

# MUMPS IN THE EASTERN BOHEMIA REGION OF THE CZECH REPUBLIC – A SEROLOGICAL SURVEY 2008–2012

Miroslav Fajfr<sup>1,2</sup>, Vlasta Štěpánová<sup>1,2</sup>, Jana Fajfrová<sup>3</sup>

<sup>1</sup>Institute of Clinical Microbiology, University Hospital, Hradec Králové, Czech Republic

<sup>2</sup>Institute of Clinical Microbiology, Faculty of Medicine in Hradec Králové, Charles University, Hradec Králové, Czech Republic

<sup>3</sup>Department of Military Internal Medicine and Military Hygiene, Faculty of Military Health Sciences, University of Defence, Hradec Králové, Czech Republic

## SUMMARY

**Background:** The incidence of mumps has decreased in many countries since the introduction of vaccination programmes, however, in the past decade a rapid increase in the disease occurrence has been reported worldwide. The reason for this situation is still not clear. We present the results of a serological survey carried out in the Eastern Bohemia Region of the Czech Republic during the years 2008–2012.

**Methods:** In total, 2,536 samples of 2,034 patients were examined during the study period. The study cohort was divided into two groups, one consisted of individuals born before the introduction of mandatory vaccination and the other one comprised individuals born after mandatory vaccination started. For the serology analyses the ELISA kits RIDASCREEN Mumpsvirus IgM and IgG (R-Biopharm®, Germany) were used.

**Results:** Out of 2,536 samples (including paired sera), 23.9% (n = 606) were positive and 12% (n = 304) had equivocal results. Most of the positive samples were obtained from patients aged 17–20 years. Significantly more ( $p < 0.05$ ) positive patients were born after the start of the national vaccination programme (patient group 2) (22.8%) compared to those born before its start (patient group 1) (13.7%). Interestingly, the analysis of data showed that 75.3% of patients falling into group 1 had anti-mumps IgG antibodies, which means that they had contracted mumps, whilst 23.5% of patients of group 2 had undetectable IgG antibodies, even though they should have been vaccinated.

**Conclusion:** The data from our study, with a low number of positive samples in the first years of the study and an increase in the last two years, could suggest the occurrence of outbreaks every 4–6 years.

**Key words:** serological survey, mumps, vaccination, ELISA, outbreak

**Address for correspondence:** M. Fajfr, Institute of Clinical Microbiology, University Hospital, Sokolská 581, 500 05, Hradec Králové, Czech Republic. E-mail: miroslav.fajfr@fnhk.cz

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## INTRODUCTION

Mumps, also called *parotitis epidemica*, is one of the vaccine-preventable diseases occurring especially in childhood in the non-vaccinated population. The infection generally has a clear clinical picture with swelling of one or both parotid glands and characteristic biochemical markers such as increased amylase activity, and mostly with high fever. In some cases, the disease may progress into meningitis, unilateral sensorineural deafness, pancreatitis or orchitis (with post-infection sterility), however, approximately one third of cases could be asymptomatic or have merely non-specific respiratory symptoms (1, 2). A vaccine was developed to prevent a severe course of the illness and its complications. Four vaccination strains have been used: Jeryl Lynn (JL), Leningrad-Zagreb (LZ), Rubini (RU), and Urabe (UR). Despite a better antibody response to L-Zagreb, the vaccination with this strain had some side effects and complications. There were cases of post-vaccination encephalitis caused by the L-Zagreb strain, and some cases of horizontal transmission of the vaccine virus were reported (3, 4). The Rubini strain was officially not recommended

by scientific authorities due to a weakened antibody response in comparison with the other vaccine strains, and its effectiveness of 33% was deemed to be inadequate (5). Therefore, the majority of countries have used the Jeryl Lynn vaccination strain in their vaccination programme. After implementation of mumps immunization programmes in many countries worldwide, the numbers of mumps outbreaks rapidly decreased. But since the end of the 1990s an increased number of mumps outbreaks have been reported in countries with an implemented mumps vaccination programme, and until now it is still not clear why the vaccination is not able to eliminate mumps outbreaks in the vaccinated population (1, 2, 6, 7). In several studies, a possibility of mumps virus re-infection in patients following “wild” mumps infection was noted (8, 9).

