DETECTION OF WEST NILE VIRUS ISOLATES IN POULTRY IN CENTRAL VIETNAM IN 2015-2019

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1. INTRODUCTION

West Nile virus (WNV) belongs to the flavivirus family and is mainly carried by mosquitoes of the genus *Culex*. The virus can cause encephalitis in humans, horses and birds. Nevertheless, humans, horses, cats, dogs, and bats are a "dead-end" pathway WNV transmission, and birds are the main reservoir leading to the circulation of the virus. Among people, the majority of infected people are asymptomatic, 20% experience mild flu-like symptoms: fever, headache, myalgia, arthralgia, weakness and nausea. In less than one person out of 150, the virus penetrates the central nervous system, which can cause meningitis, encephalitis and death, mainly older people with immunosuppression. At the same time, almost half of the recovered patients have neurological or renal complications, which may manifest after 6-12 months [1-4]. Moreover, in some patients with weakened immunity, the WNV antigen and RNA are detected in the central nervous system several years after infection, which indicates a chronic infection [5, 6] with more serious health consequences.

WNV is the most geographically widespread flavivirus: it is often found in Africa, the Middle East, South Asia, China, Australia, North America, South America, Europe, in southern parts of Russia and in Siberia. Cases of detection of WNV or antibodies to the virus were recorded in Cambodia, Malaysia, Indonesia, Myanmar and the Philippines [7-11]. At the same time, to date there is no data on the circulation of WNV in Vietnam, although the range of distribution of mosquitoes of the genus *Culex* includes this territory. In all likelihood, this is due to the fact that in most cases, no symptoms occur during infection or the disease proceeds very mildly. At the same time, the symptoms of the disease are in many ways similar to the symptoms of dengue fever and Japanese encephalitis endemic in Vietnam, which can lead to an incorrect diagnosis. In this work, for the first time, we identified WNV RNA in samples from poultry in Vietnam.

2. MATERIAL AND METHODS

2.1. Sample collection

Lung homogenates from 14 deceased domestic birds (chickens and ducks) collected in 2015-2019 on the territory of Quang Trị, Nghe An, and Ha Tinh provinces were taken into the work.

2.2. Research methods

2.2.1. Sample preparation for next generation sequencing

Lung homogenates were centrifuged at 8000 g for 5 min at 4°C, the supernatant was taken for analysis. Next, the samples were treated with Benzonaze (Sigma, USA) [12]. Total RNA was extracted using Extract RNA Reagent (Evrogen, Russia) according to the manufacturer's protocol. The aqueous phase obtained after the addition of chloroform and subsequent centrifugation was collected and diluted 1:1 with freshly prepared 70% ethanol and purified on Cleanup Mini spin columns (Evrogen, Russia). cDNA libraries for NGS were prepared using NEBNext Ultra RU Library Prep Kit first and second strand synthesis modules (New England Biolabs, USA). The obtained double-stranded DNA was purified from the products of the reaction mixture before sequencing using the Cleanup S-Cup kit (Evrogen, Russia). The concentration of dsDNA samples for NGS sequencing was at least 300 ng per sample.

2.2.2. Determination of DNA nucleotide sequences

Libraries of dsDNA were analyzed by NGS on a MiSeq sequencer using Illumina technology (Illumina, USA). Data analysis used Cutadapt (version 1.18) and SAMtools (version 0.1.18) to remove the Illumina adapters and re-read. The contigs were assembled de novo using the MIRA assembler (version 4.9.6).

2.2.3. Phylogenetic analysis

Multiple sequence alignment was performed in MEGA X (PSU, USA) using the Muscle iterative alignment method. Phylogenetic trees were constructed using the maximum likelihood method using 1000 repetitions. Nucleotide sequences of various WNV strains taken from the GenBank database were used for comparison.

