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GENETIC CHARACTERIZATION

OF AVIAN INFECTIOUS BRONCHITIS VIRUS ISOLATES RECOVERED IN CIS COUNTRIES IN 2015–2017

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SUMMARY

Avian infectious bronchitis virus is a cause of major economic losses in poultry industry. However, control of the virus is very complicated due to its high variability. The mutation frequency in the hypervariable region of the S1 gene of the virus isolated from the vaccinated birds annually amounts to 1.5%. Long-term observations of the circulation of IBV isolates detected in a number of poultry farms demonstrated that the virus genetic lineages circulating on the poultry farms could eventually change. This stipulates the need for the continuous monitoring of the virus isolates for the prevention schedule optimization. The paper demonstrates test results of 840 biological samples collected from chickens on the poultry farms in Russia and some CIS countries in 2015–2017. From 311 positive samples 147 IBV isolates were recovered, the majority of which belonged to eight genetic lines of GI genotype: GI-1, GI-12, GI-13, GI-14, GI-16, GI-19, GI-22, GI-23. Moreover, recombinant isolates were detected as well as variant isolates that belonged to none of the known genotypes.

Key words: avian infectious bronchitis virus, genetic analysis, genotype.

INTRODUCTION

Avian infectious bronchitis virus (IBV) is ubiquitous and occurs in all countries having well-developed poultry industry. It belongs to order *Nidovirales*, family *Coronaviridae*, subfamily *Coronavirinae*, genus *Gammacoronavirus*. The virus genome is a single-stranded (+)RNA. The IBV is mainly targeted at the ciliated epithelium of the upper respiratory tract and epithelial cells of the oviduct and kidneys.

There is a number of factors contributing to the enormous IBV diversity and *inter alia* the following: high density of a large number of chickens being kept on the poultry farms, use of live vaccines based on the viruses of different serotypes and co-circulation of genetically diverse virus populations on the poultry farms. The IBV evolution process is rather rapid. Continuous occurrence of point mutations, insertions and deletions as well as recombinations (in case of simultaneous reproduction of genetically different isolates in the same host) – all these result in the emergence of new virus variants [4]. Frequency of mutations within the hypervariable region of S1 gene of the IBV isolated from the vaccinated birds annually amounts to 1.5% but the virus evolution is slower in the absence of the postvaccinal immunity (0.3%) [11].

High variability of the IBV genome complicates the virus classification. New virus variants emerge continuously. Most of them cannot reproduce or can survive only for a short period of time. Some of emerging IBV variants become economically relevant and cause significant damage to poultry farms. They are capable of spreading over different regions of the world or affecting chickens within the limited geographic area.

As far as ten years ago, only several IBV genotypes were identified: Massachusetts, D274, Connecticut, Holte,