The mandatory immunization of children with the JL strain in the Czech Republic (Czechoslovak Republic) started in 1987 with a bivariate (Mopavac®, Sevapharma), and since 1995 with a combined vaccine against measles (morbilli), mumps and rubella (MMR) – vaccine Trivivac® (Sevapharma, a.s.) or in recent years with the vaccine Priorix® (GSK). Every vaccinated individual obtains two doses (the first dose in children after the

age of 15 months and a secondary catch-up dose 6–10 months later). Mumps is under national surveillance and every case must be reported to the local hygiene/epidemiological authorities. According to information from the National Institute of Public Health (NIPH) serological survey 2001, more than 97% of the Czech population born after 1987 was covered by vaccination and 75.2% of population aged 1–14 years had positive titers of IgG antibodies (10) and therefore only sporadic infections in the non-vaccinated adult population were expected. Since the beginning of 2003, rapidly increased numbers of mumps cases have been reported in the Czech Republic and the same situation has been also observed in other countries with high vaccination coverage. The rapid increase of mumps cases in vaccinated populations worldwide is still not fully explained.

We present the results of a unicentric retrospective serological survey from the Eastern Bohemia Region of the Czech Republic focused on mumps diagnosis by serological methods. The main aim of this study was the monitoring of mumps serology in our region and the comparison of the results with those from a previous study from the years 1995–2005.

## MATERIALS AND METHODS

### Samples

Samples included in the study were all serum samples which were subject to mumps serology examination (only from patients with clinical signs resembling mumps infection and on the demand of physicians) during the study period between 1/2008 and 12/2012, and overall 2,536 serum samples from 2,034 patients for serology were examined. The median of patient age was 22 years (ranging from 3 months to 98 years). All samples were examined in the Institute of Clinical Microbiology, University Hospital Hradec Králové. Samples were obtained from GPs (1,280 samples) or hospitals (1,256 samples) from the Region of Eastern Bohemia. All samples were divided into two groups. Samples from patients born before 1987 when the national mumps vaccination programme started (patients very probably not vaccinated) were allocated to Group 1. Patients born after 1987 were assigned to Group 2; the vaccination status was not verified, but according to information from NIPH the vaccination coverage was more than 97% (10). In our study, 1,545 patients with single samples and 489 patients with multiple sera (mostly paired sera, sporadically 3–4 samples) were evaluated. Paired sera were taken 2–4 weeks after the first collection.

### Serology

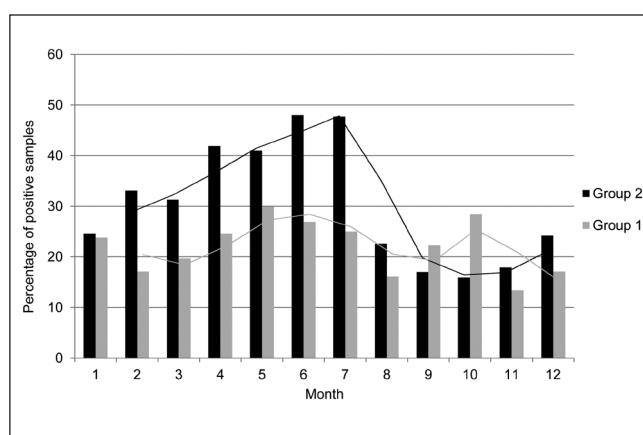
For serology the ELISA kits RIDASCREEN Mumpsvirus IgM and IgG (R-Biopharm®, Germany) were used according to manufacturer's instructions. Tests contained positive and negative control and calibrators for semi-quantitative evaluations. Final sample photo-absorption was measured on a Multiscan FC photometer (Thermo Scientific®). Measured absorbance values were recalculated according to manufacturer's instructions in units per milliliter (U/ml). Positive results in both tests were set by the manufacturer as a value of more than 24 U/ml and equivocal results in the range from 14.0 to 24.0 U/ml.

