REVISTA DO INSTITUTO ADOLFO LUTZ
Nota Científica/ Scientific Note

Acesso Aberto
Open Access

Mumps virus genotypes identified during disease outbreaks in the state of São Paulo, Brazil: 2011 – 2016

Genótipos dos vírus da caxumba identificados durante surtos da doença no estado de São Paulo, Brasil: 2011 - 2016

RIALA6/1712

Daniela Bernardes Borges da SILVA[1], Cecília Luiza Simões SANTOS[2], Katia Corrêa de Oliveira SANTOS[1], Márcia THEOBALDO[1], Renato de Sousa PAULINO[1], Norio Augusto Sasaki[1], Margarete Aparecida BENEGA[1], Terezinha Maria de PAIVA[1]*

*Corresponding author: [1]Laboratório de Vírus Respiratórios, Núcleo de Doenças Respiratórias, Centro de Virologia, Instituto Adolfo Lutz. Av. Dr. Arnaldo, 355, São Paulo, SP, Brazil. CEP: 01246-000. Tel: 11 3068 2913. E-mail: tterezinha@uol.com.br

[2]Centro de Virologia, Instituto Adolfo Lutz, São Paulo

Recebido: 06.09.2016 - Aceito para publicação: 06.12.2016

ABSTRACT

In São Paulo the mumps virus (MuV) outbreaks have been increasing from 2011 to nowadays. MuV epidemiological surveillance has been improving by using the polymerase chain reaction in real time (rRT-PCR) in addition to the specific IgM antibody (IgM-Ab) detection; in some cases, genome sequencing studies were performed. Increased virus transmission and recent outbreaks have raised interest on MuV genotyping, as a means to understand the transmission pathways and to identify the vaccine-associated cases._From January 2011 to August 2016, MuV infection was analyzed at Institute Adolfo Lutz. A total of 232 (77.33 %) throat wash samples showed positivity to mumps genome, and 68 (22.66 %) were negative when analyzed by rRT-PCR. Among 15 samples for molecular analysis, 10 serum samples from respective patients were also available for detecting anti-MuV IgM-Ab; and from these, four (40%) samples were seropositive. Vaccination status was available only for patients from Cedral and Araraquara. Phylogenetic analysis revealed the circulation of the following mumps virus genotypes in the investigated periods: 2011(M), 2012, and 2013 (K); 2014 (N); 2015 (G, K, and N); 2016 (G). Knowledge on MuV molecular epidemiology in São Paulo-Brazil could contribute to the surveillance and epidemiological program in Brazil, and globally as well.

Keywords. mumps virus, molecular epidemiology, public health.

RESUMO

No estado de São Paulo têm ocorrido surtos de caxumba desde 2011. O diagnóstico laboratorial tem sido realizado no Instituto Adolfo Lutz utilizando-se a técnica de identificação de material genético viral por meio de reação de cadeia de polimerase-em tempo real (rRT-PCR) e pela detecção de anticorpos IgM (Ac-IgM) específicos circulantes. Os recentes surtos de caxumba têm aumentado o interesse em investigar os genótipos dos vírus prevalentes para identificar os casos associadas à vacina. De janeiro de 2011 a agosto de 2016, 300 amostras de lavados da orofaringe coletadas de pacientes suspeitos de infecção foram analisadas. O material genético viral específico foi detectado em 232 (77,33 %) amostras e 68 (22,66 %) foram negativas. Das 10 amostras analisadas pelo teste sorológico, quatro (40 %) demonstraram positividade para Ac-IgM específicos anti-vírus da caxumba e seis foram negativas. Somente os municípios Cedral e Araraquara forneceram os dados referentes à vacinação. Análise filogenética mostrou a circulação dos seguintes genótipos do vírus da caxumba no período investigado: 2011 (M), 2012 e 2013 (K); 2014 (N); 2015 (GKN); 2016 (G). A vigilância virológica é mundialmente imprescindível, para identificar a diversidade e a distribuição dos diferentes genótipos, com vistas à composição de vacinas específicas.

Palavras-chave. vírus da caxumba, epidemiologia molecular, saúde pública.

