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*Article*

# Viral Detection of Suspected Sporadic Mumps in Gwangju, South Korea in 2021

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**Abstract:** Mumps is the second-most reported infectious disease in South Korea; however, due to the low pathogen confirmation rate in laboratory diagnoses, we proposed a method for reevaluating the high incidence rate via the laboratory verification of other viral diseases. In 2021, 63 cases of pharyngeal or cheek mucosal swabs of suspected mumps cases in Gwangju, South Korea, were assessed for causative pathogens using massive simultaneous pathogen testing (TaqMan™ Array Cards). More than one respiratory virus was detected in 60 cases (95.2%), 44 (73.3%) of which were co-detected. Human rhinovirus was detected in 47 cases, followed by human herpesvirus (HHV)6 in 30; HHV4 (17), human bocavirus (17), HHV5 (10), and human parainfluenza virus 3 (6) were also detected. Our findings suggest the need for further investigations on the pathogenesis of diseases mimicking mumps, which are considered to aid with appropriate public health responses, treatment, and the prevention of infectious disease outbreaks.

**Keywords**: mumps; parotitis; parotid gland extenstion; salivary gland; respiratory virus; human herpesvirus 4 (HHV4); human herpesvirus 6 (HHV6)

## 1. Introduction

Chickenpox and mumps are the most common infectious diseases in South Korea. According to the Korea Disease Control and Prevention Agency's infectious disease portal (https://www.kdca.go.kr/npt/), the number of reported cases with mumps averaged 14,352 over the past five years, the second-most after 62,356 cases of chickenpox.

Mumps is caused by a mumps virus, a single stranded negative-sense RNA virus belonging to the genus *Orthorubulavirus* of the family of *Paramyxoviridae*. [1]. Infection of humans with mumps virus results in acute illness which is usually characterized by a temporary unilateral or bilateral parotitis. Mumps has a relatively long incubation period and is highly contagious, making it difficult to eradicate [2-4]. In South Korea, the mumps vaccine has been used since the early 1980s and has reduced the burden of the disease [5]. In 1997, the Ministry of Health and Welfare began recommending two doses of MMR vaccines to be administered: one dose each at of 12–15 months and 4–6 years of age. The national vaccination coverage was very high, reaching over 95% since 1996, but the incidence of mumps has been increased steadily since 2002 [6].

Since the outbreak of the COVID-19 pandemic in December 2019, mitigation measures, such as compliance with personal hygiene regulations, social distancing, and environmental cleaning to prevent the spread of COVID-19, have affected the overall reduction of infectious diseases [7,8]. However, despite the decreasing trend, the number of

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cases with mumps reported to the Korea Disease Control and Prevention Agency in 2021 were 9,708, the second-most after 20,923 cases of chickenpox. But, it was worth noting that the reported cases were cases suspected of having clinical symptoms without laboratory diagnosis.

Among the cases reported in Gwangju, Korea from January to October 2021, nasopharyngeal or mucosal mucosal specimens from 115 cases were collected for laboratory diagnosis. Real-time Reverse Transcription Polymerase Chain Reaction (RT-PCR) targeted to the small hydrophobic gene of mumps virus was performed for the laboratory diagnosis of nasopharyngeal or cheek mucosal samples from 115 suspected patients, but no mumps virus gene was detected in any of the samples. Cases with a negative laboratory result for mumps were usually classified as suspected cases of mumps, and a differential diagnosis with other infectious agents was not routine.

In a previous study, cases identifying potential parotitis-causing pathogens were identified via the differential diagnosis of various respiratory viruses, but not the mumps virus. Human herpesvirus (HHV)4, HHV6, human parainfluenza virus 3 (HPIV3), adenovirus, and influenza virus have also been identified as parotitis-causing pathogens. Considering these points, verifying whether the mumps incidence is high is necessary via additional laboratory differentiation tests.