## Results Evaluation Criteria

The presence of specific IgM antibodies was considered as a positive serological result, confirmed by the same results or by serological-conversion in the paired sample obtained after 2–4 weeks (if it was available). In the cases of the presence of only the IgG antibody class, a fourfold increase in the IgG level in paired sera (taken within a time period of 2–4 weeks) was necessary for mumps serology confirmation. Equivocal results were defined as the presence of IgM antibodies in the equivocal range or the presence of very high IgG antibody levels, more than 150.0 U/ml. Chi-square ( $\chi^2$ ) test was used for statistical analysis in the software STATISTICA CZ 12 (StatSoft®, USA), and significance levels were determined in all analyses as  $p \leq 0.05$ . The authors did not take into account the assessment of the other determinants of risk behaviours (e.g. smoking, drug use, drunkenness, etc.)

## RESULTS

Overall 51.4% of patients ( $n=1,046$ ) were males and 48.6% of patients ( $n=988$ ) were females; 53.7% ( $n=1,362$ ) of samples were from patient Group 2 and 46.3% ( $n=1,174$ ) were from the population before the mandatory vaccination programme started (patient Group 1). Almost two thirds of all samples were from the first three decades of the patient's life. Only 37.6% of patients with ICD-10 code B26.0-9 (*Parotitis epidemica*) had serologically positive results; likewise, 21.9% of patients with a diagnosis of lymphadenitis (ICD-10 code L04 or R59) or sialadenitis (ICD-10 code K11.2) were positive or borderline positive by serological examination. Overall, 23.9% of all samples ( $n=606$ ) were positive according to the evaluation criteria mentioned above and 64.1% ( $n=1,626$ ) were negative (defined as negative for antibody or with no serological signs of ongoing disease, namely a fourfold increase of IgG antibody levels or the presence of IgM antibodies); 12.0% ( $n=304$ ) had equivocal results (according to criteria mentioned above) in the serological test. Amongst the positive samples, males (50.5%) were slightly more predominant than females (49.5%). Cases of serologically-confirmed mumps were observed mostly in March, April, May and June (53.1%) (Fig.



**Fig. 1.** Positive and borderline positive patients split according to month and vaccination history.

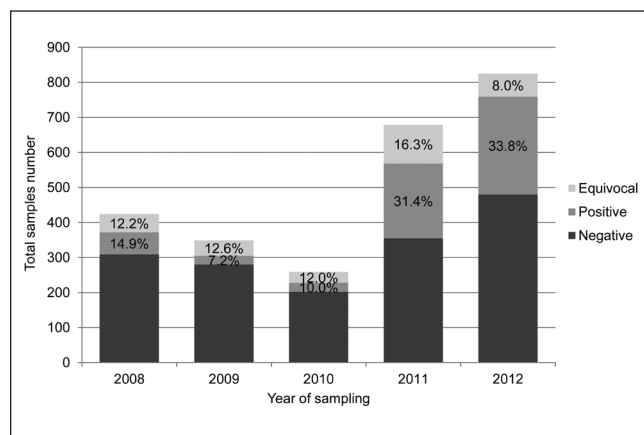
Group 1 – patients born before the introduction of national vaccination campaign; Group 2 – patients born after the national vaccination campaign. The highest number of positive patients was in the first half of the year.

1) and in patients aged between 17 and 20 years (Fig. 2). The number of samples according to each year is shown in Fig. 3. After continuous reduction in sample numbers in the first years of the study period, there was a rapid increase in both the total number of samples and the positive samples in the last two years of the survey.

