them needed hospitalization because of orchitis (9 males) and meningitis (5 patients). One patient with orchitis was treated on an outpatient basis. One female patient was admitted to hospital for a generally worsened condition, but she improved rapidly without further complications. One patient was laboratory diagnosed with mild pancreatic irritation. The need for hospitalization tended to be lower in the vaccinated patients (14.7% vs. 35.7%, p = 0.076). The study groups also differed in the severity of the disease, although insignificantly; the disease ran a severe course in 6 (17.6%) vaccinated patients and 9 (32.1%) unvaccinated patients. Standard clinical symptoms

<table>
<thead>
<tr>
<th>Table 1. Basic patients’ demographic and clinical data</th>
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<tbody>
<tr>
<td>Study cohort (N = 65)*</td>
</tr>
<tr>
<td>Mean age/median</td>
</tr>
<tr>
<td>Age range</td>
</tr>
<tr>
<td>No (%) of males</td>
</tr>
<tr>
<td>No (%) of females</td>
</tr>
<tr>
<td>No (%) of children</td>
</tr>
<tr>
<td>No (%) of hospital admissions</td>
</tr>
<tr>
<td>No (%) of patients with a severe course</td>
</tr>
<tr>
<td>meningitis</td>
</tr>
<tr>
<td>orchitis (out of males)</td>
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</table>

*including 3 patients with unknown vaccination status

<table>
<thead>
<tr>
<th>Table 2. Clinical symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study cohort (N = 65)</td>
</tr>
<tr>
<td>Clinical symptom</td>
</tr>
<tr>
<td>Pain in the area of parotid glands</td>
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<tr>
<td>Swelling in the area of one parotid gland</td>
</tr>
<tr>
<td>of both parotid glands</td>
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<tr>
<td>Fever</td>
</tr>
<tr>
<td>Headache</td>
</tr>
<tr>
<td>Meningeal symptoms</td>
</tr>
<tr>
<td>Arthralgia, myalgia</td>
</tr>
<tr>
<td>Nausea, vomiting</td>
</tr>
<tr>
<td>Swollen testis</td>
</tr>
</tbody>
</table>
Table 3. Selected laboratory data

<table>
<thead>
<tr>
<th>Laboratory indicators</th>
<th>Study cohort (N = 65) n (%)</th>
<th>Vaccinated patients (N = 34) n (%)</th>
<th>Unvaccinated patients (N = 28) n (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low (≤ 8 mg/l)</td>
<td>22 (33.8)</td>
<td>16 (47.5)</td>
<td>6 (21.4)</td>
<td>0.061</td>
</tr>
<tr>
<td>high (&gt;80 mg/l)</td>
<td>7 (10.8)</td>
<td>2 (5.9)</td>
<td>5 (17.8)</td>
<td>0.228</td>
</tr>
<tr>
<td>White blood cell count &gt; 10.0 10^9/l</td>
<td>11 (16.9)</td>
<td>3 (8.8)</td>
<td>8 (28.5)</td>
<td>0.053</td>
</tr>
<tr>
<td>Serum amylase &gt; 1.66 μkat/l</td>
<td>50 (76.9)</td>
<td>27 (79.4)</td>
<td>23 (82.1)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

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and their frequencies are summarized in Table 2. The vaccinated and unvaccinated patients did not differ significantly in basic clinical symptoms (painful swelling in the area of parotid glands, fever, joint pain, muscle pain, nausea, and vomiting). Laboratory data are given in Table 3. High CRP levels (above 80 mg/l) were found in 7 (10.8%) patients and tended to be more common in unvaccinated patients (17.8% vs. 5.9%). Elevated white blood cell counts were reported more often in unvaccinated patients (28.5% vs. 8.8%). Serum amylase was elevated in most patients, both vaccinated and unvaccinated (79.4% vs. 82.1%).