Therefore, this study aimed to identify candidates for parotitis-causing pathogens other than the mumps virus in samples of suspected mumps in Gwangju in 2021, and to determine the necessity of verifying whether the mumps incidence in South Korea is high.

## 2. Materials and Methods

### *2.1. Sample Collections*

This study assessed throat or cheek mucosal swabs obtained from patients with suspected mumps in Gwangju from January to October 2021. The samples were selected from residues frozen at -70°C after being confirmed negative for mumps via real-time RT-PCR. Sixty-three samples were randomly selected after being classified according to the age group and seasonality (Table 1).

**Table 1.** Characteristics including age, season, sex, and symptoms of suspected sporadic mumps patients.

| Month | | | Total | JAN | FEB | MAR | APR | MAY | JUN | JUL | AUG | SEP | OCT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | No. | 8 | 4 | 4 | 6 | 7 | 9 | 8 | 5 | 8 | 4 |
| Age group | | 0<Y<=5 | 22 | 3 | 2 | 2 | 2 | 2 | 1 | 1 | 3 | 2 | 4 |
| | | 5<Y<=12 | 25 | 4 | 1 | 2 | 2 | 3 | 4 | 4 | 2 | 3 | - |
| | | 12<Y<=18 | 7 | - | - | - | 2 | - | - | 2 | - | 3 | - |
| | | 18<Y<=29 | 2 | - | 1 | - | - | - | 1 | - | - | - | - |
| | | 29<Y<=49 | 6 | 1 | - | - | - | 1 | 3 | 1 | - | - | - |
| | | 49<Y<=64 | 0 | - | - | - | - | - | - | - | - | - | - |
| | | 64<Y | 1 | - | - | - | - | 1 | - | - | - | - | - |
| Sex | | Male | 32 | 7 | 2 | 3 | 4 | 1 | 5 | 2 | 3 | 5 | - |
| | | Female | 31 | 1 | 2 | 1 | 2 | 6 | 4 | 6 | 2 | 3 | 4 |
| Symptoms | Parotitis | Bilateralness | 6 | 3 | 1 | - | 1 | - | - | - | - | 1 | - |
| | | Right | 11 | - | - | 1 | 2 | 2 | 2 | 2 | - | 2 | - |
| | | Left | 5 | - | - | - | 1 | 1 | 3 | - | - | - | - |
| | | Unknown | 41 | 5 | 3 | 3 | 2 | 4 | 4 | 6 | 5 | 5 | 4 |
| | Fever | < 38°C | 13 | 1 | - | 2 | 3 | 3 | - | 1 | 1 | 1 | 1 |
| | | >= 38°C | 7 | - | - | - | - | 1 | 1 | 1 | 2 | 2 | - |
| | | Unknown | 43 | 7 | 4 | 2 | 3 | 3 | 8 | 6 | 2 | 5 | 3 |
| | Pharyngitis | | 3 | - | - | - | 1 | - | - | 1 | - | 1 | - |
| | Cough, Sputum | | 3 | - | - | - | 1 | 2 | - | - | - | - | - |
| | Etc | | 11 | 3 | - | - | 3 | 2 | 1 | - | - | 2 | - |
| | Unknown | | 46 | 5 | 4 | 4 | 1 | 3 | 8 | 7 | 5 | 5 | 4 |

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*2.2. Viral Nucleic Acid Extractions*

Following the manufacturer's instructions, nucleic acids were extracted from the samples using a QIAamp RNA kit (Qiagen, Valencia, CA, USA). We used 140 μL samples, and 60 μL final nucleic acid elutions.

*2.3. Massive Simultaneous Pathogen Testing RT-PCR With TaqMan™ Array Cards*

Forty-one respiratory tract viruses, bacteria and fungi can be searched using the TrueMark™ Respiratory Panel 2.0, TaqMan™ Array Card (Applied Biosystems, Life Technologies, CA, USA). This was used to search for parotitis-causing pathogens.