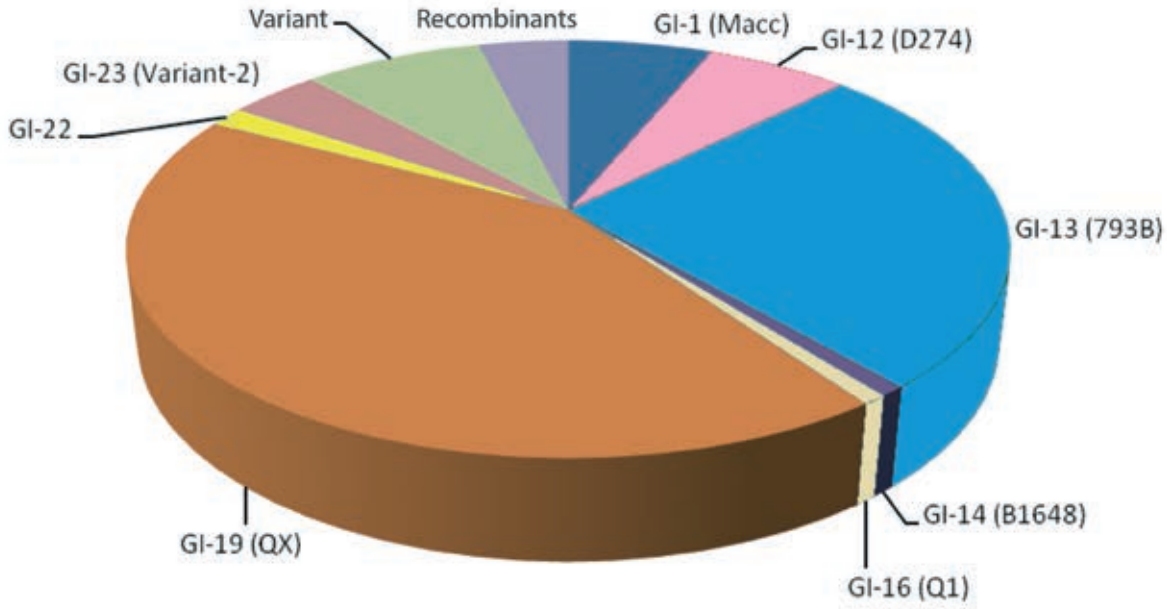

Fig. 1. Genetic lineages of IBV isolated in 2015–2017

Arkansas, Taiwan-1, Taiwan-2, Grey, 793B, QX, Italy-02, Q1, Variant-1, Variant-2, B1648, D1466, etc. Over the recent decade, the number of new genotypes significantly increased. Despite great variety of already known genotypes, new isolates are still being recovered that belong to neither of the previously identified IBV genotypes. Such isolates are called variant ones.

In 2016, V. Valastro et al. [14] suggested practical and standardized list of IBV genetic groups based on the comparison of 1286 sequences of S1 gene excluding nucleotide sequences of recombinant IBV forms. Altogether, six genotypes (GI–GVI) were identified including 32 genetic lineages of IBV.

The key goal of the work was to perform the phylogenetic analysis of IBV isolates recovered on the RF poultry farms in 2015–2017 using new classification and to identify new IBV genetic lineages emerged over the recent years for the optimal selection of the proper vaccine.

MATERIALS AND METHODS

The testing was performed using 840 samples of internal organs (lungs, trachea, kidneys, and intestines), hatching eggs and feces of chicken of different ages. The samples were received from the poultry farms located in the Russian Federation and Republic of Belarus, Tajikistan and Kazakhstan in 2015–2017.

RT-PCR and real-time RT-PCR were run according to the methodical instructions [1, 2].

Comparative analysis of about 500 bp fragment of S1 gene (position 112–653 bp of S gene of H-120 strain) was performed for IBV genotyping. Nucleotide sequences of prototype strains proposed by V. Valastro et al. were used for the analysis [14].

The nucleotide sequences were determined according to Sanger method involving fluorescence labeled chain-terminating nucleotides. The sequencing was carried out using ABI Prism 3130 sequencer (Applied Biosystems, USA) according to the manufacturer's instructions. The resulted nucleotide sequences were compared with IBV sequences deposited in the international database NCBI (http://www.ncbi.nlm.nih.gov/) using BioEdit software, version 7.0.5.3.

RESULTS AND DISCUSSION

In 2015–2017, 840 biomaterial samples collected from poultry on 50 farms in 26 regions of Russia and in the Republics of Belarus, Tajikistan and Kazakhstan were PCR tested. 420 test results were positive (50%). IBV genome was detected in eggs and in the internal organs of chickens at the age of below 578 days old.

Total of 311 RT-PCR-positive samples were selected for identification and comparative analysis of the structure of S1 gene fragment. Comparative analysis of nucleotide sequences of S1 gene fragment demonstrated that out of all positive samples 164 bore vaccine IBV strains (4/91, D274, H-120, Ma5). 147 samples contained IBV isolates belonging to various genetic lineages (Fig. 1). The phylogenetic analysis of these isolates demonstrated that their majority belonged to eight genetic lineages of GI genotype: GI-1 (Macc), GI-12 (D274), GI-13 (793B), GI-14 (B1648), GI-16 (Q1), GI-19 (QX), GI-22, GI-23 (Variant-2). Furthermore, recombinant and variant isolates were recovered that belonged to neither of the known genotypes (Fig. 1, 2). The isolates of genetic lineages GI-16 (Q1), GI-22 and GI-23 (Variant-2) were recovered on the RF poultry farms for the first time.

Genetic lineage GI-19 (QX) is the dominating one. The viruses of this lineage were isolated from the birds of different ages. The minimal age of the birds the GI-19 genotype IBV was isolated from amounted to 8 days old, the maximal – 578 days old. GI-19 (QX) virus was first isolated in China [10]. Currently (QX) virus is wide spread in Europe, South-Eastern Asia, Africa and Near East [6]. Representatives of GI-19 (QX) genetic lineage were first isolated in the Russian Federation as early as in 2001 [13].

