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Running title: Epidemiological outbreaks of measles in Kazakhstan

Keywords: Measles virus (MV); Epidemiological surveillance; Genotyping
Summary

This study was addressed to epidemiological surveillance of measles virus (MV) in the territory of Republic of Kazakhstan during 2015 – 2016 years. In our study we have detected D8 genotype of MV occurred in outbreak season of measles disease. Totally 2341 cases were registered and 19 of them were identified by genotyping. As a result, 16 samples attributed to subgroup A of D8 genotype, and 3 import cases were represented by B3 and H1 genotype of MV. Vaccination coverage analysis showed that large group of infected people were not vaccinated and did not have a reliable report on vaccination status. This issue might increase the morbidity rate among the healthy population in outbreak season. To prevent the incidence caused by this problem we have successfully introduced necessary epidemiologic arrangements in controlling of measles.

Introduction

Measles is a highly contagious infection caused by the measles virus which can be distributed among infants and children. The disease age might shift by age toward adulthood by causing serious illness and increasing mortality (1). Accelerated immunization activities played a significant role in reducing mortality from measles (2). Estimated 20.3 million deaths were prevented by vaccination between 2000 – 2015 years. Global measles mortality decreased by 74% from 535 300 cases in 2000 to 139 300 in 2010 (3). Despite significant investments in the fight against measles, over the past five years outbreaks continue to occur around the world. 30 762 cases of measles were registered for 2015 in 39 countries of the WHO European Region among 50. At the same time, 27086(88% of the total) cases were reported in four countries: Kyrgyzstan – 17 779 (58%), Bosnia and Herzegovina – 4 583 (15%), Germany – 2 383 (8%), Kazakhstan – 2 341 (8%) (4). In the Republic of Kazakhstan, vaccination against live measles vaccine as in all countries of the former Union of Soviet Socialist Republics began in 1967 (5). Planned vaccination was performed at the age of 1 to 8 years among healthy children. Nowadays measles vaccine is included in the immunization calendar according to which children are immunizing with trivalent measles-mumps-rubella vaccine at the age of 12 months, and revaccinated at 6 years old.
In 2002, epidemiological surveillance of measles and rubella was introduced in Kazakhstan in accordance of the WHO European Region strategic program for the prevention of measles and congenital rubella infection. At that time 16 regional laboratories of the Ministry of Health collected samples and sent them to the National Reference Laboratory for measles and rubella of WHO Europe region in Almaty, which conducted laboratory confirmation of measles cases using ELISA and sent samples for the PCR and genotyping study to the Regional Reference Laboratory in Moscow (G.N. Gabrichevsky Moscow Research Institute of Epidemiology and Microbiology, Russia). In the countries of the former Soviet Union, the laboratory system was created to reduce costs in a difficult economic situation in the period 1990-2000. To date, the growth of the economies of the post-Soviet countries, the emergence of modern diagnostic methods, the changing epidemic situation for measles, the need for more detailed laboratory data on circulating viruses (subtype, pathogenicity-related mutations, transmission routes, etc.), the transmission of newly emerging infections require availability of a modern, efficient laboratory network at the national level.

Kazakhstan, as one of the WHO Europe Regional Office members, needs to actively promote a common policy for improving laboratory and epidemiological surveillance of measles and rubella by introducing modern molecular-genetic typing methods in the Local National Reference Laboratory. Thus, it is very important to achieve the goals of the global measles and rubella elimination plan set by WHO. Prior to the current study, molecular-genetic typing was not carried out in the Republic of Kazakhstan. The aim of this study was to provide epidemiologic data for measles virus circulation in Kazakhstan during 2015-2016 years. In this study, we provide a regional update for epidemiologic surveillance of measles during seasonal outbreaks, in regard to circulating genotypes.

**Materials and methods**

**Definition of the measles cases**

The detection of measles is carried out by medical personnel in accordance with the following definitions – fever, maculopapular rash, cough, rhinitis, conjunctivitis. Specimen for laboratory testing (blood serum, urine) was collected during a measles outbreak from January 1, 2015 to January 30, 2016. Samples of serum were tested by ELISA to detect antibodies of IgM class using commercial kits
manufactured by Vector-Best CJSC (Russia). Blood serum was taken from 4 to 28 days after the onset of the rash.

Samples with clinical laboratory confirmation are transferred to the territorial Departments of the Committee for Public Health of the Ministry of Health. Then all the received data combined into the "Scientific and Practical Center for Sanitary and Epidemiological Expertise" of the Public Health Protection Committee of the Ministry of Health of Kazakhstan.