3. RESULTS AND DISCUSSION

3.1. Sequenceanalysis

Using random amplification in combination with next-generation sequencing, the species of birds collected in 2015-2019 on the territory of Quang trị, Nghe An, and Ha Tinh provinces was characterized. In 3 lung samples of the 14 chickens collected in Nghe An province in 2017, WNV RNA was detected. The length of the confident reading of the NS1 gene fragment was 242 nucleotides (Fig.1). In other samples, WNV RNA was not detected. Phylogenetic analysis showed that one isolate (isolate 21) belongs to lineage 1a and two isolates (isolates 6, 29) belong to lineage 2. Isolate 21 (GenBank: OP593326) had the greatest homology with isolates MN149538 (Tomsk, Russia), AY278442, AY277252, AF317203 (Volgograd, Russia), GQ851607 (Nigeria), AF260969 (Romania), MH507862 (USA). Isolate 6 (GenBank: OP593325) had the maximum homology with isolates LR743458, LR743456 (Germany), MT341471, MK473443, MN652880, MN652878, MN480795.1, MN481594.1, MH549209 (Greece), MH244513, MH244511 (Slovakia), MF984352, MF984351, MF984348 (Austria), KX375812, KT757323 (Serbia). Isolate 29 (GenBank: OP593327) was most homologous to isolate AY688948 (Israel) and to a lesser extent to isolates JX041631 (Ukraine), DQ318019 (Senegal), HM147824 (Democratic Republic of the Congo), MN481597, MN481591 (Greece), MF984346 (Austria), KT757318 (Serbia), KU206781 (Bulgaria), KT359349 (Hungary).

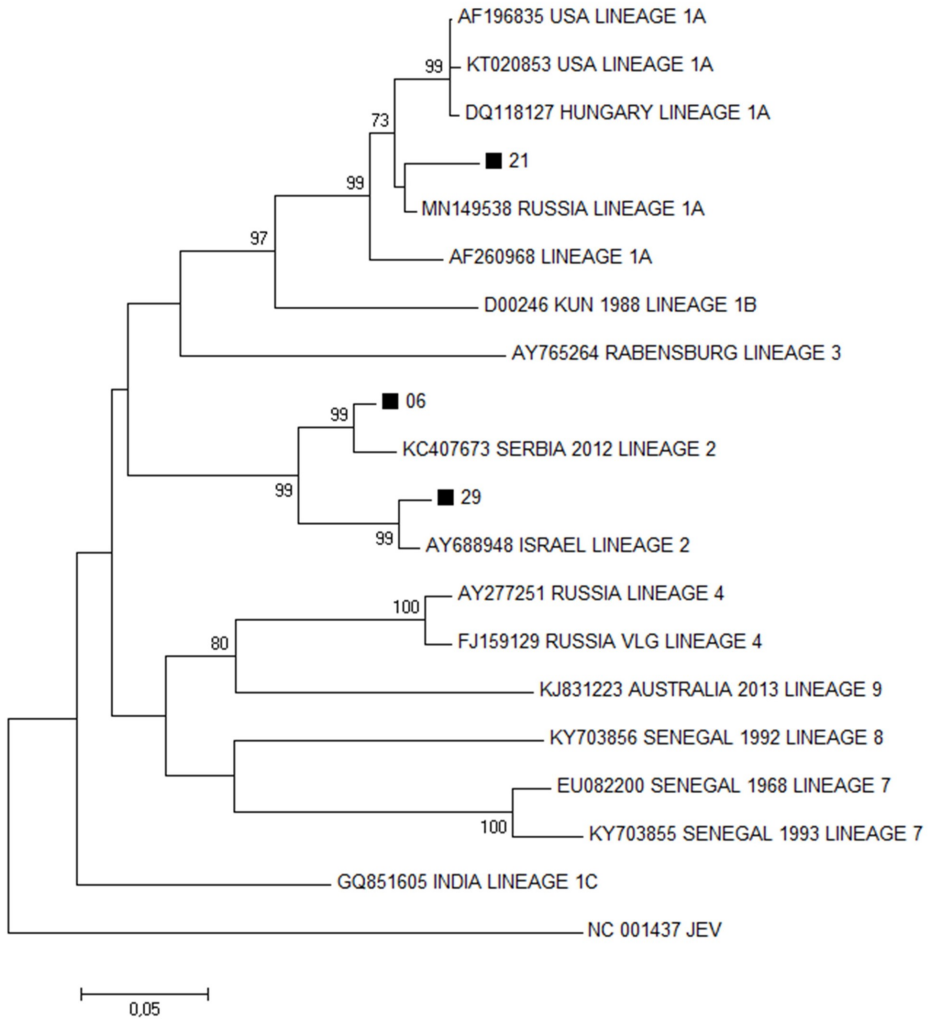

Figure 1. Phylogenetic tree constructed by the maximum likelihood method for the NS1 fragment of the West Nile virus gene, 242 nucleotides long. The studied sequences are indicated by a black square. Support indices are calculated for 1000 repetitions. Japanese encephalitis virus strain NC001437 was used to root the tree.

