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Short communication

First characterization of a Middle-East GI-23 lineage (Var2-like) of infectious bronchitis virus in Europe

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ABSTRACT

Variants assigned to GI-23 lineage of infectious bronchitis virus (IBV), formerly called Var2, have circulated for nearly 20 years only in countries of the Middle East. Strains of this lineage were first identified in Israel in 1998. More severe form of the virus appeared in 2006, when the second wave of Var2 epidemic has spread over the Middle East region. The present study describes the detection and detailed genetic characterization of the GI-23 viruses in Poland. The full-length genome of gammaCoV/Ck/Poland/G052/2016 strain consists of 27596 nucleotides and has typical organization for IBV (UTR5′-POl-S-3a-3b-E-M-4b-4c-5a-5b-N-UTR3′). The phylogenetic analysis of the complete sequence showed that it formed separate branch distinct from all of the full-length genome sequences analyzed in this study. Recombination analyses with other gammacoronaviruses revealed that Polish GI-23 strain may originate from recombination events and potential donors of build-in sequences are IBV of GI-1, GI-13 and G-19 lineages (Mass-, 793B- and QX-like strains, respectively). The 1a, 1b and N genes were involved in these recombination events. The source of virus introduction to the chicken population in Poland is unknown.

The genus *Gammacoronavirus* within the family *Coronaviridae*, in the order *Nidovirales* includes infectious bronchitis virus (IBV), turkey coronavirus (TCoV) and guinea fowl coronavirus (GfCoV) that infect different poultry species. This genus also contains other coronaviruses isolated from wild birds and from non-avian hosts (Carstens, 2010). IBV is ubiquitous in most parts of the world and is responsible for enormous economic losses in chicken production. Depending on the method used for differentiation, many different serotypes, genotypes or protectotypes of IBV have been recognized (de Wit et al., 2011). Today, genotyping based on the S1 gene fragment is the most commonly used system for IBV classification, although the lack of standard rules concerning the S1 gene region to be analysed and the absence of uniformity in the nomenclature of the genetic groups, makes interpretation very difficult. Recently, a new classification based on the whole S1 gene (about 1600 nt) analysis has been proposed. It distinguished and named 32 lineages, comprised into 6 genotypes (GI to GVI) (Valastro et al., 2016). Some of these lineages are widely distributed in several continents or countries as GI-1 (formerly Mass-like), GI-13 (793B) and GI-19 (QX), whereas others, like the GI-16 (Q1), GI-21 (Italy02) and GI-23 (Var2), are more narrowly distributed (de Wit et al., 2011; Valastro et al., 2016).

Variants assigned to GI-23 lineage of IBV have circulated only in the Middle Eastern countries for nearly 20 years. Strains of this lineage were first identified in Israel in 1998, just two years after the occurrence of first IBV outbreak in the country caused by Var1 (Callison et al., 2001), currently classified as GI-13 lineage. In subsequent years, a new strain genetically similar to Var2, so-called IS/720-like, was found in chickens suffering respiratory and urinary health problems in this country (Meir et al., 2004). Over the years, the number of cases of the disease caused by IS/720-like variant gradually decreased until 2006, when the second wave of GI-23 (Var2) epidemic occurred. The new GI-23 viruses were more pathogenic and caused enormous losses in Israeli poultry industry, forcing the implementation of a new homologous vaccine (with IS/1494/06 strain) in 2010 (Even-Chen et al., 2014). The GI-23 IBV strain rapidly spread to other Middle Eastern countries. In 2009, it appeared in Jordan and in northern Iraq (Seger et al., 2016). Molecular monitoring of IBV strains circulating in Iran revealed that most detected strains belonged to IS/720 and Var2 (both GI-23), which were responsible for 18.4% and 17.2% of clinical cases of IB, respectively (Hosseini et al., 2015). In Egypt, this IBV lineage has been circulating since 2010 and by 2012 has dominated among other strains (Hussein et al., 2014; Selim et al., 2013; Zanaty et al., 2016). In

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2011 and 2012 the GI-23 strains has been identified in Turkey and Libya, respectively (Awad et al., 2014; Kahya et al., 2013; Yilmaz et al., 2016). Later on, the presence of IBV GI-23 was found in Saudi Arabia, Kuwait, Bahrain, Armenia, and since 2015 also in Russia, Lithuania and Ukraine (personal information from Ashash U., Israel). The IBV GI-23 lineage was detected in unvaccinated and vaccinated broilers, layers and broiler breeders and caused respiratory system disease, kidney damage and drop in egg production (Hussein et al., 2014; Kahya et al., 2013; Selim et al., 2013). Intensive international trade, uncontrolled movement of people and animals as well as of wild birds across the borders of the Middle Eastern countries were suspected as the cause of the spread of the GI-23 lineage (Domanska-Blicharz et al., 2014; Hussein et al., 2014; Kahya et al., 2013). In this short communication, we report the results from studies on IBV circulating in Poland conducted over the period of six months and also from sequencing of the complete genome of IBV belonging to the GI-23 lineage identified for the first time in Poland.

