

# Recombinant turkey coronavirus: are some S gene structures of gammacoronaviruses especially prone to exchange?

Katarzyna Domanska-Blicharz,<sup>1</sup> and Joanna Sajewicz-Krukowska

*Department of Poultry Diseases, National Veterinary Research Institute, 24-100 Puławy, Poland*

**ABSTRACT** The objective of the present study was to characterize the atypical turkey coronavirus strain detected in a commercial meat turkey farm in Poland. Using the viral metagenomics approach, we obtained a complete genome sequence of coronavirus, isolated from duodenum samples of animals suffering from acute enteritis. The nearly full-length genome consisted of 27,614 nucleotides and presented a typical genetic organization similar to that of Polish infectious bronchitis virus (IBV) or French turkey coronavirus/guinea fowl coronavirus strains. Phylogenetic analysis based on both the full-length genome and the whole S gene suggested that gCoV/Tk/Poland/G160/2016 is related to turkey and

guinea fowl coronavirus and not IBV strains. Sequence analysis of the genome revealed unique genetic characteristics of the present strain, demonstrating that the virus emerged as a result of the exchange of the S gene of IBV GI-19 lineage with the S gene related to the North American turkey coronaviruses and French guinea fowl coronaviruses. Analysis of earlier, similar recombinations suggests that both the S gene structures may be particularly mobile, willingly switching between different gammacoronavirus genomic backbones. The identified recombinant caused a severe course of the disease, which may imply that it is in the first phase of breaking the barriers between different bird species.

**Key words:** turkey coronavirus, recombination, phylogenetic analysis, Poland

2021 Poultry Science 100:101018  
<https://doi.org/10.1016/j.psj.2021.101018>

## INTRODUCTION

Members of the Orthocoronavirinae subfamily in the Coronaviridae family and the Nidovirales order are enveloped viruses with a positive-sense, single-strand, nonsegmented RNA genome of approximately 27–32 kb in size. This subfamily consists of 4 genera—*Alphacoronavirus*, *Betacoronavirus*, *Gammacoronavirus*, and *Deltacoronavirus*. Only gammacoronaviruses and deltacoronaviruses infect bird species, but some of them can also infect mammals (Cui et al., 2019). Currently, 2 of the existing 3 *Gammacoronavirus* subgenera—*Igacovirus* and *Brangacovirus*—were identified in birds, and the third—*Cegacovirus*—is identified in a marine mammal, beluga whale (SW1 virus) (ICTV 2020). The main representative of avian coronavirus (ACoV) species of the *Igacovirus* subgenus is the infectious

bronchitis virus (IBV). This is the first coronavirus discovered in the world since the disease was described in 1931, with viral etiology confirmed 5 yr later (Schalk 1931; Beach and Schalm 1936). Subsequent members of ACoV are turkey coronavirus (TCoV) and guinea fowl coronavirus (GfCoV) (Jackwood et al., 2010; Liais et al., 2014).

Infectious bronchitis virus is a highly contagious virus responsible for respiratory diseases, nephritis, reproductive disorders, and sometimes digestive tract disorders in chickens. Despite the research on the virus that has been carried out for over 84 yr and the availability of many live attenuated and inactivated vaccines, the disease still causes large economic losses in the poultry industry worldwide (de Wit et al., 2011; Jackwood 2012; Jordan 2017). The disease of young turkeys was first identified in the 1970s of the last century in the USA, where for years, it was known as blue comb disease, as mud fever, or more recently as turkey coronavirus (Guy, 2013). Turkey coronavirus is responsible for enteritis called poult enteritis complex or a more acute form of enteritis known as poult enteritis mortality syndrome. In Europe, the first TCoV was isolated in 2008 from turkey poultlets exhibiting clinical signs compatible with poult enteritis complex (Maurel et al., 2011). Guinea fowl coronaviruses were identified for the first time in 2011 in France as the

© 2021 Published by Elsevier Inc. on behalf of Poultry Science Association Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Received June 2, 2020.

Accepted January 19, 2021.

