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Unnoticeable Mumps Infection in India: Does MMR Vaccine Protect against Circulating Mumps Virus Genotype C?

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***Abstract*—**MMR vaccine failure had been reported globally and here we report that it occurs now in India. Samples were collected from clinically suspected mumps cases were subjected for anti mumps antibodies, virus isolation, RT-PCR, sequencing and phylogenetic tree analysis. 56 samples collected from men and women belonging to various age groups. 30 had been vaccinated and the status of 26 patients was unknown. 28 out of 30 samples were found to be symptomatic and positive for Mumps IgM, indicating active mumps infection in 93.4% of the vaccinated population. A phylogenetic tree comparison of the clinical isolate is shown to be genotype C which is distinct from vaccine strain. Our study clearly sending warning signs that MMR vaccine is a failure and it needs to be revamped for the human use by increasing its efficacy and efficiency.

***Keywords*—**Genotype C, Mumps virus, MMR vaccine, Sero types.

I. Introduction

MUMPS is an acute, highly contagious, viral infection found throughout the world, characterized by parotitis of salivary glands, aseptic meningitis, transient deafness and encephalitis. Other clinical features include orchitis, oophoritis and respiratory symptoms. Mumps is a vaccine preventable childhood disease that tends to be mild; about 30% of infection is asymptomatic and more common in adults. Transmission occurs through inhalation of respiratory droplets or by direct person-to-person contact. Until recently it was believed that after MMR vaccination offered complete protection against Mumps but emerging data is disturbing and suggestive that MMR vaccine failed to protect us from primary infections or reinfections [1], [2].

The mumps virus (MuV) belongs to genus Rubulavirus, family Paramyxoviridae. It is an enveloped RNA virus with a non-segmented single-stranded negative-sense genome of 15,384 nucleotides (nt). The genome contains seven transcription units that encode open reading frames for the nucleocapsid (N), phospho (P), matrix (M), fusion (F), small hydrophobic (SH), hemagglutinin-neuraminidase (HN), and large (L) proteins. Since the SH gene is most genetically divergent, its sequence data have been mainly used for phylogenetic analyses of MuVs. However, it may not necessarily reflect the antigenic properties of MuVs [1], [3]. Although it is generally believed that MuV is serologically monotypic, distinct genetic lineages of wild-type MuVs have been described and reported to be co-circulating globally. Genotype assignment for MuV is based on sequence analysis of the entire 316 nucleotides of the small hydrophobic (SH) gene. To facilitate studies on the molecular epidemiology of MuV, a standard nomenclature was proposed in 2012 [1]. Much remains to be learnt about the global distribution of MuVs as genotype information has been reported from only 38/194 countries, of which 34 have reports since 2005. There have been no reports on the prevalence of mumps infection or its genotype of MuVs in India in unvaccinated and vaccinated populations, and a majority of cases go unnoticed [1], [4], [5]. Here we report a minor epidemic of acute mumps infection among MMR vaccinated individuals. Sequencing and phylogenetic analysis indicate that the circulating genotype in t he community is MuV genotype C which has least homology with vaccine strain. This report is a warning sign of vaccine failure but it could be only a tip of the iceberg. MMR vaccine needs to be stopped and revamped with quality control (QC) and quality assurance (QA) before administered to the general public.

II. Methods

A. Patient Recruitment and Sample Collection

WHO guidelines were adopted for selection of mumps cases. A proforma containing following information was obtained from each patient which included date of birth, gender, occupation, date of disease onset, signs and symptoms, date of diagnosis, MMR immunization status, and reinfections. An informed consent and human ethical

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clearance for sample collection was obtained. Samples from 56 patients with clinically diagnosed mumps were collected over a period of 11 months (July 2011 to May 2012) from different parts of Chennai, India. Blood (for antibody analysis) was collected from all patients, throat swab (for virus isolation and RT-PCR) were collected from 5 patients. The sample was collected on days 0 to 7 after onset (3 days on average) and stored at -86°C until use.

