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Letter to the Editor

Horizontal transmission of the Leningrad-Zagreb mumps vaccine strain: A report of three cases

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Systematic use of the live attenuared mumps vaccines for the last four decades significantly reduced the incidence of disease and even resulted in its eradication in some parts of the world (1, 2). However, reactogenicity due to a poor attenuation, the possibility of vaccine strain shedding and horizontal transmission represent some problems related with some mumps vaccine strains (3, 4, 5, 6, 7). Although virus shedding following immunization with certain live attenuated mumps vaccine strain has been well documented, it is generally accepted that horizontal transmission of vaccine strains is a rare event, and to the best of our knowledge until now there have been only two reports in whom symptomatic disease developed as a consequence (6, 7). Sawada et al. described in 1993 a horizontal transmission of the Urabe AM9 vaccine strain from a symptomatic vaccinee to her younger sister (6), while recently a horizontal transmission of the Leningrad-3 mumps vaccine strain from healthy vaccinees to six previously vaccinated contacts resulting in symptomatic infection, has been described (7). In both studies, parotitis developed as a unique consequence of horizontal transmission of mumps vaccine strains. Neither neurological, nor other extrasalivary abnormalities were seen among those patients, and none of them had to be hospitalized. All patients were in childhood age (6, 7).

In Croatia the measles, mumps and rubella (MMR) vaccine containing Leningrad-Zagreb (L-Z) mumps vaccine strain has been a part of routine immunization programme for thirty-one years (9). L-Z, originating from the Russian Leningrad-3 vaccine strain, has been successfully used in other parts of the former Yugoslavia, as well as in some of the developing countries (1, 10). L-Z is a very immunogenic and efficacious strain, but even serious adverse reactions (e.g. aseptic meningitis), especially after primovaccination have been reported (9, 10, 11, 12). Although reactogenicity of L-Z strain, has been confirmed, and viremia and viral shedding among vaccinees is a probable event, not a single case of horizontal transmission of L-Z mumps vaccine strain has been reported to date in peer-review journals.

After two cases of parotitis in adults temporarily associated with recent MMR vaccination of their children were recorded in Croatia during 2004 and 2005 (data not published), we started a prospective observational study of all parotitis cases at the University Hospital for Infectious Diseases (UHID), in Zagreb, Croatia in order to confirm the existence of horizontal transmission of the L-Z vaccine strain. The study

started on November 1, 2006 and finished on October 31, 2007. In the observed period 16 patients with clinically evident parotitis were seen at the UHID, of whom four were suspected of becoming infected by a horizontal transmission of mumps vaccine strain. During the study period 16 600 doses of MMR vaccine containing L-Z vaccinal strain have been administered in Zagreb and its vicinity.

Patients eligible for this study were selected on the basis of the following criteria: (i) no history of recent vaccination against mumps; (ii) contact with a recently vaccinated person, i. e. within 45 days prior to development of first symptoms of disease and (iii) negative results of parainfluenza virus types 1, 2 and 3 and Epstein-Barr virus (EBV) serology (13). In one patient all samples tested negative for mumps virus RNA, while in three patients (P1 – P3) with suspected horizontal transmission, an infection with L-Z strain was confirmed (Table 1). A possible source of infection in all three cases were their own children recently vaccinated with L-Z containing vaccine. Vaccination histories of all patients were collected through interviews. P1 and P2 were vaccinated in early childhood with one dose of MMR containing L-Z vaccine strain, while P3 was previously unvaccinated (Table 1).

All three patients included in the study were adults. Both P2 and P3 had parotitis while P1 had parotitis, orchitis and AM. P2 and P3 were treated as outpatientswhile P1 was hospitalized for five days. No neurological sequelae were seen nor subjective complaints registered at the follow-up visit of P1 two months after the discharge.

In P1, urine and CSF samples for molecular analysis, and serum for serology were taken on the 2nd day of hospitalization. Throat swabs, urine samples and serum taken at the first visit were obtained from P2 and P3 (Table 1).

Samples for mumps virus detection (throat swabs, urine and CSF) were stored at -80°C and then transferred to the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia. A sample of freeze-dried L-Z mumps vaccine, batch No. 119R (Institute of Immunology, Zagreb, Croatia), reconstituted in 500µl of sterile water prior to RNA extraction, was used as a positive control. Viral RNA was extracted from clinical and vaccine samples using QiaAmp Viral RNA mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Briefly, 560 µl of AVL buffer containing carrier RNA was added to 140 µl of throat swab suspension, urine, CSF or vaccine sample and incubated 10 minutes

at room temperature. After the addition of 96% ethanol, each sample was transferred into a QIAamp Mini spin column, centrifuged for 1 min at 8,000 rpm, and then washed with AW1 and AW2 buffers. Bound RNA was double eluted with 40 µl of AVE buffer. Prior to RNA extraction urine samples were centrifuged for 10 min at 1500 x g and only the supernatant was used.