The nucleotide sequence data reported in this article have been submitted to the GenBank database and have been assigned the accession number MT367412.

<sup>1</sup>Corresponding author: [domanska@piwet.pulawy.pl](mailto:domanska@piwet.pulawy.pl)

causative agent responsible for peracute enteritis called fulminating disease (Liais et al., 2014).

Avian coronaviruses have similar phylogenetic relationships and genomic structures. Their genome consists of 15 nonstructural proteins encoded by open reading frame (ORF) 1a/b at the 5'-end, followed by the structural proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N) encoded by other ORF at the 3'-end. This part of the genome also encodes a few low-molecular-weight accessory proteins whose number and nature vary depending on the species and even the ACoV strain. These proteins would be 3a, 3b, 4b, 4c, 5a, and 5b, although TCoV, GfCoV, and some IBV strains also have an ORF NS6 located downstream of the nucleocapsid protein encoding the 6b protein (Maurel et al., 2011; Ducatez et al., 2015; Brown et al., 2016; Domanska-Blicharz et al., 2020). In addition to their similar genome structure, these strains also have a close genetic relationship as nucleotide similarity of whole genomes between TCoV/GfCoV and IBV strains is higher than 86%. The most different part of the genome between these viruses is the S gene that shares at most 36% identity (Jackwood et al., 2012; Brown et al., 2016). Deep molecular analysis suggests that TCoV and GfCoV might emerge from recombination events between IBV and another unknown coronavirus, the donor of the S gene (Jackwood et al., 2010; Brown et al., 2016). Moreover, a comparison of the full-length genomes of the only European (French) and North American TCoV strains indicates that they would have different evolutionary pathways in North America and Europe (Brown et al., 2016). The different S genes affected the tropism of the virus as acquiring such a structure of this gene caused an affinity switch from the respiratory/renal system observed in the case of IBV to the digestive system in the case of TCoV/GfCoV (Wickramasinghe et al., 2015; Bouwman et al., 2019).

The presence of IBV was identified for the first time in the chicken population in Poland at the end of the 1960s, and then, a few waves of epidemics caused by different IBV variants appeared every 8–11 yr: GI-1 in 1988, GI-13 in 1997, GI-19 in 2004, and GI-23 in 2015. During this period, IBV strains described as unique early Polish variants and recombinants were also detected (Domanska-Blicharz et al., 2020). In turn, the prevalence of TCoV in the Polish turkey population was estimated at the level of about 6.5%. The S gene structure of Polish TCoV was similar to other French/European TCoV. So far, no viruses similar to GfCoV have been detected. The objective of the present study was to characterize the atypical TCoV strain detected in a Polish turkey farm.