B. Measurement of Mumps Specific IgM, IgG and Rubella specific IgG:

An IgM and IgG EIA kit (Labor Diagnostika Nord GmbH & Co. KG, Germany) was used to determine mumps specific IgM and IgG levels in patient sera. Both assays were performed and interpreted according to the manufacturer's instructions. The IgM and IgG antibody titer was designated as an antibody index by calculating the ratio between the average optical density (OD) value of the sample and that of the cut-off, provided with the kit. An antibody index exceeding 1.1 was determined as positive. Rubella specific quantitative IgG EIA (Techno Genetics, Italy) was done to check MMR induced rubella specific IgG antibody in all 56 cases, in order to confirm if they had been vaccinated. Rubella IgG antibody titers were calculated from the sample absorbance and the reference standard curve generated from the reference sera provided with the kit. IgG antibody titers of >15 IU/mL were defined as positive.

C. Virus Isolation and Hemadsorption Assay

MuV was isolated from the throat swab soaked in viral transport medium, were centrifuged at 1,500 rpm for 10 min. After sterilization through a 0.2µm syringe filter, the supernatant was used for virus infection onto Vero cells maintained in Minimum Essentials Medium with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin and 100µg/ml streptomycin. The cells were cultured at 37°C at 5% CO2, observed for 10 days and harvested when the cytopathic effects (CPE) became prominent. The culture harvests were aliquoted and stored at −86° C until processed further. Two blind passages were performed on all CPE-negative tissue cultures. For the Hemadsorption assay, Vero cells were grown in microplates. Two days later, the cellular medium was removed from young monolayer, infected with clinical isolate of mumps virus, incubated at 37°C, at 5% CO2 for 90 minutes. The cells were washed with PBS and a suspension of 4% guinea pig red cells was added to the cells and incubated for 1 h at 4°C. The cells were extensively washed with PBS and observed under an inverted microscope.

D. PCR Amplification and Automated DNA Sequencing

The total RNA was extracted as per Jelena Ivancic et.al. [6]. Briefly, viral genomic RNA was extracted from 140µl of throat swab samples, culture supernatant, and MMR vaccine by using a QIAamp viral RNA minikit according to the manufacturer's protocol. The extracted total RNA was quantified by using nanodrop 2000, to amplify the SH & HN gene region followed by reverse transcription of viral RNA into cDNA by using an Omni script RT kit according to the manufacturer's instructions. Full length or partial sequences of the SH, HN genes were amplified from cDNA by using specific primers. To obtain the complete SH gene, a primer of 506 bp was designed from L-Zagreb vaccine strain (AY685920)(SHLZ-F:5'-CAAGTAGTGTCGATGATCTCAT CAGG-3'/SHLZ-R:5'GTGAAGAGTTTCGAGGGCTCATC-3'). A novel HN gene primer of 567 bp length was designed found in the conserved region of all genotypes, which was listed in the WHO recent update (HNCD-F: 5'AGCTGCTCRATTGCAACAGTCCCT-3'/ HNCD-R: 5'-AGTTCATACGGCCACCAGCT-3') [1]. Each PCR mixture included 1U of Taq DNA polymerase, 5xPCR buffer (50mM KCl, 1.5mM MgCl2, 10mM Tris-HCl, pH 9.0), 20 pmol of each primer and 10mM dNTP mix in a total volume of 10µl. All the PCR chemicals were purchased from Fermentas, USA. PCR was performed by 35 cycles of denaturation at 94 ° C for 45 s, annealing at 66 ° C for 45 s, extension at 72 ° C for 45 min, and a final extension step of 3 min at 72 ° C. The cycle sequencing reaction was performed using BigDye terminator V3.1 cycle sequencing Kit containing AmpliTac polymerase (from Applied Biosystems, P/N: 4337457). The sequencing reaction-mix was prepared by adding 1 µl of BigDye v3.1, 2µl of 5x sequencing buffer and 1µl of 50% DMSO. To 4µl of sequencing reaction-mix, 4 pico moles of primer (2µl) and sufficient amount of plasmid was added. The constituted reaction was denatured at 95°C for 5 minutes. Cycling began with denaturation at 90°C for 30s, annealing at 52°C for 30s and extension for 4 minutes at 60°C and repetition for a total 30 cycles in a MWG thermocycler. The reaction was then purified on sepheadex plate (Edge Biosystems) by centrifugation to remove unbound labelled and unlabelled nucleotides and salts. The purified reaction was loaded on to the 96 capillary ABI 3700 DNA analyzer and electrophoresis was carried out for 4 hours.