Comparative analysis of IBV isolates recovered from 2015 to 2017 demonstrated that beside genetic lineage GI-19 (QX), every year GI-1 (Macc), GI-12 (D274), GI-13 (793B) isolates were recovered (Fig. 2). Part of the isolates belonging to these three genetic lineages was likely to be

the derivatives of the vaccine strains used in the RF poultry farms for many years. In the most general use are live vaccines based on strains H-120, MA5, D274 and 4/91.

Genotype GI-14 (B1648) isolates are sporadically recovered on the Russian poultry farms. Nephropathogenic IBV strain GI-14 (B1648) was first isolated in Belgium in 1984. Despite mass vaccination, GI-14 (B1648) and its variants are still circulating in Europe and Central Africa [8].

In 2015, virus of genetic lineage GI-23 (Variant-2) was detected on poultry farms in Kazakhstan, and in 2016 – in the Russian Federation. IBV of such genotype had never been detected in these two countries before. Lineage GI-23 is a unique cluster, which is geographically confined within the Middle East countries. The strains belonging to this lineage were detected in Israel in 1998 and continue their circulation in this region [7, 12]. Some of the strains became prevailing on the majority of the farms and induced respiratory and renal pathology in chickens [9].

In 2017, representatives of two, novel for Russia, IBV genetic lineages emerged: GI-16 (Q1) and GI-22. IBV genetic lineage GI-16 (Q1) was detected in chickens in Italy, Spain, and China, as well as in the majority of the countries in Latin America and Near East [6]. Results of the experiments involving GI-16 (Q1) virus were first published by L. Yu et al. [5]. The virus was isolated from proventriculus of young layers. Experimental infection of birds resulted in respiratory signs, diarrhea and proventriculus lesions. The mortality reached very high level (75100%). No lesions were reported in kidneys. Herewith, experiments involving Chilean isolates of genetic lineage GI-16 (Q1) demonstrated no virus replication in the proventriculus but indicated the virus-induced lesions in the respiratory tract and kidneys.

Isolates of genetic lineage GI-22 were recovered in China during IB outbreak in broilers and layers demonstrating kidney lesions. Numerous epidemic investigations performed in China demonstrated prevalence of genetic lineages GI-22 and GI-19 in the country.

Variant IBV isolates with S1 gene structure different from all other known genetic lineages being detected on the poultry farms are indicative of a wide genetic diversity of the circulating IBV isolates. Variant IBV isolates were detected in the Krasnoyarsk Krai, Leningrad and Vladimir Oblasts as well as in Kazakhstan and Tajikistan. In 2016, a variant isolate IBV24-16 was recovered from the samples of internal organs collected from chickens on one of the farms in the Leningrad Oblast. The isolate was recovered together with the vaccine strains 4/91 and H-120. The repeated testing performed on the same farm in three and nine months demonstrated that the recovered variant isolates IBV37-16 and IBV08-17 were different from IBV24-16 by 1.5 and 3%, respectively.The variant isolate IBV19-16 recovered on the poultry farm in the Vladimir Oblast has been circulating here for at least ten years. No similar isolates were detected on other poultry farms of the Russian Federation.

The variant isolates demonstrate capacities to infect birds despite vaccination. Prevalence of some variants is endemic in some geographic regions. They can circulate in the poultry herd for some time and then disappear.

Other variants can become prevailing and they can rapidly spread over the long distances.

Over the recent years, combinations of various vaccines are used for IB prevention and the number of vaccinations is increased. Repeated vaccination with different strains