## MATERIALS AND METHODS

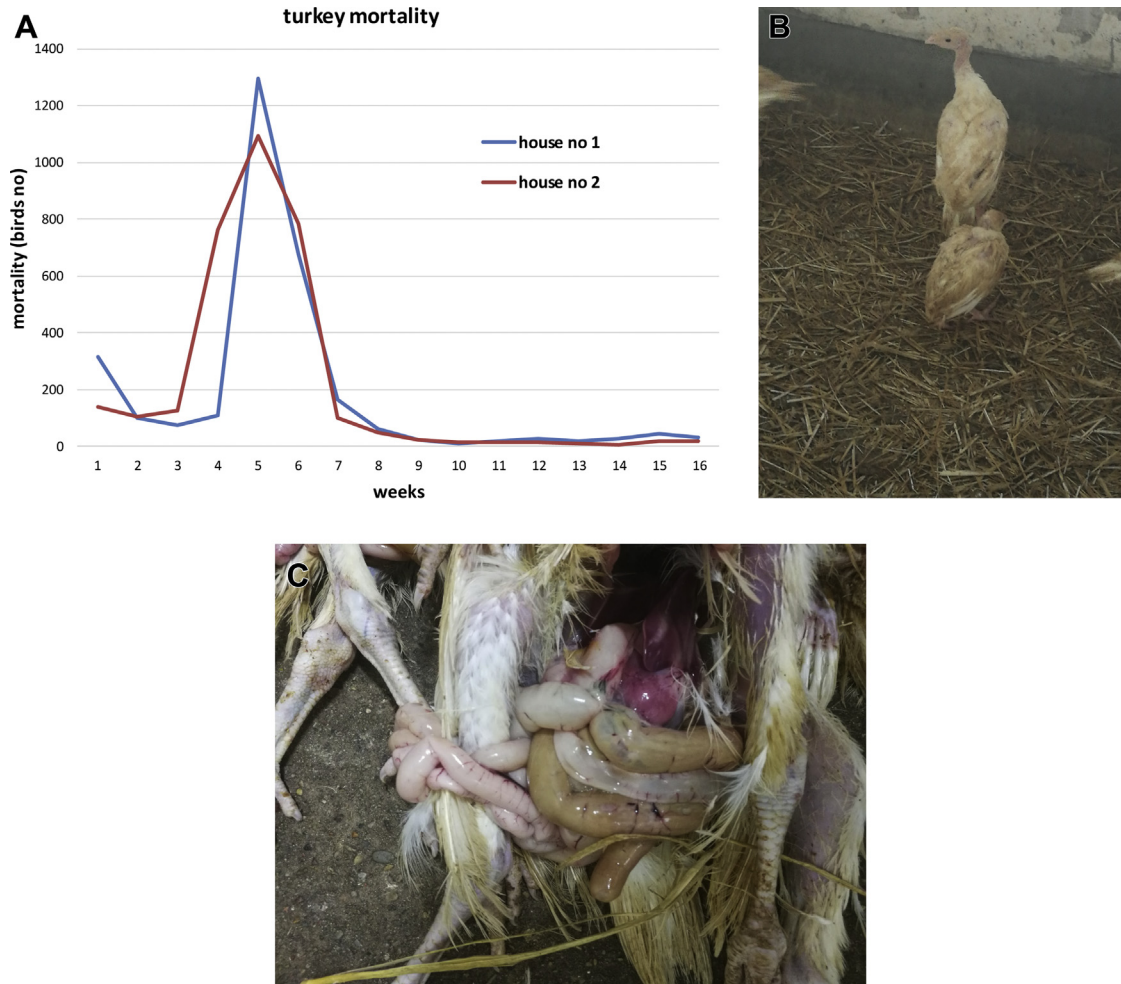
### Flock and Sample Collection

The meat turkeys (Big6) in the number of 29,042 females were reared in a commercial farm consisting of 2 houses. In the hatchery and on their 22nd day of life,

the turkeys were vaccinated against turkey rhinotracheitis (Poultac TRT; Zoetis Sp. z o.o., Warsaw, Poland) by spray. In addition, birds were immunized for the third time against turkey rhinotracheitis using the vaccine Aviffa RTI (MERIAL S.A.S., Lyon, France) on day 63 and against Newcastle disease using the vaccine Cevac Vitapest L (Ceva Santé Animale, Libourne, France) on day 71 via drinking water. In June 2016, an increase in mortality (on the 21st day of age in the first house and 28th day in the second house) began to be observed, which lasted for 4–5 wk. In the beginning, about 100–130 birds per week were found dead, but 2 wk later, on the turkeys' 35th day of life, this number increased dramatically to over 1,000 birds per week. Later on, the mortality of birds began to decline, and 3 wk later, it reached 20–30 birds per day (Figure 1A). Infected birds were apathetic and stunted (Figure 1B). In addition, they showed ruffled feathers and reduced feed and water intake, and their feces were watery and foamy with a greenish brown color, which sometimes contained mucosal fragments and urates. The consequence of the birds' diarrhea was dehydration. There was also a disturbance in thermoregulation—birds gathered around a heat source and clumped into groups. The postmortem examination of dead turkeys at the farm mainly revealed swollen, congested but sometimes also pale and thin-walled intestines, which were filled with gasified, watery content (Figure 1C). The total mortality rate was 21.6%. The overall performance of the turkey flock has deteriorated because the BW of the 15-wk-old bird was reduced by 4% (8.9 kg), with a 2.5% higher feed conversion rate (2.6 kg/kg of BW). Fragments of the duodenum collected from several (4–5) birds at 5, 9, and 14 wk of age were sent to the laboratory for testing.