E. Phylogenetic Analysis

The SH and HN genes sequences of virus were compared with WHO mumps update 2012 [1] and previously published mumps virus sequences by MEGA5 program [7] that uses the neighbour-joining method. The resulting dendrograms were used to verify previously proposed genotype assignments and identify areas for clarification. The parameter employed was Kimura 2-parameter model and the robustness of the internal branches was determined by 500 bootstrap replications. The phylogenetic tree is displayed.

III. Results

A. Results of Clinical Specimens

Of the 56 samples collected from various age groups, a majority of 30 were from paediatric patients. Gender-wise, males predominated with 55% and the rest were females. The MMR vaccination details have been tabulated [Table I]; 30 had been vaccinated (MMR group) and the status of 26 patients was unknown (MMR-X group). Results showed that 48 (86%) out of 56 were positve for mumps IgM and 52 (93%) out of 56 were negative for Mumps IgG. These facts convincingly suggest that MMR vaccine failed to offer

protection in vaccinated individuals against mumps infection. 47 (84%) samples tested positive for rubella specific IgG. Testing for measles IgG was not performed as a separate measles vaccine is given at 9 months of age, prior to the MMR vaccine (15 months and 5 years) and thus would lead to inaccurate results [8].

B. Mumps in MMR Vaccinated Cases

Among the 56 samples, a total of 30 were MMR vaccinated cases, with 23 samples from the paediatric age group. 28 out of 30 samples were found to be with acute mumps infection and positive for Mumps IgM, indicating active mumps infection in 93.4% of the vaccinated population. These results demonstrate the failure of MMR vaccine to provide immunity against mumps infection.

C. Isolation and Genotypic Analysis of the MuVi/Chennai.IND [C] Strain

Typical CPE was observed in Vero cells, which showed rounding up and increased light reflection, detachment from the culture dish, cell lysis and finally death, when incubated with throat swabs from 5 cases. Isolated MuVs were confirmed by haemadsorbtion test. Haemagglutinins incorporated on the surface of the Vero cell cause RBCs to adsorb onto the cells when incubated with an RBC suspension [Fig. 1]. The cDNA was amplified by RT-PCR from throat swab and the culture supernatant for direct sequencing. Notably, the virus was demonstrated to be mumps virus genotype C and was named as MuVi/Chennai.IND [C] [Fig. 2]. The present study sequences were matched with recent WHO reference strains [Table II]. Analysis of the 316 bp gene revealed 100% identity between two isolated sequences found in the present study (GenBank accession number: JX392385, JX392386). The present study genotype C sequences from India were most similar (98%) to WHO reference strain genotype C (GenBank accession number: EU370206, JQ945268) [1] and less closely related (87%) to the C strains identified in UK, but had originated in India (GenBank accession number: AF142765) [9]. For a more precise phylogenetic position, additional analyses were performed based HN gene sequence, of the isolate in the present study (GenBank accession number: JX456273, JX456274) by comparison of sequences available in the WHO update 2012 [1] [Fig. 3]. A phylogenetic tree comparison of the SH gene and HN gene sequences of MuV isolates around the world is shown in Fig. 2 and Fig. 3 using WHO reference strains.

D. Amino Acid Sequences of SH Gene

The consensus sequence having 57 amino acids of genotype C mumps virus from reference strains MuVi/Zagreb.HRV/39.98 [C] (9218/Zg98), MuVi/Stockholm.SWE/46.84 [C] (V34) and MuVi strain Goa1/India98 [C] were compared with that of present study isolates MuVi/Chennai.IND [C]. It was found that these two genes were identical, except for valine and glycine in the 38th position respectively. The variations in sequences between the reference strains and study isolates have been highlighted in Table III.

IV. Discussion

Mumps virus infections are often self limiting however when it take a severe course it could cause a devestating consequences. MMR vaccine was believed to be protecting us from mumps by the scenario is drastically changing and alarming now. We collected sera from 56 acute mumps cases who were previously MMR vaccinated (confirmed with rubella IgG). 93% of these patients had no mumps IgG and 86% showed mumps IgM. Phylogetic analysis revealed that the clinical isolates were indeed genotype-C which is distinct from L-Zagreb strain found in MMR vccine. This distinction could have contributed to the non-protection of mumps by MMR.A careful overview of litrature suggest that this is probably the first report of recent time which questions the efficacy of MMR vaccine.