A slightly modified nested SH/LZ RT-PCR assay, specific for mumps virus SH gene, was used to detect mumps RNA using the outer primer set SH1/SH2 for the first PCR amplification and the inner primer set LZ1/LZ2 for the second step, as described previously (14). Briefly, the first PCR was carried out using OneStep RT-PCR Kit (Qiagen) in a 25 µl reaction volume, that included 5 µl of extracted RNA, 5 µl of 5x QIAGEN OneStep RT-PCR buffer with 12.5 $MgCl_2$, 400 µM dNTP, 1 µl of QIAGEN OneStep RT-PCR Enzyme Mix, 4 U of RNase inhibitor and 600 nM of each SH primer. The cycling conditions used were 50°C for 30 min, 95°C for 15 min, followed by 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 2 min. This was followed by a final extension step at 72°C for 10 min, and the amplification reaction mixtures were cooled to 4°C. The second PCR was performed using HotStar Taq Plus DNA Polymerase kit (Qiagen) in a 50 µl reaction volume that included 2 µl of the first-step PCR product, 5 µl 10x CoralLoad PCR buffer with 15 mM $MgCl_2$, 200 µM dNTP, 2.5 U of HotStar Taq Plus DNA Polymerase and 200 nM of each LZ primer. The cycling conditions used were 95°C for 5 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 2 min, followed by a final extension step at 72°C for 10 min, and the amplification reaction mixtures were stored at 4°C. The amplified PCR products were purified by QIAquick PCR Purification Kit (Qiagen) and sequenced directly using BigDye Terminator v1.1 Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA) with LZ1/LZ2 primers. Sequencing products were purified of unincorporated dye labeled dideoxynucleotides by processing through DyeEx™ 2.0 Spin Kit spin columns (Qiagen). Sequence analysis was performed using ABI PRISM™ 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA) and the complementary sequences were aligned and edited with BioEdit Sequence Alignment Editor (North Carolina State University, Raleigh, NC). A comparison of the obtained nucleotide sequences with those available in GenBank was performed using the BLAST server (http://www.ncbi.nlm.nih.gov/BLAST). Results from the BLAST comparisons were confirmed additionally by ClustalW (http://www.ebi.ac.uk/Tools/clustalw/) multiple alignments using the sequence of

interest and reference sequences of L-Z vaccine (GenBank accession number AY685920) and master seed strain (GenBank accession number AY685921).

The patients` sera were processed at the serological laboratory of the UHID and at Virology Department of the Croatian National Institute of Public Health. Recent infection with parainfluenza viruses and Epstein-Barr virus (EBV) was excluded using commercial Virotech ParaScreen ELISA kit (Genzyme Virotech GmbH, Russelsheim, Germany) and LIAISON EBV kit (DiaSorin, Saluggia, Italy), respectively. Infection with parainfluenza virus types 1 – 3 was excluded if specific IgM and IgA antibodies were not detected. Recent EBV infection was excluded if IgM to viral capsid antigen (EBV VCA) and/or IgG to early antigen (EBV EA) were not detected.

Serological detection of anti-mumps virus IgM and IgG antibodies was done by Virotech Mumps ELISA kit (Genzyme Virotech GmbH, Russelsheim, Germany). Quantitative IgM and IgG results were classified as positive, equivocal or negative according to the manufacturer`s recommendations: > 11 Virotech Units (VE) positive; 9 – 11 VE equivocal and < 9 VE negative.

Neutralizing anti-mumps antibodies were determined by the procedure described by Örvell et al., slightly modified to the microtechnique (15). Briefly, serial twofold dilutions of the patients' sera in a volume of 0.05 ml were mixed with an equal volume of L-Z mumps vaccine strain (100 $TCID_{50}$/0.1ml) in flat-bottomed cell culture microtitre plate. Two wells were inoculated per one serum dilution, with dilution range of 1:4 – 1:128. Serum/virus mixtures were incubated at 37°C, 5% CO2 for 1 hour. During this incubation period a suspension of approximately 1.5 x 10^{-5} cells per ml of Vero cells was prepared in MEM (Invitrogen, Paisley, UK) with hepes, supplemented with 2% foetal bovine serum, and distributed 0.1 ml of cell suspension into all wells. Plates were incubated at 37°C, 5% CO2, and daily inspected for cytopathic effect with final readings after 5 days of incubation. For each serum, the neutralizing antibody titer was determined as the highest dilution in which cytopathic effect was not observed. The cut-off for seropositivity was a neutralizing antibody titer ≥ 1:4.