When analyzing the sequences closest to the ones we found in BLAST, it turned out that in isolate 6, more than 250 of the closest sequences homology of more than 97%. In isolate 21, the homology of the closest sequences varied from 96.69% to 95.04%. For isolate 29, only one isolate had a homology of 98.35%, three isolates had a homology of 93.39%, among the other more than 250 closest sequences, the homology was below 93%. The presence of only one isolate with high homology with such a short fragment may indicate that this isolate does not have evolutionary advantages that allow it to actively spread. However, to test this hypothesis, it is necessary to obtain genome-wide sequences.

3.2. WNV distribution of in Southeast Asia

The data obtained in the work indicated that in 2017, isolates of WNV lineages 1a, 2 were circulating in the territory of Nghe An province. WNV strains of lineages 1a and 2 are the most epidemiologically significant and cause the greatest number of human diseases in Europe, Asia and Africa. This is the first data on the detection of WNV in Vietnam, which may indicate its possible penetration from nearby countries, since cases of detection of WNV or antibodies to the WNV were recorded in many countries of Southeast Asia. In Indonesia, in the serum of a 15-year-old boy taken in 2005, a WNV isolate belonging to lineage 2 was detected. At the same time, the highest homology was noted with isolates not from Australia, but from Uganda, which indicates an ever wider geographical distribution of arboviruses [10]. WNV lineage 1b was isolated in Malaysia in the state of Sarawak in 1966 [8], while this strain is so different from other lineage 1b strains identified in Australia that it is now classified as a lineage 6 strain. This may indicate that this strain has diverged from Australian strains for a long time and could have been circulating in this territory for a long time [9]. Currently, GenBank has deposited at least 25 WNV sequences from Malaysia found in *Culex* mosquitoes, wild birds, and bats. A study using serological tests performed in 2016 showed the presence of neutralizing antibodies to WNV in domestic ducks and chickens in three provinces of Cambodia [7]. Vietnam and Cambodia share a common border, respectively, infected mosquitoes and ticks, which are less common, but also involved in the transmission of WNV [13], can travel between the two countries by means of transport, contributing to the spread of the virus. The introduction of WNV into the territory of Vietnam by wild birds is also possible. Unlike humans, horses, cats, dogs, and bats, which represent a dead-end path, birds have a high viremia, due to which they are able to transmit WNV not only to mosquitoes, but also to other birds [14]. It is possible that WNV has been circulating in Vietnam for a long time and remained undetected due to the predominantly asymptomatic or very mild course of the disease. The health care system is focusing on other flaviviruses that are very endemic in Vietnam: dengue virus and Japanese encephalitis virus. The similarity of symptoms in a large number of patients infected with these viruses may interfere with the accurate differential diagnosis of WNV. In addition, these viruses belong to the same family, and in ELISA tests and in rapid tests, false positive results are possible when the WNV antigen can interact with antibodies to dengue and Japanese encephalitis viruses, which, accordingly, will lead to an incorrect diagnosis.

Although most cases of West Nile fever are asymptomatic or mild, the identified isolates are of lineages 1a and 2, which are pathogenic to humans and are responsible for outbreaks in Europe and North America. Currently, novaccine against WNV has been developed, there is no specific treatment, therefore, only anti-epidemic measures are applicable for control, which, in particular, require the creation of effective test systems for accurate diagnosis and determination of the distribution area of this virus.

4. CONCLUSIONS

For the first time, data were obtained on the detection of West Nile virus RNA in deceased birds using the NGS method in Vietnam. WNV RNA was found in three lung samples of chickens from Nghe An province collected in 2017. One sequence belonged to lineage 1a, two sequences belonged to lineage 2. The isolates of lineages 1a and 2 are responsible for many WNV outbreaks in Europe and North America and therefore require close attention. Further monitoring of WNV in mosquitos, birds and humans is needed in Vietnam.

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SUMMARY

DETECTION OF WEST NILE VIRUS ISOLATES IN POULTRY IN CENTRAL VIETNAM IN 2015-2019

Cases of detection of RNA or ELISA markers of West Nile virus (WNV) have been recorded in many countries of Southeast Asia. At the same time, there were no reports of outbreaks of fevers caused by WNV. Perhaps this is due to the asymptomatic or very mild course of the disease or to the focus of the health systems of these countries on other viral fevers. In this work, for the first time in Vietnam, West Nile virus (WNV) RNA was detected in poultry. WNV RNA was found in three lung samples of chickens from Nghe An province collected in 2017. The isolates belonged to lineages 1a and 2, responsible for many WNV outbreaks in Europe and North America and therefore require close attention.

Keywords: *Transmissible infections, West Nile virus, Vietnam.*

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