Introduction - Mumps (or epidemic parotitis) is an acute infectious disease caused by mumps virus (MuV) characterized by parotid gland swelling. Although the main clinical manifestation of mumps are parotitis (90 % of cases), several other clinical complications as aseptic meningitis, transient deafness, encephalitis, orchitis, oofhoritis have been observed[1]. At the time when no specific vaccine product was available, the aseptic meningitis was the most common clinical evidence of mumps. The live attenuated mumps vaccine was first licensed in the USA in 1967; and it has been widely used as a component of the trivalent measles-mumps-rubella (MMR) vaccine ever since. In the state of São Paulo-Brazil, the MMR vaccine program was introduced in 1992 for immunizing children from 01 to 10 years old. The literature data show that the campaign for MuV vaccination has substantially contributed to the decrease of this disease incidence[2]. The lifelong protection for many years due to the natural infection, and in addition to the widespread vaccination appeared to be an approach to control the disease. Re-infection with MuV could occur worldwide by means of natural infection or after vaccination; and owing to these characteristics, this infectious disease is considered as a global Public Health issue. Increased transmissions and recent outbreaks[3-5] emphasize the relevance of the MuV surveillance activities for generating the baseline genetic data. This information aimed at mapping the genotype distribution in the different continents, to identify the vaccine-associated cases[1]. The review published by Li Jin et al[6] showed that only six of the 12 MuV genotypes have been circulating since 2010, including genotypes G (52 %), H (16 %), C (12 %), F (8 %), K (8 %) and D (4 %), based on the baseline genetic data provide by 25 countries. In São Paulo state the MuV outbreaks have been notified by the Epidemiological Surveillance Center of the State Department of Health, São Paulo-Brazil. Laboratory confirmation is based on the detection of MuV-specific IgM antibodies in acute–phase serum samples, and the MuV isolation is performed by cell culture methodology. Virus isolation is a time-consuming technique considering the needs for achieving a rapid diagnosis. The MuV-specific IgM antibody might be undetectable in the early samples collected at the time less than three days after the mumps symptoms onset, and in samples collected from previously vaccinated individuals[7,8]. Thus, Boddicker et al[9] developed and validated a mumps real-time RT-PCR (rRT-PCR) for improving the sensitivity of PCR assay to detect MuV in clinical samples from patients. This molecular methodology is considered as a valuable tool to detect the MuV RNA directly from clinical samples, as throat wash, collected from patients presenting MuV-specific clinical symptoms. MuV outbreaks have been notified by several countries including Brazil. The present study aimed at investigating the MuV genotypes involved in the mumps outbreaks occurred in the state of São Paulo, Brazil. The obtained results were compared with those described by authors from other countries, taking into account the limited data globally available.

Material and methods - From June 2011 to August 2016 the Respiratory Virus Laboratory of the Respiratory Disease Department-Virology Center - Institute Adolfo Lutz (IAL) received 300 throat wash samples collected from patients with suspected mumps. Virus identification was performed by means of the Centers for Disease Control and Prevention - polymerase chain reaction in real time (CDC-rRT-PCR) protocol, kindly provided by Dr. Paul Rota. The virus sequencing reaction was performed as described elsewere[9], by using 3130 Applied Biosystems. Nucleotide sequences were aligned using the multiple sequence alignment method implemented in CLUSTAL X[9]. Considering that the molecular assay provides rapid diagnosis, the physicians worldwide prefer to perform the patients follow-up diagnosis by using rRT-PCR. The laboratory diagnosis of mumps has been carried out at IAL only, and being a Public Health Institute, in addition to the rRT-PCR molecular technique, the health centers of some municipalities of the São Paulo state use to sent the blood samples for performing the serological testing for parotitis diagnosis. This assay detects the anti-MuV-specific IgM antibodies in acute-phase

Publicação contínua *on line*: número de páginas sem efeito para citação
On line continuous publishing: page number not for citation purposes

serum samples. Therefore, the received 10 serum samples and stored at -70 °C were analyzed by serological assay, using commercial kit Enzygnost ELISA (anti-parotitis virus/IgM - Siemens, Germany).