### RNA Extraction and Molecular Methods

Samples delivered to the laboratory were kept refrigerated until testing. They were grounded and suspended (w/v) in phosphate-buffered saline. The suspensions were centrifuged at 3,000 × *g* for 15 min, and 200 μL of the obtained supernatants was used for nucleic acid isolation, which was carried out using the QIAamp cador Pathogen Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Few different molecular assays were applied for detection of viruses regarded as those that are mainly responsible for enteritis in turkeys. Real-time reverse transcription PCR (RT-PCR) was carried out as previously described for the amplification of a 143-bp 5'-UTR fragment of the coronavirus of the *Igacovirus* subgenus (Callison et al., 2006). The presence of astroviruses was detected by RT-PCR aimed at the RNA-dependent RNA polymerase gene fragment (Tang et al., 2005). For parvovirus identification, the amplification of NS1 of the *Aveparvovirus* gene was applied (Zsak et al., 2009). The amplification of the highly conserved NSP4 gene region was used for rotavirus A detection (Day et al., 2007). We also applied a set of primers previously used for S gene amplification of European TCoV (Maurel et al., 2011). In addition, Illumina



**Figure 1.** Disease symptoms of turkeys recorded in the infected flock: (A) mortality course observed for a 16-wk period; (B) turkey stunting noted at 10 wk of age; (C) changes in intestines observed during necropsy of dead turkeys: swollen and congested intestines.

MiSeq technology (Illumina Inc., San Diego) offered by the Department of Microbiology, National Veterinary Institute (SVA), Uppsala, Sweden, was applied for complete genome amplification. Shortly, 180  $\mu$ L of the filtered supernatant was treated with 20  $\mu$ L of TURBO DNase (Invitrogen, Waltham) and 2  $\mu$ L of RNase One (Promega, Madison) at 37°C for 30 min. RNA was extracted from 200  $\mu$ L of the treated supernatant using a combination of TRI (Sigma, Darmstadt, Germany) and Direct-zol RNA MiniPrep (Zymo Research, Irvine) and eluted in 30  $\mu$ L of DNase/RNase-free water. The first-strand synthesis was performed using the SuperScript IV First-Strand cDNA Synthesis Kit (Invitrogen, Waltham) according to the manufacturer's instructions. After treatment with RNase H at 37°C for 20 min, the second-strand synthesis was performed with the addition of the Klenow fragment (New England Biolabs, Ipswich) to the first-strand cDNA. The reaction was run at 37°C for 1 h before a 10-min termination step at 75°C. A DNA library was prepared using a Nextera XT sample preparation kit (Illumina Inc., San Diego) and then validated and quantified using a Bioanalyzer (Agilent, Santa Clara). Sequencing was performed on a MiSeq instrument using the MiSeq Reagent kit v3 (Illumina Inc.,

San Diego), according to the manufacturer's instructions.

### **Molecular Data Processing**

The sequencing data were processed using the CLC Genomics Workbench (Qiagen, Hilden, Germany). The sequencing reads were trimmed based on quality and *de novo* assembled into contigs, with which BLAST (National Center for Biotechnology Information, Bethesda) against a virus database was performed to identify matching virus species. The reads were also mapped to the available reference sequences, and then, the consensus sequence was extracted. The phylogenetic analysis of the complete genome was conducted to investigate the relationship of the obtained virus with different coronaviruses downloaded from GenBank including North American and French TCoV, GfCoV, and numerous IBV from different regions such as Poland, the USA, China, and Korea. For a comparison of S genes, more available sequences of avian gammacoronaviruses detected in turkeys, guinea fowl, and chickens were included. Multiple sequence alignments were generated using the Geneious 2020.1.1 program (Biomatters