Studies on detection of antibodies to measles virus, measles RNA, genotyping and phylogenetic analysis were conducted in the National Reference Laboratory for Measles and Rubella of WHO Europe regional bureau "Scientific and Practical Center for Sanitary and Epidemiological Expertise" of the Ministry of Health of the Republic of Kazakhstan in Almaty city.

Genotyping

All urine samples for genotyping were selected in the first 5 days after the onset of the rash. Isolation and amplification of the viral RNA was performed according to the manufacturer's instructions using the commercial kit from QIAGEN (Cat. No 52906).

Genotyping was carried out based on the C terminal sequence of 450 nucleotides length fragment of N gene recommended for the measles virus genotyping (6). Phylogenetic trees were constructed in Mega 7.0 software using the maximum likelihood method, Kimura two-parameter model, gamma distribution and Bootstrap analysis (500 repetitions) (7). Sequences were introduced and compared to other sequences of measles viruses in the WHO - MeaNS database using the BLAST algorithm (8). Identification was carried out relative to the inventory numbers of the nucleotide sequences having the maximum coincidence.

Epidemiological studies

The studies were conducted during the outbreak of measles in Kazakhstan from January 1, 2015 to January 30, 2016. We conducted an observational, descriptive, one-stage analysis of measles cases by determining the prevalence, occurrence, proportions.

The morbidity rates by age groups and regions were calculated for cases in Kazakhstan using 2015-2016 data from official sources.
Results

Epidemiologic surveillance for measles

Measles outbreaks started to be registered from beginning 2015 in all 16 regions of the country. 2679 cases suspected of measles were detected in the period from January 1, 2015 to January 30, 2016, of which 338 were unconfirmed cases, 1815 laboratory confirmed and among them 3 imported cases, 526 epidemiologically related cases. Thus 2341 cases of measles were established in total.

Data on registration of measles cases with a large number of cases obtained from Astana city (n=396, 15%), East Kazakhstan region (n=333, 12%), Mangistau region (n=292, 11%), South Kazakhstan region (n=269, 10%), Almaty city (n=150, 6%) and Almaty region (n=127, 5%) (Fig. 1).

In January 2015, transmission of measles virus infection has been increased in 10 regions of the country. The total number of detected cases was 220, mainly in Astana city (n = 81; 36.9%), East Kazakhstan region (n = 82; 37%), Mangistau region (n = 17; 8%), South-Kazakhstan region (n = 17; 8%). In February, regional centers reported 485 measles cases in 16 regions of the country. In general, the morbidity rate in the country amounted 13.53 cases per 100 thousand populations, and the morbidity peak was in April 2015 when 515 incidences were registered. After rapidly increasing infection started to slowly drop down by decreasing in August 2015 up to 24 cases and continued until December 2015 (Fig. 2).

Young people at the age of 20-29 (n = 719; 31%), adults from 30 and more years (n = 615; 26%), children under 1 year old (n = 576; 25%), adolescent and young people aged 15-19 (n = 300; 13%) were the most susceptible to the disease. The incidence was predominantly registered, among persons older than 14 years (n = 1634; 70%).

Analysis of the vaccination status of measles cases in Kazakhstan shows that the vast majority of people are not vaccinated against measles and those who do not have reliable data on vaccination. Of the 2341 total number of reported measles cases almost half did not have vaccination data (n = 1093). About one-third were not immunized (n = 628), and most of them were infants up to 1 year (n = 560) who did not receive the vaccine. Among the 620 vaccinated cases prevailed those who received two or more doses of the vaccine (n = 527). Out of the total number of vaccinated persons, a larger proportion comes from
people aged 20-29 years (n = 266) and 15-19 years (n = 207). The distribution of measles cases by age group and the status of vaccination are shown in Table 1.

Vaccination coverage

Data analysis over the past four years has shown that the average vaccine coverage for two doses of the MMR trivalent vaccine against measles, rubella and mumps in children aged 2 years and 6 years was more than 95% (Table 2).

It should be noted that since 2014 in Kazakhstan monitoring of vaccine waivers has been introduced in the regions at the national level. Annually in the Republic of Kazakhstan, there is a tendency of growth for waivers from vaccination. So, if in 2013 the number of waivers from vaccination was 500 cases, then in 2016 it became more than 10 000.