Mumps virus RNA was detected in vaccine sample and in four (66.7%) out of six clinical samples tested (Table 1). In P1 viral RNA was detected in CSF sample only. In P2, both the throat swab and urine sample were positive for viral RNA, while in P3 mumps virus RNA was successfully amplified from throat swab sample.

Direct sequencing of the approximately 330-bp long LZ PCR product of four mumps virus RNA positive samples revealed that the all three patients were infected with the L-Z mumps vaccine strain. The SH sequences obtained from al three patients matched completely (100%) the SH reference sequences of the L-Z vaccine and master seed strains, available in GenBank.

Specific IgG and IgM antibody levels as well as neutralizing antibody titers of all patients are presented in Table 1. All three patients were IgM-negative but had detectable levels of specific anti-mumps IgG antibodies. Neutralizing antibodies were detected only in serum sample of the P1, although the titer was low (1:8).

Horizontal transmission of mumps vaccine strains is a very rare event, and to the best of our knowledge until now only transmission among persons of childhood age has been described. All subjects in whom symptomatic disease developed as a consequence of horizontal transmission of vaccine strain had parotitis of benign course (7, 8). No single case of symptomatic disease following horizontal transmission of mumps vaccine in an adult person has been reported to date, as well as no case of extrasalivary localization of infection. In contrast to previously reported cases, all three patients described in the present study were adult persons, one of whom developed two extrasalivary complications requiring hospitalization.

The possible source of infection in our patients was their own children who were recently primovaccinated with L-Z containing MMR vaccine. First symptoms of disease in patients appeared within 33 – 44 days after the vaccination of their children. Taking into account the duration of incubation period in natural mumps infection, as well as the timing of clinical presentation of previously described L-Z vaccinal complications, our data, although scarce, may suggest that viral shedding might have happened two to four weeks after the vaccination, which is consistent with the published data (8, 12, 16).

In all our patients, the infection with L-Z was confirmed using RT-PCR targeting SH gene. Viral RNA was detected in throat swabs of P2 and P3 and in urine and CSF samples of P2 and P1, respectively. Interestingly, viral RNA was detected in only one out of three urine samples tested, although viruria in mumps infection is a frequent and long lasting event (16). The presence of variable amounts of PCR inhibitors in the eluate could be the reason why the detection of mumps virus RNA in urine was less successful than in other clinical samples (17).

All three patients in our study had detectable anti-mumps IgG antibodies. The most plausible explanation for the presence of specific anti-mumps antibodies in P1 and P2 might be the immunization with L-Z in early childhood, although all our patients were born in the period when epidemic parotitis was common in Croatian population, and previous asymptomatic infection with a wild strain can be another possibility (9). Despite the presence of anti-mumps IgG antibodies, P1 had a very low titer (1:8), while P2 and P3 had undetectable neutralizing antibody levels. Our results thus support previously known facts that total IgG is not an appropriate correlate of protection to reinfection with mumps virus, and that ELISA is capable to detect a wide spectrum of anti-mumps antibodies, of which only a small proportion are able to neutralize the virus (7, 18). Low, or undetectable level of neutralizing anti-mumps antibodies provided the explanation why contact with a vaccine strain resulted in symptomatic infection.

In conclusion, the present report describes the first virologically confirmed cases of symptomatic mumps following horizontal transmission of mumps vaccine strain in adults. Our data suggest that the L-Z mumps vaccine strain can be transmitted horizontally, causing parotitis, as well as extrasalivary manifestations of the disease including meningitis, even among previously vaccinated subjects. Thus, a close monitoring of vaccinated children family members, especially mumps virus naive or those who had been vaccinated long time ago is highly advisable.

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Table 1

Epidemiologic, clinical, RT-PCR and serologic data of patients with confirmed L-Z mumps infection

Patient	Age (years)	Sex	Previously vaccinated	Clinical presentation	Source of infection	Date of child`s vaccination	Date of first presentation	Date of sampling	RT-PCR throat swab	RT-PCR urine	RT-PCR CSF	ELISA IgM (VE)	ELISA IgG (VE)	NT (titer)
P 1	29	M	yes	Pa, O, AM	son	May, 10	June, 12	June, 17	NC	neg	pos	neg	43	1:8
P 2	30	M	yes	Pa	son	July, 12	August, 25	August, 26	pos	pos	NC	neg	48	<1:4
P 3	37	F	no	Pa	daughter	August, 28	October, 11	October, 12	pos	neg	NC	neg	51	<1:4

Pa, parotitis; O, orchitis; AM, aseptic meningitis; NC, not collected; M, male; F, female; neg, negative; pos, positive.