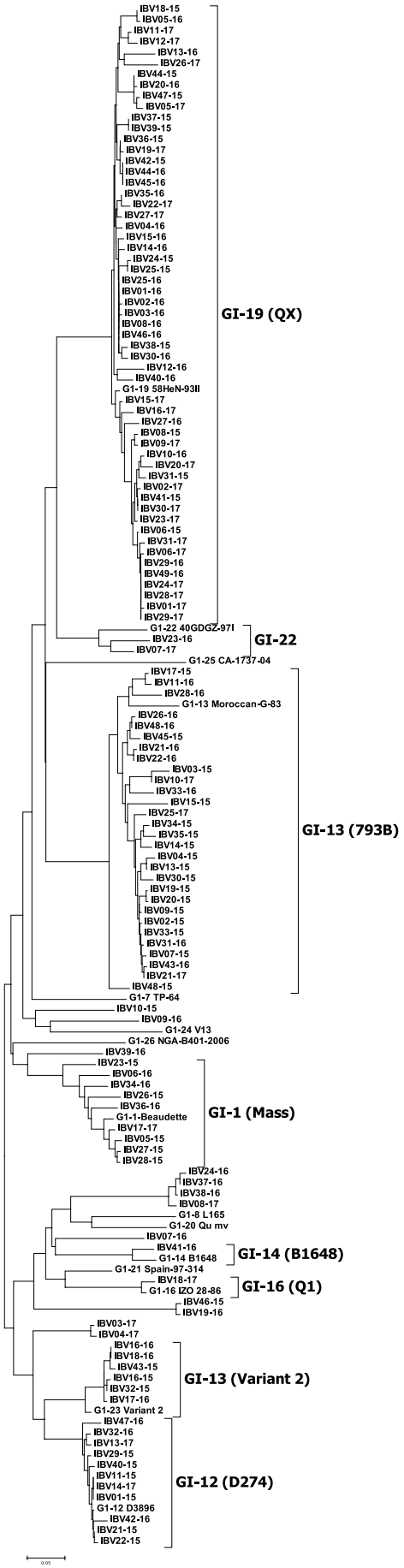

Fig. 2. Phylogenetic relations of IBV strains and isolates based on the analysis of S1 gene fragment

Fig. 3. Sequence alignment of S1 gene fragment of recombinant isolates (IBV03-17 and IBV04-17) and supposed parents (vaccine strains H-120 and D274)

Recombination point is shown in the figure.

is considered to have the potential for inducing more effective protection against vast variety of antigenic variants of IBV.

Thus, the vaccine strains along with the isolates can be detected in the same sample. In 30% of cases, the vaccine strains of genetic lineages GI-1 (Macc) or GI-13 (793B) were detected along with GI-19 (QX) isolates. One of the samples simultaneously demonstrated two vaccine strains H-120 – GI-1 (Macc) and 4/91 GI-13 (793B) – and isolate GI-19 (QX).

Simultaneous replication of IBV of different lineages in the same host can result in recombination leading to new virus variant emergence.

In 2015–2017, three viruses were detected, whose nucleotide sequence in S1 gene consisted of two fragments of two parent viruses: GI-13 (4/91) and GI-12 (D274), GI-13 (4/91) and GI-1 (Macc), GI-1 (Macc) and GI-12 (D274). There were previously reported cases of recombinant detections, where the parent viruses were represented by the field isolates of genotypes QX and Macc, as well as by the vaccine strains D274, 4/91 and H-120 in different combinations including combination of H-120 and D274 [3].

In 2017, two samples from poultry farm in Mari-El demonstrated two IBV isolates (IBV03-17 and IBV04-17). Part of S1 gene of these isolates originated from the virus of genetic lineage GI-13 (D274), and the other part originated from GI-1 (Macc) (Fig. 3). The nucleotide sequences of S1 gene fragments of the isolates differed only by ten bp and out of them eight nucleotide substitutions were nonsynonymous. Of interest is the fact that isolate IBV03-17 was recovered from the sample of the internal organs of 224-day-old layer and isolate BV04-17 – from hatching egg. Even if the virus of some genotype is currently circulating in the chicken population, however, the virus population is not homogenous. In the infected host the IBV exists as a set of virions bearing slightly changed but closely related genomes, the so called quasispecies. The quasispecies are the result of mutations and recombinations occurring due to the error of the virus genome replication. The genetic changes actively occurring in the population allow rapid IBV adaptation to the host through the selection of the most suitable virus population. Such selection allows for the long survival of the virus in the host itself and in the host population and results in the changes of the virus pathogenesis and emergence of new IBV variants.

CONCLUSION

Results of the phylogenetic analysis of the IBV isolates recovered in 2015–2017 demonstrated that field isolates of eight genetic lineages of GI genotype including GI-1 (Macc), GI-12 (D274), GI-13 (793B), GI-14 (B1648), GI-16 (Q1), GI-19 (QX), GI-22, GI-23 (Variant-2)) circulated in the Russian Federation. The isolates of genetic lineages GI-16

(Q1), GI-22 and GI-23 (Variant-2) were detected on the RF poultry farms for the first time. Besides, some recombinant isolates and variant isolates were recovered that belonged to neither of the known genotypes.

Despite the repeated IB vaccination using strains H-120 and 4/91, the spread of the isolates belonging to genetic lineage GI-19 (QX) over the poultry farms in the Russian Federation is still continuing. The IB vaccination strategy should be reviewed on the poultry farms where isolates GI-19 (QX) are detected.

Analysis of the IBV isolates recovered on some poultry farms during several years demonstrated that change of the virus genetic lineages on the farm in the course of time preconditions the need for continuous monitoring of circulating IBV isolates for further optimization of the prevention scheme.

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