**Table 1.** Region/gene positions and lengths (nt and aa) of the gCoV/Tk/Poland/G160/2016 strain.

| Region/gene | 5'-UTR | ORF1a      | ORF1ab     | S             | 3a            | 3b            | E             | M             | 4b            | 4c            | 5a            | 5b            | N             | 6b            | 3'-UTR        |
|-------------|--------|------------|------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Position    | 1-574  | 575-12,427 | 575-20,466 | 20,411-24,025 | 24,025-24,198 | 24,198-24,392 | 24,373-24,696 | 24,674-25,345 | 25,346-25,630 | 25,551-25,721 | 25,705-25,902 | 25,899-26,147 | 26,090-27,319 | 27,328-27,549 | 27,550-27,614 |
| Length (nt) | 574    | 11,853     | 19,818     | 3,615         | 174           | 195           | 324           | 672           | 285           | 171           | 198           | 249           | 1,230         | 222           | 64            |
| Length (aa) | -      | 3,950      | 6,606      | 1,204         | 57            | 64            | 107           | 223           | 94            | 56            | 65            | 82            | 409           | 73            | -             |

Abbreviation: ORF, open reading frame.

Ltd., Auckland, New Zealand), and the percentage of nucleotide and amino acid sequence similarities was assessed using the same software program. Phylogenetic trees of the S gene and the complete genome were generated from the aligned nucleotide sequences using the maximum likelihood method in MEGA version 7 (Molecular Evolutionary Genetics Analysis, Pennsylvania) using the best-fitting nucleotide substitution models. Bootstrap analyses of the resultant trees were performed using 1,000 replicates (Tamura et al., 2013). To detect any recombination events, the complete genome of the detected TCoV, and selected the most similar sequences were analyzed using 9 different methods available in RDP package version 4 (Center for Microbial Ecology, Michigan; RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan3Seq, LARD, and PhyPro) (Martin et al., 2015). Only the recombination events identified by at least 3 methods with a  $P$ -value lower than  $1.0 \times 10E-30$  were considered true. In addition, to visualize the similarity of particular fragments of the genome to different gammacoronaviruses, SimPlot version 3.5 (Phylip; <https://bio.tools/simplot>) analysis was also conducted. In all analyses performed using both software programs, the window and step sizes of 200 and 20, respectively, were used.