From 29 of 51 PCR positive patients, the virus was isolated in Vero cells. These tissue isolates along with 22 swab specimens from patients where the virus isolation attempt was unsuccessful were the source materials for mumps virus DNA sequencing. The sequencing was successfully completed on 30 specimens, i.e. on 23/29 tissue isolates and 7/22 swab specimens. All 30 sequenced mumps virus strains were assigned to group G (Fig. 1). The mumps virus strains isolated in the Czech Republic in 2012 were assigned to genotype G (Fig. 1) as well. Within this genotype, based on the nucleotide sequence encoding SH protein, the isolates were differentiated into three groups linked to the region of infection – Karlovy Vary, Pardubice, and Teplice (Fig. 2). The phylogenetic comparison of the isolates from 2012 and 2013 did not show any significant differences, only single point mutations without any significant phenotypic repercussion, so no specific clinical symptoms or epidemiological links were established. The strains from 2013 were assigned either to the Karlovy Vary group or to the Pardubice group, with none of them belonging to the Teplice group (Fig. 2). The 30 sequenceable specimens were collected from patients from different regions, mostly from Prague (20 specimens), followed by the Liberec Region (2 specimens), Central Bohemia Region (2 specimens), Hradec Králové Region (1 specimen), South Moravia Region (1 specimen), and Ústí nad Labem Region (1 specimen). For three patients, these data are not available. Most specimens were collected within the first six months of 2013, with the highest number obtained in April which correlates with the distribution of reported mumps cases by month in CR, based on the EPIDAT data.

Epidemiological Analysis of Mumps Cases in the Czech Republic in 2013

In 2013, 1,553 cases of mumps were reported to the EPIDAT system. Of these, 640 were laboratory confirmed. The patients were 871 males (56.1%) and 682 females (43.9%). The most affected age groups were 10–14 years (425 cases, i.e. 27.4%) and 15–19 years (381 cases, i.e. 24.5%). A high percentage of the patients were fully vaccinated with two doses: 1,245 patients, i.e. 80.2% of all reported cases. However, the rates of complications are statistically significantly higher in the unvaccinated than in the vaccinated group. The most often reported complications were orchitis (90 cases – 10.3%) and meningitis (21 cases – 1.4%). Orchitis was diagnosed in 30.3% of the unvaccinated and in 6.4% of the vaccinated males (p < 0.001). Meningitis was reported in 3.1% of the unvaccinated and in 1.0% of the vaccinated patients.
In 2013, most cases were sporadic, with only 53 cases being reported within three outbreaks in the Ústí nad Labem Region. The highest number of cases occurred in the first half of the year, with a peak of 243 cases reported in April.

**DISCUSSION**

Data on reported cases of mumps in CR have been available since 1955. In the pre-vaccination period, 48,000 cases of mumps were reported annually on average (20,000 to 100,000 cases depending on about three-year epidemic cycles). The results of the serological survey of 2013 showed the following percentages of seropositivity for IgG antibodies: 53% in children from 5–9 years of age, 35% in 10–19 year olds, and only 38% in 20–29 year olds. Among the naturally immunised population, the seropositivity rate is higher (80%) but still remains below the 95% level required for herd immunity (10). The discrepancy between the long-term high vaccination coverage of above 98% and the antibody levels which were not high enough in any age group is considered as alarming (10) and is seen as one of the reasons behind the rise in mumps cases despite the vaccination programme. The most affected age groups in 2013 were 10–14 years and 15–19 years, which is consistent with the conclusions of the serological survey (10).

The present study confirmed the positive effect of vaccination on the incidence of complications. Nearly one third (32.1%) of the unvaccinated group were diagnosed with meningitis or orchitis in comparison with 17.6% of the vaccinated patients. Similar proportions of complications were also reported from other countries where genotype G is circulating: Macedonia, The Netherlands, Bavaria, or Scotland (11–14). In the study cohort, 42.9% of the unvaccinated and 17.4% of the vaccinated patients were diagnosed with orchitis. Even more compelling data are provided by the EPIDAT system, with orchitis being reported in 30.3% of the unvaccinated male patients and in 6.4% only of their vaccinated counterparts. In comparison with the EPIDAT data on meningitis in unvaccinated and vaccinated patients (3.1% and 1.0%, respectively), this study found a somewhat higher rates of this complication in both groups (10.7% and 5.9%, respectively). Nevertheless, the differences can be explained by the small number of patients in the study cohort in comparison with the nation-wide database. Another fact to be considered is that patients with uncomplicated mumps who are treated on an outpatient basis by their general practitioners may not be referred to the reference laboratory. Differences between the vaccinated and unvaccinated patients were found in the inflammatory markers (white blood cell count and CRP) and serum amylase. The vaccinated patients had lower levels of inflammatory markers. Elevated CRP levels (> 80 mg/l) were detected in 7 patients, mainly in those with orchitis (in 4 unvaccinated males and 1 vaccinated male). Elevated CRP levels in mumps orchitis have been reported by others (15, 16) as well. Unvaccinated patients also showed substantial higher serum amylase levels than the vaccinated ones; nevertheless, it should be reminded here that serum amylase levels vary with the time from the onset of the disease. In the study cohort, no statistically significant differences were observed in the incidence of particular clinical symptoms between the vaccinated and unvaccinated patients, but vaccination appeared to have an unambiguously positive effect on the incidence of complications and the need for hospital admissions.