Results - Of 300 throat wash samples, 232 (77.33 %) and 68 (22.66 %) showed positive and negative results on rRT-PCR, respectively. Among 15 (100 %) patients included in the **Table**, the respective serum samples from 10 (66.70 %) were picked out and sent to IAL, and they were analyzed by serological testing. Of these 10 samples, four (40 %) were positive for anti-MuV-specific IgM antibodies and the respective throat-wash sample was also positive on MuV-rRT-PCR. And six (60 %) sera were anti-MuV-specific IgM negative, but MuV infection was confirmed by rRT-PCR technique. Of 300 specimens received at IAL from 2011 to 2016, 212 (79.69 %) were collected from January 2015 to August 2016. Nine genotypes were identified in this period, five (60 %), two (20 %) and two (20 %) corresponded to the G, K, and N genotypes, respectively. Vaccination data were available for patients from Cedral and Araraquara municipalities only (**Table**). Although the vaccination information were available for three (20 %) patients, all of them (100 %) showed positive rRT-PCR for MuV. The phylogenetic analysis revealed the circulation of the following mumps genotypes in the investigated period: 2011 (M), 2012 and 2013 (K); 2014 (N); 2015 (G, K, and N); 2016 (G). The common symptoms of parotitis and fever among the investigated patients were clinically confirmed.

Table. Origin of Brazilian mumps virus investigated in this study

Samples identification*	Location	Patients age (year/sex)	Date of onset (month/year)	Specimen	Vaccine status	IgM serology
MuVi/Assis-SP.BRA/NK.11[K]	Assis	15/F	May/12	Throat wash	NK	NK
MuVi/Guarulhos-SP.BRA/37.12/2[K]	Guarulhos	37/M	Sep/12	Throat wash	NK	NK
MuVi/Guarulhos-SP.BRA/37.12/2[K]	Guarulhos	23/M	Sep/12	Throat wash	NK	NK
MuVi/São Paulo -SP.BRA/37.13/2[K]	São Paulo	15/M	Sep/13	Throat wash	NK	NEG
MuVi/São Paulo -SP.BRA/37.13/2[K]	São Paulo	14/M	Sep/13	Throat wash	NK	POS
MuVi/São Paulo -SP.BRA/44.14[N]	São Paulo	14/M	Nov/14	Throat wash	NK	NEG
MuVi/Florianópolis -SC.BRA/11.15[G]	Florianópolis	20/M	Mar/15	Throat wash	NK	NK
MuVi/Cedral -SP.BRA/28.15[G]	Cedral	15/F	Jul/15	Throat wash	Yes	NEG
MuVi/Campinas -SP.BRA/39.15[G]	Campinas	20/M	Out/15	Throat wash	NK	NEG
MuVi/São Paulo -SP.BRA/31.15[N]	São Paulo	19/F	NK	Throat wash	NK	POS
MuVi/São Paulo -SP.BRA/32.15[N]	São Paulo	27/F	Aug/15	Throat wash	NK	POS
MuVi/Itatiba-SP.BRA/43.15[G]	Itatiba	10/F	Out/15	Throat wash	NK	POS
MuVi/Araraquara-SP.BRA/42.15[K]	Araraquara	19/F	Nov/15	Throat wash	Yes	NEG
MuVi/Araraquara-SP.BRA/41.15[K]	Araraquara	20/F	Nov/15	Throat wash	Yes	NK
MuVi/Campinas-SP.BRA/52.15[G]	Campinas	20/M	Jan/16	Throat wash	NK	NEG

NK, not know. *Assigned according to the standardized nomenclature proposed by WHO, 2012

Discussion - The present study reports the circulation of MuV genotype N, in the state of São Paulo during the period from 2014 to 2015, which has not been included in the recent review focusing on the baseline genetic data provided by 25 countries[6]. Genotype G has been predominating in Brazil, and this genotype has been circulating in the state of São Paulo in 2015 and in the early of 2016; and according to Li Jin et al[6] the genotype G has been the predominating MuV worldwide, until now. The increase of MuV outbreaks worldwide and the diversity of MuV genotypes arises a question on the cross-protection among the MuV vaccines, accordingly to their genotypes composition and the circulating mumps wild viruses; and investigations on this issue are warranted[10]. Limitations regarding the MuV-specific IgM antibody detection have already observed. Of the 10 serum samples received for performing the serologic testing, four (40 %) were positive for anti-MuV specific IgM antibody and also MuV-rRT-PCR was positive; and six (60 %) sera showed negative results in this serological assay. However, the MuV infection was confirmed by rRT-PCR technique, as shown in **Table.** These results highlight the advice of collecting the blood samples three days or more after the clinical symptoms onset, for improving the sensitivity and specificity of MuV-specific IgM antibody detection, as recommended in the previous studies[1]. Also, limitation regarding the vaccination data was evidenced in this study. Of three (20 %) vaccinated patient, the throat-wash samples collected from all of them showed positive results on MuV/rRT-PCR, and these data revealed MuV re-infection. There is a consensus on the need to follow the studies on genotype cross-neutralization, aiming at establishing whether the genetic variation could lead to the vaccine failure[10]. Of 300 specimens sent to IAL from 2011 – 2016, 212 (79.69 %) were collected from January 2015 to August 2016. In this period, of nine identified genotypes, five (60 %), two (20 %), two (20 %) corresponded to genotypes G, K, N, respectively. These data highlighted a similar distribution of MuV genotype G in the state of São Paulo, when compared with a recent review on MuV genotypes circulating worldwide, even at limited baseline genetic data considering the global context[1,6].