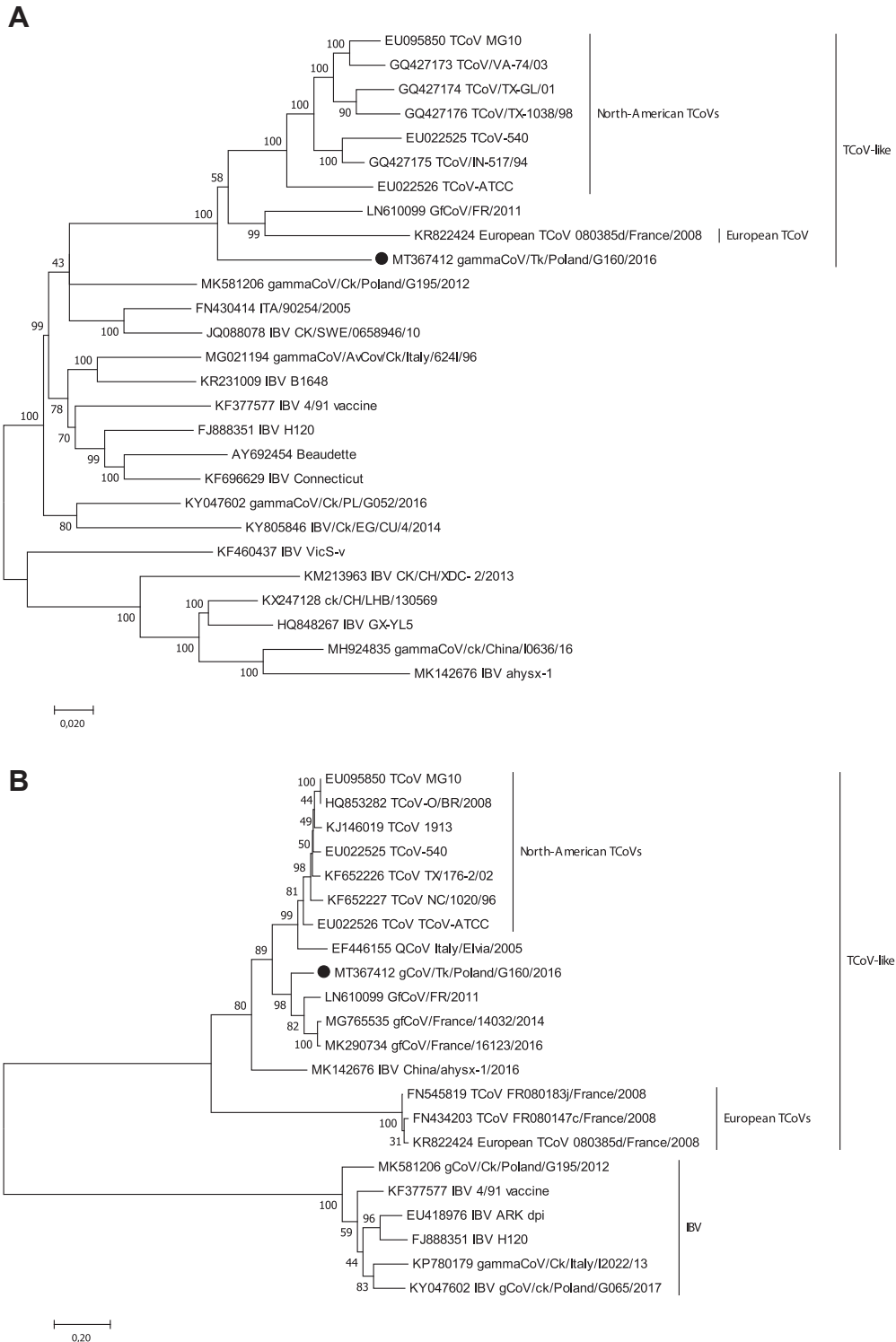
## RESULTS

### *Molecular Investigation*

Astroviruses, parvoviruses, and rotaviruses A were not identified, whereas real-time RT-PCR results for specimens from all 3 sampling times revealed the presence of gammacoronavirus. However, the threshold values obtained for samples collected from 5-, 9-, and 14-wk-old turkeys were different (16.7, 30.5, and 33.9, respectively). Unfortunately, the conventional molecular methods used to determine the S gene sequence of the detected coronavirus failed to produce the expected amplicons, despite the large amounts of virus indicated by the low Ct values obtained from 5-wk-old turkeys. The virus detected in 5-wk-old birds was named gamma-CoV/Tk/Poland/G160/2016 and further processed for whole-genome sequencing.

### *Genome Organization and Phylogenetic Analysis*

The nearly full-length genome consisted of 27,614 nucleotides and began from 5'-UTR, followed by 2 large ORF that encode polyproteins 1a (pp1a) and 1b (pp1b), then ORF S, 3a, 3b, E, M, 4b, 4c, 5a, 5b, N, and 6b, and ending with 3'-UTR (Table 1). The phylogeny based on the complete genome sequence revealed that the gCoV/Tk/Poland/G160/2016 strain was in the same branch of the phylogenetic tree as gammacoronaviruses isolated from turkeys and guinea fowl, which was different from IBV (Figure 2A). The full-length nucleotide genome of gCoV/Tk/Poland/G160/2016 had the closest similarity to the genome of GfCoV/



**Figure 2.** Phylogenetic analysis of (A) the complete genomes and (B) the S gene of gammacoronaviruses constructed using MEGA.