The first case of IBV belonging to the GI-23 IBV lineage (gammaCoV/Ck/Poland/G229/2015) was identified in Poland in December 2015 and occurred in a commercial Ross broiler flock vaccinated with IBV Mass-like (GI-1) strain at the hatchery. Clinical signs of depression, sudden decrease in feed consumption, diarrhea and increased mortality (7%) were observed in 6 week-old chickens. Postmortem examination revealed enlarged and congested livers and kidneys. Sample supernatants of kidneys suspensions (10% w/v) prepared in sterile phosphate-bufferred saline (PBS) were used for viral RNA isolation (QIAamp Viral RNA Mini Kit, Qiagen, Germany) according to the manufacturer's instructions. Real-time RT-PCR targeting the 5′ untranslated region (5′UTR) fragment, as previously described (Callison et al., 2006), was conducted using the QuantiTect Probe RT-PCR Kit (Qiagen, Germany) in a 7500 Real Time PCR System (Applied Biosystems, USA). For preliminary genotype determination adopted in our laboratory for diagnostic purposes, primer set mixed with chemistry offered by the One-Step RT-PCR kit (Qiagen, Germany) which enabled amplification of approximately 400-bp nucleotide sequence covering one of the hypervariable region of S1 gene (aa 274–387) was used (Worthington et al., 2008). Obtained amplicons were sequenced in both directions using Sanger sequencing technology by Genomed Sp. z o.o. (Warsaw, Poland). The forward and reverse nucleotide sequences were edited and aligned in the final consensus sequence using the SeqMan program (DNASTAR, Madison, WI). They were then compared with sequences published in GenBank database using Basic Local Alignment Search Tool (BLAST). The partial S1 gene of gammaCoV/Ck/Poland/G229/2015, obtained from RNA isolated from infected kidneys, revealed 99% nucleotide identity with the Var2 IBV IS/1494/06 strain of the GI-23 lineage (GenBank accession number HM131453). During the next six months up to June 2016 another organ/tissues samples from forty-nine chicken flocks, both commercial layers and broilers, were submitted to the Department of Poultry Diseases, National Veterinary Research Institute, Pulawy, Poland for disease diagnosis and thirty-nine (79.6%) of them were IBV positive. For all positives, IBV genotype determination using above described method was applied. BLAST analysis of obtained about 400-nt fragments of the S1 gene revealed that ten (25.6%) of the positive flocks were infected with the Var2 variant. Furthermore, in nineteen (48.7%) and ten (25.5%) flocks 793B-like and QX-like IBV strains, respectively were identified. Symptoms like mild to medium respiratory signs and watery diarrhea were observed in most of Var2-infected chickens. To confirm the classification of the Var2 virus according to the recently proposed rules (Valastro et al., 2016), the whole S1 gene of 6 samples with high virus load in infected tissues was amplified with set of specific primers (File S1). Multiple alignments of sequences were performed using Clustal W. Maximum likelihood (ML) and Neighbor-joining (NJ) phylogenetic analyses of each gene and of the complete genome, were conducted in MEGA v6 using the best-fitting nucleotide substitution models. Bootstrap analysis of the resultant trees was performed using 1000 replicates

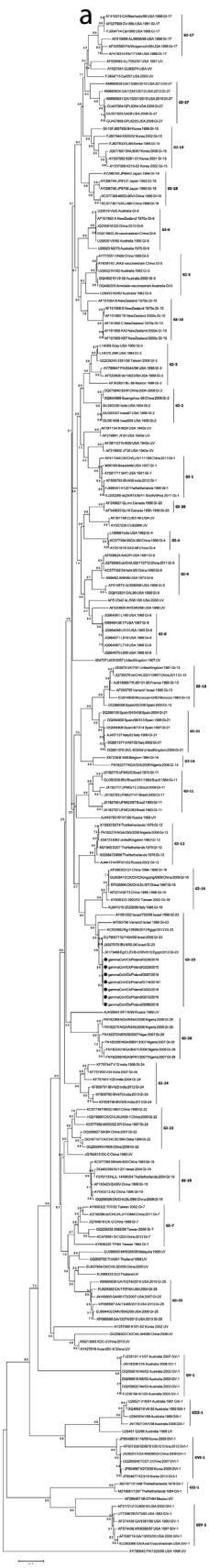

Fig. 1. Phylogenetic tree of complete S1 nucleotide sequences (1456 nt) of 199 reference and 7 Polish IBV strains (with black dots) (a). Separate subtree of GI-23 lineage of IBV (b).