Genotyping of measles virus

During 2015-2016, blood samples of 2679 people were analyzed by ELISA for confirmation of measles, of which 1815 samples contained anti-measles IgM antibodies. 19 urine samples were examined by RT-PCR, and RNA of MV was detected in all samples. These samples were delivered from North Kazakhstan (n = 7), Akmola (n = 3), South Kazakhstan (n = 7) regions, Shymkent city (n = 1) and Almaty city (n = 1). Fragments of 450 bp long N gene were amplified in all 19 samples to perform genotyping of MV. 16 genotypes were endemic and 3 genotypes were imported.

In the results of 15 strains genotyping we have determined that all strains belong to genotype D8, to subgroup A, which were divided into 5 variants. Among them 11 strains belong to variant 1 of which MVi/Villupuram.Ind/03.07 was the representative. The one strain belonged to variant 2 similar to MVs/Frankfurt Main.DEU/17.11, and one strain to variant 3 with representative MVs/Rostov on Don.RUS/47.13/2, two strains were similar and related to variant 4 MVs/Pretoria.ZAF (19.09), one strain belong to variant 5 and was similar to MVs/Sohar.OMN/23.12 (Fig. 3).

In this period 3 import cases of measles from Russia and China were registered. So in April 2015, 2 cases of measles in the North-Kazakhstan region were registered among persons who visited Russian Federation. The results of these samples genotyping showed that measles disease was caused by a virus belonging to the MVs/Kansas.USA/1.12/B3 genotype. In January 2016 in Almaty city 1 case of measles
were detected in people visiting Mongolia. The results of genotyping showed that they belong to the MVs/Hong Kong.CHN/49.12/H1 genotype (Fig. 3). Strains of measles viruses like MVs/Kansas.USA/1.12/B3 and MVs/Hong Kong.CHN/49.12/H1 had not previously circulated in Kazakhstan.

Epidemiologic surveillance arrangements

After assessing the risk of the situation associated with a measles disease spreading in all regions of the country in 2015 and the constant threat of infection import, by the Ministry of Health of the Republic of Kazakhstan were taken an additional activities. Therefore in October 2015, an additional immunization against measles with a monovalent live attenuated measles vaccine for adolescents aged 15-19 years (school, university students, soldiers and others) were carried out. The campaign for additional immunization was carried out at vaccination points in 16 territorial health organizations (rural medical outpatient clinics, district and central district hospitals, city hospitals) and educational organizations (schools, universities).

A totally 351 286 (99.4%) children aged 1 year were vaccinated against measles in a planned way and 314 108 (98.3%) children at the age of 6 years were revaccinated against measles. The proportion of those vaccinated persons was 99.9% from the total number of the target group as part of the campaign for additional immunization against measles. The vaccination process was additionally ensured by the organization of 4120 visiting vaccination teams work. The necessary conditions for the organization and conduct of anti-epidemic arrangements were created to prevent the spread of infections.

Discussion

In accordance with WHO strategic plan, it was planned to eliminate measles and rubella at least in five regions of the world by the end of 2015. Elimination implied the absence of endemic circulation of measles and rubella viruses in a certain territory for at least 12 months, with an effective system of epidemiological surveillance (9).

Due to an increase in population immunity by overall vaccination program, there has been pronounced tendency to reduce the incidence of measles. However, since 2013 there is an increase in the incidence of measles induced by outbreaks in most regions of the world. 31520 measles cases were
registered in European Region registered in 2013 which is higher in 4386 cases than in 2012 (n = 27134) (10). In Kazakhstan during the 2013-2014 periods were registered 73 and 321 suspicious measles cases, respectively (data not shown). In our study, we have recorded 2341 measles cases during 2015-2016 outbreak seasons. The incidence of measles affected all 16 regions of Kazakhstan. In general, the morbidity rate per 100 thousand people in our country was 13.53, which is 7 times higher than the values of 2014 (1.88) and 30 times higher than the indicators of 2013 (0.43). The incidence peak occurred in April 2015 and decreasing was observed from August 2015 to the end of December 2015.

Analysis of vaccination data among measles cases in Kazakhstan showed that the vast majority of them are persons not vaccinated against measles, most of whom were children under 1 year of age and those without reliable data about vaccination. Therefore, in order to prevent the occurrence of measles outbreaks, it is necessary to carry out a large-scale screening of persons over the age of 14 vaccinated and not vaccinated for the presence of post-vaccination antibodies, since during their vaccination there was no proper immunization reports and quality control of vaccines in Kazakhstan. Another potential issue may be raised by persons who avoids from vaccination. Hence it is necessary to solve all problems connected with vaccination risks.