First, pre-amplification was performed using TrueMark™ Respiratory Panel 2.0, Pre-Amp primers. Additionally, 1:20 dilutions of pre-amplified products were inoculated into an array card according to the manufacturer's instructions, and real-time RT-PCR was performed using the QuantStudio 12 K Flex System (Applied Biosystems, Life Technologies, CA, USA). The assay includes control assays for Human 18S ribosome RNA gene to confirm the quality of the swabs and to verify assay performance.

## 3. Results

*3.1. Etiology of Viral Pathogen for Parotitis*

Six respiratory viruses were identified in the 63 samples: human rhinovirus (HRV), HHV6, human boca virus (HBoV), HHV4, HHV5 and HPIV3 in 47, 30, 17, 17, 10 and 6 samples, respectively.

Only one respiratory virus was detected in 16 samples (25.4%), including HRV in 10 (15.9%), HHV6 in three (4.8%), and HHV5, HPIV3, and HBoV in one sample each. However, HHV4 was not identified as a single marker.

Co-detected viruses were detected in 44 cases (69.8%), while dual, triple, and quadruple detections were observed in 24 (38.1%), 17 (27.0%), and three (4.8%) cases, respectively. HRV was the most common virus associated with co-detection. Respiratory viruses detected with HRV included nine cases of HHV6 (14.3%) and six of HHV4 (9.5%), and four of HHV6 and HBoV (6.3%). No respiratory viruses were detected in the three samples (Table 2).

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**Table 2.** Types of virus detected in patients with suspected sporadic mumps. HRV, human Rhinovirus; HBoV, human Bocavirus; HPIV3, human parainfluenzavirus 3; HHV4, human herpesvirus 4 (Epstein-Barr virus); HHV5, human herpesvirus (Cytomegalovirus); HHV6, human herpesvirus 6.

| | HRV | HHV6 | HHV5 | HHV4 | HPIV3 | HBoV | No. of cases (%) |
|---|---|---|---|---|---|---|---|
| **Single** | + | - | - | - | - | - | 10 (15.9) |
| | - | + | - | - | - | - | 3 (4.8) |
| | - | - | + | - | - | - | 1 (1.6) |
| | - | - | - | - | + | - | 1 (1.6) |
| | - | - | - | - | - | + | 1 (1.6) |
| | - | - | - | + | - | - | 0 (0) |
| **Co-detection (Dual)** | + | + | - | - | - | - | 9 (14.3) |
| | + | - | - | + | - | - | 6 (9.5) |
| | + | - | - | - | - | + | 2 (3.2) |
| | - | + | - | + | - | - | 2 (3.2) |
| | - | + | - | - | - | + | 2 (3.2) |
| | + | - | + | - | - | - | 1 (1.6) |
| | + | - | - | - | + | - | 1 (1.6) |
| | - | + | - | - | + | - | 1 (1.6) |
| **Co-detection (Triple)** | + | + | - | - | - | + | 4 (6.3) |
| | + | + | + | - | - | - | 3 (4.8) |
| | + | + | - | + | - | - | 2 (3.2) |
| | + | - | + | - | - | + | 2 (3.2) |
| | + | - | - | + | - | + | 1 (1.6) |
| | + | + | - | - | + | - | 1 (1.6) |
| | + | - | + | - | + | - | 1 (1.6) |
| | + | - | - | + | + | - | 1 (1.6) |
| | - | + | - | + | - | + | 1 (1.6) |
| | - | - | + | + | - | + | 1 (1.6) |
| **Co-detection (Quadruple)** | + | + | - | + | - | + | 2 (3.2) |
| | + | - | + | + | - | + | 1 (1.6) |
| **No virus** | - | - | - | - | - | - | 3 (4.8) |