Conclusion - Phylogenetic analysis revealed the circulation of the following mumps genotypes in the state of São Paulo during the period from 2011 to 2016: 2011 (M), 2012 and 2013 (K); 2014 (N); 2015 (G, K, N); 2016 (G). Li Jin et al[6] review showed that only six of the 12 mumps genotypes have been circulating since 2010, including the genotypes G, H, C, F, K, D based on the analyzed mumps viruses by 25 countries. The present study showed that the genotype N has also been circulating in the state of São Paulo, Brazil, evidencing that how is important to participate in the MuV surveillance in order to improve the worldwide MuV baseline genetic data. In addition, taking into account the vaccine era, a global effort to follow mumps virus surveillance is warranted.

ACKNOWLEDGEMENTS

Our gratitude to Nilva Matias dos Reis, Ana Lúcía de Carvalho Avelino for technical support. We also thank the personnel of the Epidemiological Surveillance Center of the State Department of Health, São Paulo, Brazil

REFERENCES

1. WHO. Mumps virus nomenclature update: 2012. Wkly Epidemiol Rec. 2012;87 (22):217–24.

2. Hviid A, Rubin S, Mühlemann K. Mumps. Lancet. 2008;371:932-44. [DOI:10.1016/S0140-6736(08)60419-5].

3. Albertson JP, Clegg WJ, Reid HD, Arbise BS, Pryde J, Vaid A, et al. Mumps Outbreak at a University and Recommendation for a Third Dose of Measles-Mumps-Rubella Vaccine — Illinois, 2015–2016. MMWR Morb.Mortal. Wkly Rep. 2016;65(29):731–34. [DOI:10.15585/mmwr.mm6529a2].

4. Kutty PK, McLean HQ, Lawler J, Schulte C, Hudson JM, Blog D, et al. Risk factors for transmission of mumps in a highly vaccinated population in Orange County, NY, 2009-2010. Pediatr Infect Dis J. 2014;33(2):121-5. [DOI: 10.1097/INF.0000000000000020].

5. Park SH. Resurgence of mumps in Korea. Infect Chemother. 2015;47(1):1-11. [DOI:10.3947/ic.2015.47.1.1].

6. Jin L, Örvell C, Myers R, Rota PA, Nakayama T, Forcic D, et al. Genomic diversity of mumps virus and global distribution of the 12 genotypes. Rev Med Virol. 2015;25:85-101. [DOI:10.1002/rmv.1819].

7. Jin L, Brown DW, Litton PA, White JM. Genetic diversity of mumps virus in oral fluid specimens: application to mumps epidemiological study. J Infect Dis. 2004;189 (6):1001–8. [DOI:10.1086/382134].

8. Jin L, Vyse A, Brown DW. The role of RTPCR assay of oral fluid for diagnosis and surveillance of measles, mumps and rubella. Bull World Health Organ. 2002;80(1):76 –7.

9. Boddicker JD, Rota PA, Kreman T, Wangeman A, Lowe L, Hummel KB, et al. Real – time reverse transcription – PCR assay for detection of mumps virus RNA in clinical specimens. J Clin Microbiol. 2007;45(9): 2902–8. [DOI:10.1128/JCM.00614-07].

10. Echevarría JE, Castellanos A, Sanz JC, Martinez de Aragón MV, Peña Rey I, Mosquera M, et al. Mumps vírus genotyping: Basis and Known circulating genotypes. Open Vaccine J. 2010;3:37–41.