The measles cases identified in Kazakhstan in 2006 were belonged to D6 genotype (11). The morbidity situation changed with outbreak in 2010-2013 by circulation of D4 genotype of genetic line «MVi/Bandarabas.IRN/05.10/2». Since in 2013-2014 global distribution of D8 genotype of the /MVi/Villupuram.IND/03.07/ were observed in the world and it touched our country (data not shown).

In order to implement the WHO recommendations in 2015, the National Reference Laboratory for measles and rubella of the WHO/Europe region in Almaty city introduced molecular genetic methods for the detection and identification of measles viruses. Obtained results of nucleotide sequences of 19 samples were compared to WHO database (MeanS) reference strains. It was determined that the incidence between January1, 2015 and January 30, 2016 was caused by 5 variants of MV, subgroup A, genotype D8. In addition, we detected imported MV strains similar to MVs/Kansas.USA/1.12/ (n = 2) of B3 genotype and MVs/Hong Kong.CHN/49.12/ (n = 1) of H1 genotype which previously not circulated in territories of the country (Fig. 3).
The National Commission ascertained the effectiveness and timeliness of the epidemic activities for additional immunization against measles among adolescents aged 15-19 with vaccination coverage of more than 95%. The implementation of the above activities resulted in a marked decrease in the incidence of measles in Kazakhstan in 2016 (n = 122) (data not shown) by more than 19 times, compared to 2015 (n = 2341). However, it should be emphasized that it is necessary to conduct screening studies for the presence of postvaccinal antibodies and carry out “catch-up” vaccinations in risk groups (vaccinated and not vaccinated over 14 years) in order to prevent measles outbreaks.

This study demonstrates importance and effectiveness of MV determination by molecular genetic methods. Epidemiological surveillance of measles is important because of many countries continue to move towards elimination of MV, therefore demonstrating lack of sustained transmission in the indigenous population. Identification of measles virus in combination with effective anti-epidemic arrangements can limit the further spread of the disease with endemic circulation and import of infection.

Studies on the genotyping of MV strains were carried out within the framework of the project "Development of molecular genetic tests for the diagnosis and monitoring of measles and rubella in the territory of Kazakhstan" grant funding for scientific research for 2015-2017 of the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan. (5412/GF-4)

**Conflict of interest**

None to declare.

**References**


Figure 1 – Number of measles cases by regions of Kazakhstan in 2015.

Bold numbers represents detected measles cases divided by region

Figure 2 - Number of registered measles cases by month in Kazakhstan, from January 1, 2015 to January 30, 2016 (n = 2341).

Figure 3 - Phylogenetic tree of MV genotypes identified in Kazakhstan during 2015-2016 years outbreak season.

●,▼,▲, ■,♦ - sequence of MV reference strains; ○ - sequences of MV strains isolated in Kazakhstan.

Phylogenetic tree of H1 and B3 genotypes of MV, identified in Kazakhstan (2015-2016).
Table 1 - Age and vaccination status of patients with laboratory confirmed, epidemiologically related measles cases (excluding imported cases) from January 1, 2015 to January 30, 2016 (n = 2341).

Table 2 - Coverage of routine vaccination in Kazakhstan from 2013 to 2016.
Table 1

<table>
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<th>MMR vaccine</th>
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<th></th>
<th></th>
<th></th>
<th>Total</th>
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<td>&lt;1 year</td>
<td>1-4 years</td>
<td>5-9 years</td>
<td>10-14 years</td>
<td>15-19 years</td>
<td>20-29 years</td>
<td>30 years and older</td>
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<td>13</td>
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<td>628</td>
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<td>6</td>
<td>3</td>
<td>14</td>
<td>29</td>
<td>27</td>
<td>93</td>
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<tr>
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<td>15</td>
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<td>554</td>
<td>1093</td>
</tr>
<tr>
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<td>9</td>
<td>55</td>
<td>67</td>
<td>300</td>
<td>719</td>
<td>615</td>
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Table 2

<table>
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<th>2013 (%)</th>
<th>2014 (%)</th>
<th>2015 (%)</th>
<th>2016 (%)</th>
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<tr>
<td>MMR vaccine, 1st dose</td>
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<td>99,2</td>
<td>100</td>
<td>99,4</td>
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<tr>
<td>MMR vaccine, 2nd dose</td>
<td>99,3</td>
<td>99</td>
<td>98,4</td>
<td>99,3</td>
</tr>
</tbody>
</table>
Figure 3