*3.2. Age-specific Prevalence Trends*

In the 0<y≤5 age group, HRV exhibited the highest detection rates in 17 (77.3%) cases, followed by HHV6 in 14 (63.6%), HHV5 in six (27.3%), HBoV and HPIV3 in five (22.7%), and HHV4 in two (9.1%). The HRV detection cases were also the highest at 16 (64.0%) in the 5<y≤12 age group, followed by HHV6 in 11 (44.0%), HBoV in 10 (40.0%), HHV4 in six (24.0%), HHV5 in three (12.0%), and HPIV3 in one (4.0%). Even in the 12<y≤18 age group, HRV was detected in six (85.7%) cases and HHV6 in four (57.1%), followed by HBoV in two (28.6%), HHV4 in two (28.6%) and HHV5 in one (14.3%). In the 29<y≤49 age group, five (83.3%), four (66.7%), and one (16.7%) case revealed HRV, HHV4, and HHV6, respectively (Fig 1).

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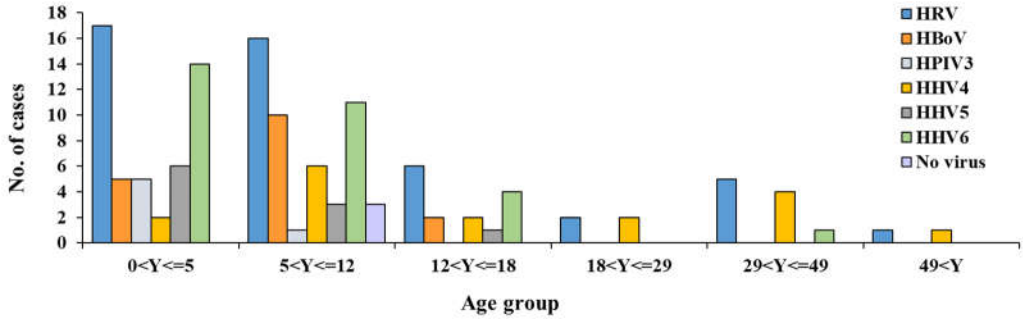


**Figure 1. Age-distribution of viruses identified in patients with suspected sporadic mumps.** Detected viruses were counted individually and co-detection was not considered. HRV, human Rhinovirus; HBoV, human bocavirus; HPIV3, human parainfluenza virus 3; HHV4, human herpesvirus 4 (Epstein-Barr virus); HHV5, human herpesvirus (cytomegalovirus); HHV6, human herpesvirus 6.

In the distribution of virus detection by age group, dual detection was the highest at 45.5% in the 0<y≤5 age group, followed by triple (31.8%) and quadruple (4.5%) detections. The proportion of single-detected viruses, including HRV, HPIV3, and HHV6, was 18.2%. In the 5<y≤12 age group, triple detections were the highest at 36.0%, followed by single (32.0%), dual (16.0%), and quadruple (4.0%) detections. In the 12<y≤18 age group, dual detections (42.9%) were more frequent than single detections (28.6%), and the triple and quadruple detections accounted for 14.3% each. In the >18 years age group, dual detection was identified in most cases (Fig 2).