The vaccine strains were derived from clinical isolates that were common in the 1960s and 1970s and are classified into different genotypes. For instance, the Jeryl-Lynn and Rubini strains are genotype A, Leningrad 3 and L-Zagreb strains are genotype D, and Urabe Am9 strain is genotype B. In the EU countries and in the USA, a derivative of the Jeryl-Lynn strain is in use. In the Czech Republic, genotype G was detected previously (17, 18) and the situation did not change substantially.

A long-term programme aimed at population health improvement in CR called Health for all in the 21st century set as one of the goals to be achieved by 2010 to reduce the incidence of mumps to less than one case per 100,000 population, but this goal has not yet been met. Prerequisites for reducing the incidence of mumps in the population with high vaccine coverage are an appropriate vaccination strategy, early and reliable diagnosis, and effective preventive measures. Nevertheless, prophylactic vaccination against mumps in the focus of infection does not work during the incubation period and may not provide protection against
the disease. However, if given during the incubation period, the vaccine does not cause higher rates of post-vaccination complications or a more severe course of the disease, if not prevented (19–22). This approach is supported by the lesson learned from the mumps outbreak in the Ústí nad Labem Region in 2011 where in the focus of infection, the most affected age groups (10–14 and 15–19) received extra doses of the vaccine. In total, 18,865 vaccine doses were used and within two weeks, mumps cases declined and further spread to other cities in the administrative region was prevented. Only as few as 10 of the 18,865 vaccinated persons developed mumps (23) and are highly likely to have been in the incubation period when vaccinated. Despite the long-term high mumps vaccine coverage, mumps cases have recently been on the rise in CR, with post-vaccination cases accounting for about 80% of the reported cases (9). A question arises of the efficacy of the vaccine used. The considered primary vaccine failure as a result of genotype mismatch between the vaccine strain and wild-type mumps virus strains, in accordance with the literature data, is not likely to play an important role (24, 25) and the laboratory results obtained from our ongoing grant study do not support this assumption either (data under publication). Neither is improper storage or handling assumed to be the reason behind a lower vaccine effect. A secondary vaccine failure due to waning immunity seems to be a more plausible explanation. To remedy the adverse epidemiological situation, one of the possible steps is to change the vaccination strategy by adding a third dose at the adolescent age (26–28). Although globally discussed, this strategy is not supported by the immune status data for the Czech population from the last two years and, therefore, regular immunological surveys would be really helpful in obtaining such data. In the Czech Republic, the third vaccine dose at the adolescent age has not been envisaged so far; however, a change to the immunisation schedule, by prolonging the interval between doses, has been considered. Conducting the vaccine coverage and wild-type mumps virus genotype surveys is, therefore, crucial for adjusting the immunisation schedule to the actual needs as well as for adopting other preventive measures.

CONCLUSION

Despite the emergence of mumps cases among the vaccinated population, the present study has confirmed the positive effect of the mumps vaccine, particularly on the incidence of complications and inflammatory markers. All 30 sequenced mumps virus strains were assigned to group G. A secondary vaccine failure due to waning immunity seems to be a plausible explanation for the rise in mumps cases. Since 2005, the main trends in mumps cases (slight prevalence of cases in males, a high proportion of cases in vaccinated patients, effect of vaccination on the incidence of complications, orchitis in particular, and a shift towards higher age groups) have remained unchanged or similar.

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