Within a decade of the implementation of the mumps vaccine in 1967 in the United States, by 2001, the disease was nearly eliminated, with less than 0.1 case per 100,000. Similar success in the control of mumps has been achieved in other countries; however, over the past 6 years, mumps has made a global resurgence, including vaccinated populations, even in the US which recently experienced its largest outbreak since 1987 [10], [11], but in India there is no such report regarding the statistics of mumps infection and its outbreak is yet to be noticed.

Mumps, though historically a disease of childhood, present outbreaks of mumps predominantly involves young adults, nearly all of whom had been vaccinated, most with the two-dose schedule. While these data are suggestive of waning immunity, it has also been postulated that antigenic differences between the vaccine and strains causing outbreak may result in a deficient immune response conferred by the vaccine [12], [10], [11]. Our study also shows the same kind of results where near 50% patients are adults, among which 71% were positive, though vaccinated. There have been very few reports in India that describe the seroprevalance of MMR vaccine and mumps mediated meningitis, which do not account to expose the current scenario of mumps in India [13], [14], [15]. Our study shows the state of mumps infection among different age groups, male and female ratio, and in MMR vaccinated, unvaccinated populations and indicated active mumps infection in 93.4% of the vaccinated population. Mumps is a potentially eradicatable disease through the use of MMR vaccine, and the World Health Organization, the Advisory Committee on Immunization Practices as well as the Indian Academy of Pediatrics, have recommended including two doses for immunization. But there is a need of data to document its impact in developing countries including India, where mumps is still a major health problem due to absence of effective vaccination programs [14], [16], [8]. An important fact that needs mention is that currently the MMR vaccine is not routinely administered to children in government healthcare centres [13], [14], [17]. The epidemiology and magnitude of mumps in India is still not fully appreciated and it is suggested that outbreak occurs at intervals of 5 to 10 years [15] and incidence of mumps infection occurs during January to March of each year [13]. Previous studies have indicated that children are commonly affected with

preponderance in males in addition to reports of meningo encephalitis and orchitis in vaccinated patients [13], [14], [15], [18]. Our study is most recent and shows the current status of mumps infections in MMR vaccinated cases in India, especially in Chennai. The efficacy of MMR vaccine in preventing mumps is equivocal; our study shows questioning of protection against mumps by MMR vaccination. It needs mention that the rubella component of MMR vaccine is efficient, evidenced by 84% of samples were positive for rubella IgG. A study conducted by Bakker et al., from Canada showed an incidence of mumps among 15% among vaccine recipients. In the affected males, attack rate of orchitis was 21% suggesting the causal role of inadequately attenuated mumps vaccine [19]. In comparison, our study showed an incidence of mumps in 93.4 % of vaccine recipients and 3 patients showed symptoms of testicular pain following mumps infection. It needs to be reiterated that the literature on mumps virus mediated protection rates and emergence of infertility among the Indian population is scanty and thus evaluation of mumps in the Indian subcontinent is mandatory and need of the hour. Outbreaks of mumps infection have been witnessed globally and a recent study in China based on developing a novel vaccine, conferring immunity against all genotypes reflects the necessity for its effective prevention [20].

MuV is serologically monotypic; however, distinct genetic lineages of wild-type MuVs have been described and reported to be co-circulating globally. The situation in India is yet unknown. Our study was initially aimed to study mumps infection in MMR vaccinated population and hence only serological analyses were done. A disturbing fact was that 93.4% positivity in those cases led us to explore the genetic basis behind it, and thus only two samples could be obtained from acute cases. With this notion phylogenetic analyses were done and it was found that isolates belonged to genotype C and was named MuVi/Chennai.IND [C] as per WHO protocol [1]. The SH gene sequences (316 nt) obtained from these isolates form a single cluster, which consists of two different sequences, and their genetic divergences are 2% compared to the WHO reference strains most closely related to genotype C (EU370206, JQ945268) and 13% compared to the strain (AF142765) isolated by Li Jin et.al., in Goa, India in 1999 [9]. Once thought to be completely preventable, outbreaks with some serious complications suggest otherwise. The MMR vaccine is not efficient in preventing mumps as rubella infection. Our study sheds light on the current scenario of mumps infection in India, especially in vaccinated individuals. It is also the first report indicating its genotype circulating in Chennai, its variations and phylogenetic analysis. Mumps is one among the infections which warrants attention in present times. Thus, adequate measures need to be undertaken for effective prevention and complete eradication of mumps infection.