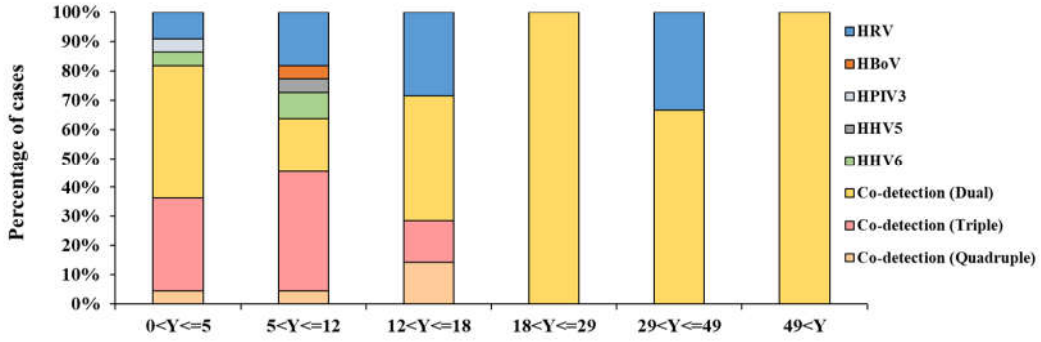


**Figure 2. Proportion of single detection or co-detection of viruses in different age groups.** Single detection and co-detection were divided and the single detection of each virus was counted. HRV, human rhinovirus; HBoV, human bocavirus; HPIV3, human parainfluenza virus 3; HHV4, human herpesvirus 4 (Epstein-Barr virus); HHV5, human herpesvirus (cytomegalovirus); HHV6, human herpesvirus 6.

*3.3. Seasonal Distribution*

In the monthly detection distribution of the viruses identified in this study, no pathogens showing special seasonality were identified, except for HPIV3. However, HPIV3 was observed only from September to October in 2021 (Fig 3).

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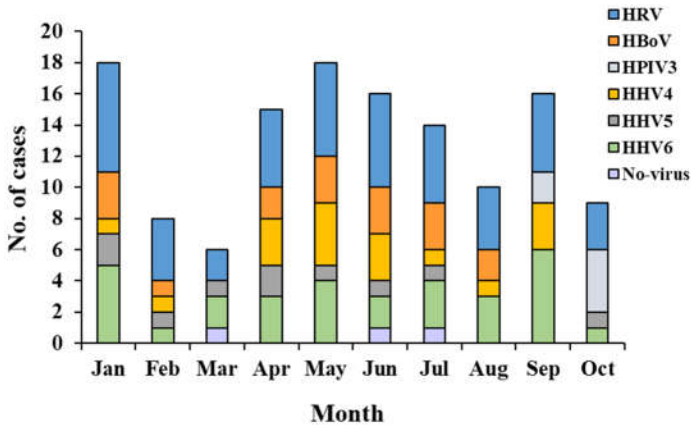


**Figure 3. Seasonal variation of viruses identified in patients with suspected sporadic mumps.** Detected viruses were counted individually and co-detection was not considered. The monthly distribution of six types of viruses was confirmed. HRV, human rhinovirus; HBoV, human bocavirus; HPIV3, human parainfluenza virus 3; HHV4, human herpesvirus 4 (Epstein-Barr virus); HHV5, human herpesvirus (cytomegalovirus); HHV6, human herpesvirus 6.

## 4. Discussion

This study was conducted to retrospectively identify the inconsistency associated with the negative diagnostic laboratory results of most samples of sporadic mumps cases reported by clinical medical institutions in Gwangju, South Korea. This is because other pathogens have been suggested to cause parotitis despite the commonly known correlation between mumps and parotitis. Respiratory viruses, such as HHV4, HHV5, HHV6, HPIV1, HPIV3, and influenza virus, have been reported as causative pathogens of acute viral non-mumps parotitis.

Symptoms similar to parotitis, but with unclear pathogens may be mistaken for mumps caused by the mumps viruses, and consequently managed as a legal infectious disease, which may result in inaccurate official pathogen statistics. In the testing of pathogens exhibiting identical symptoms, the detection of the causative pathogen has been attempted using a method that can simultaneously detect pathogens.

We attempted to detect 41 viruses, including the mumps virus, bacteria, and fungi, using TaqMan™ Array Cards in 63 samples from suspected mumps patients, which tested negative in laboratory diagnostic examinations [9]. Resultantly, six viruses were detected, and 60 (95.2%) of the tested samples were positive for at least one virus. The most frequently detected virus was HRV, detected in 47 cases (74.6%). HRV is the most common RNA virus that causes more than 50% of upper respiratory tract diseases worldwide and is typically co-detected with other viruses [10,11]. Moreover, general clinical symptoms include runny nose, cough, and sore throat, and do not include salivary or parotid gland extension [12].