In Group 1 a total of 1,174 samples from 1,028 patients were evaluated. Among them, 17.8% (n=209) of samples were positive, 11.4% (n=134) were in the equivocal range, and 70.8% (n=831) were negative. The median of patient age in Group 1 was 43 years (with the range from 23 to 88 years). Of the examined patients from Group 1, 13.7% (n=141) were serologically positive and 12.5% (n=129) had equivocal results. A total of 75.3% (n=774) of patients had detectable IgG antibodies which meant they had contracted mumps in the past. In the positive samples in Group 1, females (52.3%) predominated over males. In Group 2 a total of 1,362 samples from 1,006 patients were evaluated, 55.9% from males (n=562) and 44.1% from females (n=444). The median age of patients in Group 2 was 16 years (with the range from 6 months to 25 years). Surprisingly, 23.5% (n=236) of patients from Group 2 had non-detectable levels of IgG antibodies and only 53.4% (n=537) of positive samples were from patients with a mumps diagnosis. Overall 21.9% (n=220) of patients from Group 2 developed positive serological results and 15.0% of them had equivocal results (n=151). In Group 2 there were statistically significantly more positive patients than in Group 1, both between males (p=0.0011) and females (p=0.0001).

## DISCUSSION

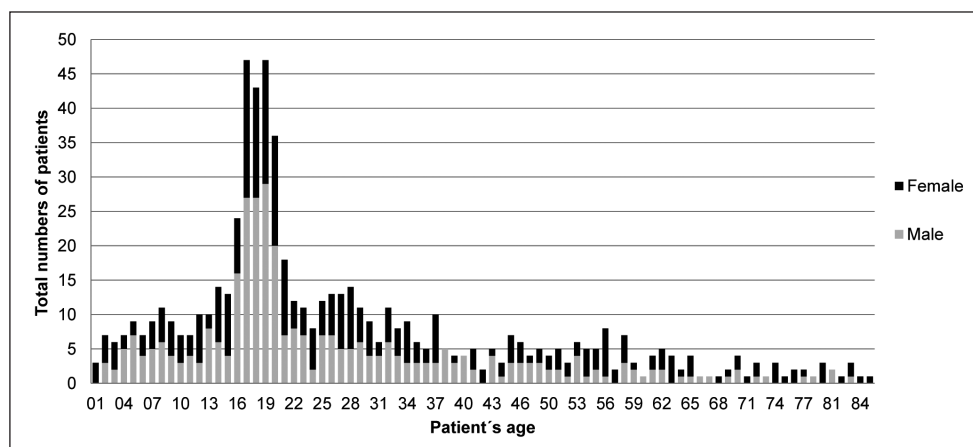
In the previous study (11) from our region which evaluated the period between the years 1997 and 2005, an increasing incidence of mumps was observed from 2003 until its culmination in 2005 when the mumps outbreak lost its seasonal characteristic and a high number of positive patients were found throughout the whole year. In this outbreak, the most positives were reported in January, May, June and October. In our current study the most positives were found in March–June, and 70.3% of all positive patients were found in the first half of the year, which rather imitates the course of the first outbreak in 2003, when the majority of positive results



**Fig. 3.** Overall samples split according to years and test results.

This graph well documents the rapid increase of both sample numbers and positive samples in the years 2011 and 2012. (Equivocal results means detection of IgM antibody level in the equivocal range or high IgG level (more than 150 U/ml) in single serum samples; negative means no antibodies or no serological signs of ongoing infection).

were found in the spring. Our findings of low numbers of overall samples and positive samples in the years 2008–2010 together with a rapid increase in the years 2011 and 2012 may suggest the return of a recurrent mumps outbreak every 4–6 years. The data from our study were fully in congruence with the data from the mumps serology surveillance system of NIPH from the whole Czech Republic. According to previous data, the most frequently positive samples were from patients aged between 17 and 20 years and our recent data confirm the same (11). In another study from the Czech Republic, the most positive samples were from the age group 15–19 years, and the authors confirmed relatively high vaccination cover in this group because 87.1% of cases had a two-dose vaccination history (12). All these findings may suggest an insufficient protection of our two-dose vaccine scheme beyond 15 years after vaccination, and were in congruence with other studies. They support the possibility of a change in the vaccination scheme with one dose in the teenage years (13, 14). On the other hand, the vaccination coverage decrease in the Czech population from 97–100% in 2001 to 87.1% (in population aged 15–19) in 2005/6 and the reason for recurring outbreaks could be an insufficient population vaccination coverage which is not