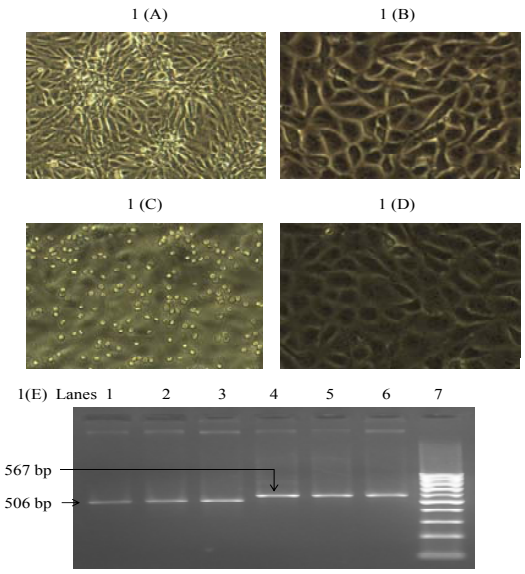

Fig. 1 Result of virus isolation and RT-PCR: 1A - CPE of mumps virus rounding with vacuolation, 1B – control. 1C – Hemadsorption test (RBC's adsorb onto the virus infected cells) 1D – control. 1E - The polymerase chain reaction products of the SH gene (506bp) and HN gene (567bp), lane 1, 2, 3 for SH gene, Lane – 4, 5, 6 for HN gene, lane 7 – 100bp DNA marker. Lane – 1, 2, 4, 5 were clinical isolates, lane - 3, 6 were L-Zagreb vaccine strain (control)

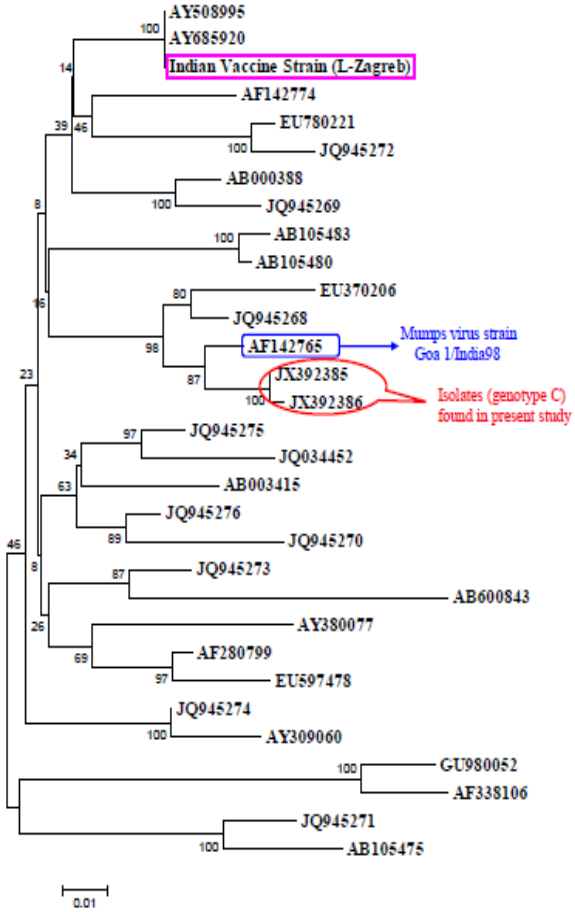

Fig. 2 Phylogenetic trees of MuV genotypes based on the 316 nucleotides of the entire SH gene: Neighbour-joining method of MEGA5 program were used. The parameter employed was Kimura 2-parameter model and the robustness of the internal branches was determined by 500 bootstrap replications. The horizontal length of the bar denotes percentage difference between sequences (see scale at bottom) and the bootstrap numbers (%) are given at each node