HHV6 and HHV4, which were identified as major pathogens of non-mumps parotitis in previous studies, were detected in 30 (47.6%) and 17 (27.0 %) cases, respectively, in the present study. A previous South Korean study was conducted on children aged between 3 months and 16 years, with HHV4 being the most common, followed by HHV6, and rarely with hPIV3, adenovirus, and HBoV [13]. In a U.S. study conducted during 2014 and 2015, HHV6, influenza virus A, and HHV4 were primarily identified in non-mumps patients exhibiting acute parotitis or other salivary gland swellings [14]. In a Spanish study, HHV4 was mainly identified in patient samples with unilateral swelling for an average of 4.3 days, followed by PIV3, PIV2, and adenovirus [15]. Furthermore, HHV6 and HHV4 have been reported as major pathogens of non-mumps parotitis [16,17].

The association between HBoV, detected at the same frequency as that of HHV4, and parotitis is rare. However, according to Calvo et al. [18], HBoV was identified as a

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symptom of left sinusitis, high fever, and dyspnea in a 17-month-old male infant, suggesting that it should be suspected as a cause of acute non-mumps parotitis in young children.

Similar to HHV4 and HHV6, HHV5 belongs to the herpes virus group and is the most common virus that causes infectious mononucleosis characterized by fever, pharyngitis, and lymphadenopathy [19]. HPIV3 has been reported to be a major cause of bronchitis and pneumonia in infants under 6 months of age in spring and early summer [20]. In our study, HPIV3 was confirmed in six cases (9.5%) in September and October, which can be considered as a late epidemic of HPIV3 that can be the effect of the COVID19 pandemic in South Korea in 2021, occurring from September to November [21]. Except for HPIV3, most viruses identified in this study were detected sporadically during the study period. Likewise our study, the number of patients with suspected mumps reported to the KDCA could not ascertain seasonality and, only in October showed a significant increase.

Patients with suspected mumps were reported to KDCA by clinicians based on clinical symptoms. The age of the reported patients is from 5 to 9 years, most of them were under 14 years. Our cases were some of these reported cases. The mainly age indentified in our study was also under 12 years.

In our study, the co-detection of two or more viruses was commonly found in 44 (73.3%) of the 60 samples in which respiratory viruses were detected. In particular, co-detection was the highest at 40.9% in the group under 5 years of age. In a study by Goka et al. [22], the association between single or co-infection of respiratory viruses, including RSV, HRV, and HPIV, in patients aged 0 to 105 years was investigated, and the risk of co-infection was higher for those <5 years of age than for other age groups. Studies by Danfeng et al. [23] also showed that the frequency of co-infection between the ages of 3 and 6 years was higher than that in other age groups. Respiratory virus infection is considered highly contagious, making it easy for young people with weak immunity to become infected [7,24].

Our study had several limitations because it was retrospectively conducted. First, there were limitations in obtaining accurate clinical information, such as the date of edema, site of edema, and history of MMR vaccination for the collected samples. Second, it was difficult to accurately determine the timing of sample collection, which may affect the sensitivity of the RT-PCR method used for the diagnosis of mumps [15,25]. Third, various bacterial infections and other factors related to sinusitis were not considered [26].

Nevertheless, we suggest that for suspected sporadic mumps cases, respiratory viral infections other than mumps should be considered, and the actual scale of domestic mumps should be re-evaluated. Consequently, first, as a results of previous studies and this study, respiratory viruses, including HHV6 and HHV4, may cause non-mumps parotitis. Second, most people experience pharyngitis following respiratory virus infections, and pharyngitis with swollen tonsils and sore throat is difficult to distinguish from parotitis, which may indicate mumps [12]. Finally, further investigations of diseases mimicking mumps should enable a better understanding of the etiology and clinical and epidemiological features of outbreak-related and sporadic cases, leading to appropriate public health responses and treatments.

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**Institutional Review Board Statement:** This study used routine national surveillance data. Therefore, no ethical approval was required. The subset of the data extracted for analysis was fully anonymized before it was accessed for analysis.

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**Conflicts of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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