**Fig. 2.** Positive and borderline samples split according to patient age and gender.

The highest number of positive/borderline positive samples in patients aged 17–20 is well documented.

high enough to achieve collective protection (12). Also, in our study 23.5% of patients born after the start of national vaccination had undetectable IgG antibodies. However, the reason for this finding is unclear, it is probably a combination of a decrease in vaccination cover and the waning of post-vaccination immunity. The fact that there was only 42.3% congruence of positive serological results in patients with a definite mumps diagnosis exposed frequent misinterpretation of clinical pictures resembling those of *Parotitis epidemica*.

Mumps serology evaluation presents a substantial challenge for a high percentage of the vaccinated population in our country, especially with only single-sample examination (in our recent study approximately half of all samples). In situations where the first serological examination was done during fully developed disease, a high level of IgG antibodies had already been produced, hence evaluation of the serological result was made very difficult because of the requirement for a fourfold increase in antibody levels. In the cases where only a single sample is obtained, the dynamics of antibody response cannot be followed and serological evaluation is impossible or at best very difficult. A possible helpful diagnostic tool could be avidity testing for mumps virus specific IgG, which is able to generate a result from a single sample (15, 16). PCR from blood, saliva or cerebrospinal fluid samples could be also used for confirmation of uncertain mumps infection, but has not been used routinely in our country because of its high cost.

The reason for the statistically significantly more numerous cases of mumps in the vaccinated population is not clear. One possible reason could be the behaviour of the young population (close contact, large numbers of people in a small place, etc.) which facilitates the spread of infection via small droplets. The closer contact among people in the colder months could explain the higher positivity of mumps in these months. A much-discussed possibility for mumps vaccination failure is the phenomenon of antibody level waning, which refers to the progressive decrease in antibody level with time after vaccination. In a Finnish study a decrease of IgG antibody avidity was confirmed over time after vaccination, resulting in lower protection of post-vaccination antibodies. The authors of that study concluded the positive effect of a third booster vaccination (16).

One of the most often discussed hypotheses of why vaccination does not protect against wild viruses is the genetic variability of the mumps virus. Wild virus could be responsible for outbreaks of mumps in the vaccinated population. The Jeryl Lynn vaccine strain belongs to genotype A (Leningrad-Zagreb belongs to genotype B), and according to some studies the wild strain which is responsible for outbreaks belongs mainly to other genotypes. In the Western hemisphere including the Czech Republic, the most isolated genotype has been G (17), in the Asia-Pacific region genotypes J and F, and in the Middle East genotype H. In several studies surprising findings confirmed that the serum from those vaccinated with JL vaccine neutralized 7 other genotype strains. However, it was confirmed that this interesting vaccine “side-effect” was highly dependent on the time after vaccination, and titers of virus-neutralizing antibodies decreased with post-vaccination time (2, 6). This could refer to the decreased effectiveness of the vaccine after a post-vaccination decade, and it also confirms the validity of the notion of a booster vaccination in the teenage populations. This hypothesis was in congruence with another study which refers to the probable need for a periodical 4–8 years

booster vaccination for the preservation of vaccine effectiveness (14). The northern European countries and some other European countries have a different vaccination scheme for the MMR vaccine, with the first dose given at the age of 14–18 months and the second booster dose at the age of 4–8 years. According to some studies, this scheme probably offers a better long-term disease protection than that in which both doses are given in early childhood (16, 18).

## CONCLUSION

Our results validate the continuance of mumps serology in our region, as the recent results were in congruence with the results from the previous study. The data from our study with a low number of positive samples in the first years of the study and an increased number in the last two years could suggest a recurrence of outbreaks every 4–6 years. The appearance of mumps infection predominantly in the teen population more than 15 years after the catch-up vaccine dose could lead to revision of the vaccination programme.

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## Conflict of Interests

None declared

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