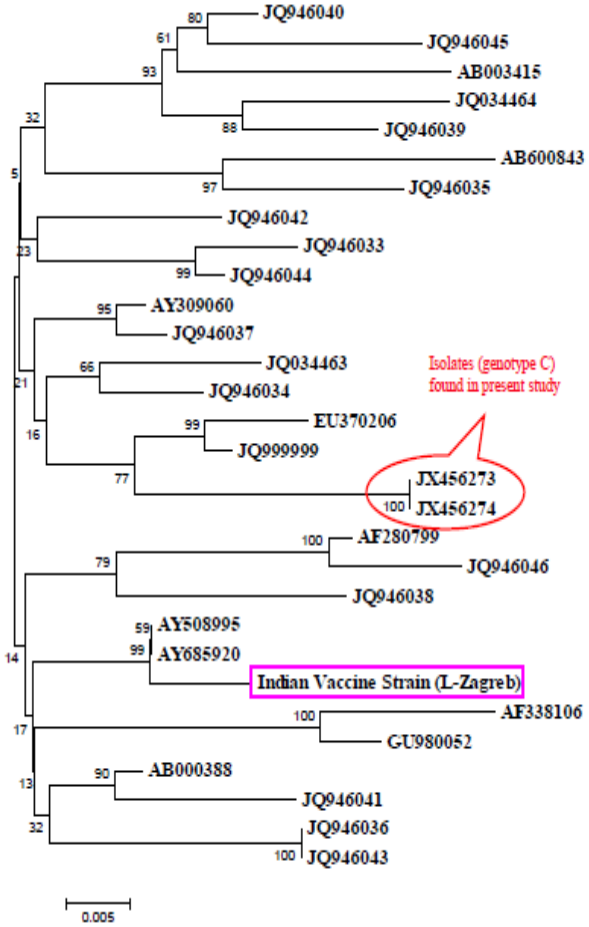

Fig. 3 Phylogenetic trees of MuV genotypes based on the 567 nucleotides of the conserved region of HN gene: Neighbour-joining method of MEGA5 program were used. The parameter employed was Kimura 2-parameter model and the robustness of the internal branches was determined by 500 bootstrap replications. The horizontal length of the bar denotes percentage difference between sequences (see scale at bottom) and the bootstrap numbers (%) are given at each node

TABLE I
RESULT OF CLINICAL SPECIMENS AND MMR VACCINATION FAILURE AGAINST MUMPS INFECTION

Results of clinical specimens

Patient age group	Total no of case	Gender		MMR vaccination details				Tested for					
								Mumps IgM		Mumps IgG		Rubella IgG	
		Male	Female	1st dose alone	2nd dose alone	1st & 2nd dose	unknown	No. of positive	No. of negative	No. of positive	No. of negative	No. of positive	No. of negative
< 12 years	30	19	11	11	2	10	7	30	Nil	Nil	30	21	9
13-18 years	9	3	6	4	Nil	Nil	5	7	2	1	8	9	Nil
19-25 years	10	4	6	1	Nil	2	7	6	4	2	8	10	Nil
25- 45 years	7	5	2	Nil	Nil	Nil	7	5	2	1	6	7	Nil
Total	56	31	25	16	2	12	26	48	8	4	52	47	9
Percentage (%)		55	45	29	4	21	46	86	14	7	93	84	16

Result of MMR vaccination failure

Patient age group	Total no of case	Total no of MMR vaccinated case	Total no of mumps parotitis+IgM positive	Percentage (%)
< 12 years	30	23	23	100
13-18 years	9	4	3	75
19-25 years	10	3	2	67
25- 45 years	7	Nil	Nil	Nil
Total	56	30	28	93.4

TABLE II
PRESENT STUDY ISOLATES ALONG WITH WHO MUMPS GENOTYPE REFERENCE STRAINS

Genotype	WHO Reference strain	GenBank accession number	
		SH	HN
A	MuVi/Boston.USA/0.45 (Enders/USA45)	GU980052	GU980052*
	MuVi/Pennsylvannia.USA/13.63[A] (VAC) (Jeryl Lynn 5)	AF338106	AF338106*
B	MuVi/Urabe.JPN/0.67[B] (Urabe AM-9)	AB000388	AB000388*
	MuVi/Himeji.JPN/24.00[B] (Himeji89)	JQ945269	JQ946041*
C	MuVi/Zagreb.HRV/39.98[C] (9218/Zg98)	EU370206	EU370206*
	MuVi/Stockholm.SWE/46.84[C] (V34)	JQ945268	JQ999999*
D	MuVi/Ge9.DEU/0.77[D] (Ge9)	JQ945275	JQ946039*
	MuVi/Nottingham.GBR/19.04[D]	JQ034452	JQ034464*
F	MuVi/Shandong.CHN/4.05[F] (SD9)	EU780221	JQ034463*
	MuVi/Zhejiang.CHN/11.06/1[F] (ZJ06-1)	JQ945272	JQ946034*
G	MuVi/Gloucester.GBR/32.96[G] (Glouc1/UK96)	AF280799	AF280799*
	MuVi/Sheffield.GBR/1.05[G]	EU597478	JQ946046*
H	MuVi/Bedford.GBR/0.89[H] (Be1)	JQ945273	JQ946035*
	MuVi/Ulaanbaatar.MNG/22.09[H] (MNG09-024)	AB600843	AB600843*
I	MuVi/Akita.JPN/42.93[I] (Odate1)	JQ945274	JQ946037*
	MuVi/Dg1062.KOR/46.98[I] (Dg1062/Korea/98)	AY309060	AY309060*
J	MuVi/Leeds.GBR/9.04[J]	JQ945271	JQ946033*
	MuVi/Sapporo.JPN/12.00[J] (Sapporo K-4)	AB105475	JQ946044*
K	MuVi/RW154.USA/0.70s[K] (RW154)	JQ945276	JQ946040*
	MuVi/Stockholm.SWE/26.83[K] (V28)	JQ945270	JQ946045*
L	MuVi/Fukuoka.JPN/41.00[L] (Fukuoka49)	AB105483	JQ946036*
	MuVi/Tokyo.JPN/6.01[L] (TokyoS-III-10)	AB105480	JQ946043*
N	MuVi/Vector.RUS/0.53[N] (VAC) (L3/Russia/Vector)	AY508995	AY508995*
	MuVi/L-Zagreb.HRV/0.71[N] (VAC) (L-Zagreb)	AY685920	AY685920*
	MuVi/Chennai.IND/49.11 [C] – Present study isolate	JX392385	JX456273*

C	**MuVi/Chennai.IND/18.12 [C] – Present study isolate**	JX392386	JX456274*
C	MuVi strain Goa1/India98[C]	AF142765	-

As per WHO mumps virus sequence divided into 12 genotypes designated A-N based on SH gene (316 nt) and HN gene (1749 nt) genotyping. In present study SH gene (316 nt) and HN (567 nt) based genotyping were done. * - A novel primer was designed from conserved region of all reference HN gene strains to detect all reference genotype of mumps virus, as well as from clinical samples.

TABLE III
ALIGNMENT OF DEDUCED AMINO ACID SEQUENCE OF SH GENE OF INDIAN STRAINS AND GENOTYPE C MUMPS VIRUSES

Reference strain	**Genotype**	**Genbank accession number**	**Consensus amino acid sequence**					
			1.....10	11.....20	21.....30	31.....40	41.....50	51.....57
MuVi/Zagreb.HRV/39.98[C] (9218/Zg98)	C	EU370206	MPAIQPPLYL	TFLLLILLYL	IITLYVWVVS	TITYKTAVRH	AALHQRSPSR	WSFDHSL
MuVi/Stockholm.SWE/46.84[C] (V34)	C	JQ945268	MPAIQPPLYL	TFLLLILLYL	IITLYVWVVS	TITYKTAVRH	AALYQRSLFR	WSFDHSL
MuVi/Chennai.IND/49.11 [C]	C	JX392385	MPAIQPLLYL	TFLLLILLYL	IITLYIWVIS	TITYKTAVRH	AVLYQRSLLR	WSFDHSL
MuVi/Chennai.IND/18.12 [C]	C	JX392386	MPAIQPLLYL	TFLLLILLYL	IITLYIWVIS	TITYKTAGRH	AVLYQRSLLR	WSFDHSL
MuVi strain Goa1/India98[C]	C	AF142765	MPAIQPLLYL	TFLLLILLYL	IITLYIWVIS	TITYKTAVRH	AVLYQRSLFR	WSFDHSL
Indian Vaccine strain (L-Zagreb)	N	JX894237	MPAIQPHLYL	TFLLLILLYL	IITLYVWIIL	TITYKTAVRH	AALHQRSFFR	WSFDHSL

The sequences representing each genotype C strains having 57 amino acids are listed in this table. In order to highlight the specific differences in present study isolates (JX392385, JX392386) sequences, a genotype C sequence (EU370206, JQ945268) was adopted as a consensus amino acid sequence. The numbers above the consensus amino acid sequence show the amino acid residue numbers. Colour less alignment exhibit amino acids identical to those to the consensus sequence.

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