FR/2011 at the level of 88.3%. The next gammacoronaviruses with high similarity to gCoV/Tk/Poland/G160/2016 were North American TCoV, with a nucleotide sequence identity of 88.1–88.7%. The nucleotide identities with IBV strains ranged from 79.5 to 86.9% with CK/CH/XDC-2/2013 and gammaCoV/Ck/Poland/G195/2012, respectively. The homology to the whole genome of the Chinese ahysx-1/2016 strain was 81.4%. Similar alignment was obtained in the S gene

phylogenetic tree, with gCoV/Tk/Poland/G160/2016 being the most closely related, with 83.5–84% nucleotide identity to GfCoV/FR/2011 and other French GfCoV strains from 2014–2016 (Figure 2B). The next gammacoronaviruses with high similarity to gCoV/Tk/Poland/G160/2016 were North and South American TCoV strains, with the nucleotide sequence identity between 77.4 and 80.2%. In turn, the studied strain showed lower identity to Italian quail coronavirus and French

**Table 2.** Confirmation of the recombination event in the genome of the gCoV/Tk/Poland/G160/2016 strain.

| Methods  | Average $P$ -value, E-10 ( $10N^1$ ) |
|----------|--------------------------------------|
| RDP      | 3.038E-159                           |
| GENECONV | 2.664-173                            |
| BootScan | 1.639-123                            |
| MaxChi   | 1.312-55                             |
| Chimaera | 4.558-33                             |
| SiScan   | 4.164-83                             |
| 3Seq     | 1.165-14                             |

Average  $P$ -values obtained in the 7 methods available in RDP software.  
<sup>1</sup>Exponent in scientific notation.

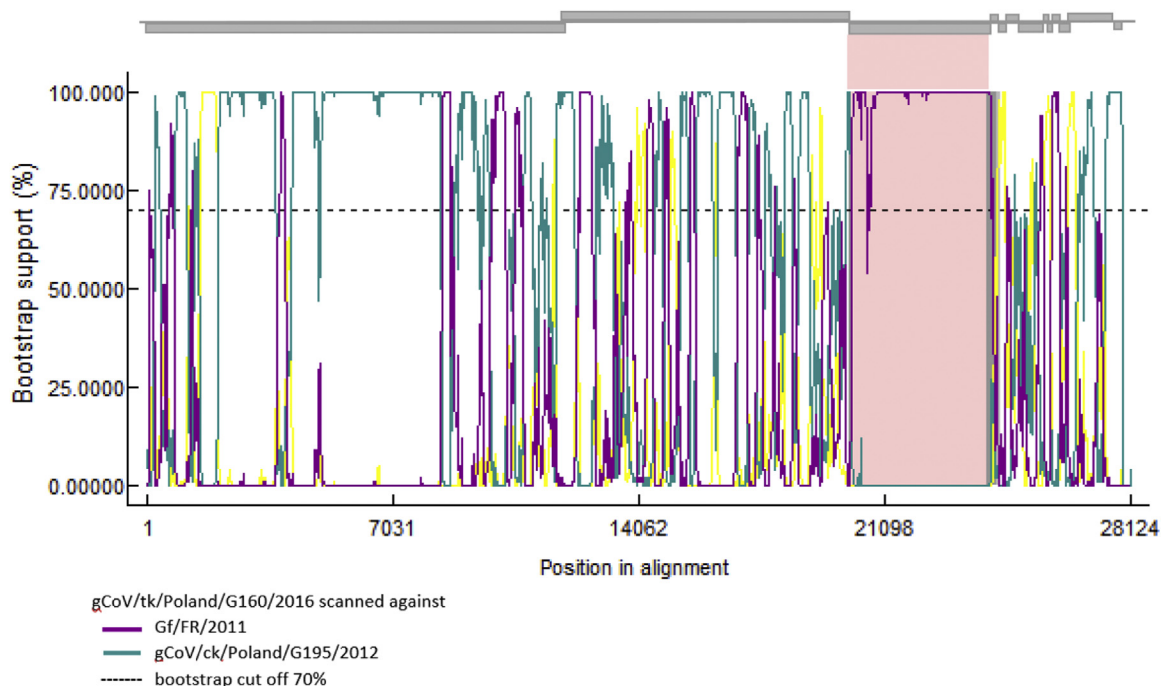
TCoV, at the level of 74.7 and 62.9–63%, respectively. The only IBV with a higher identity of 76.6% to the gCoV/Tk/Poland/G160/2016 strain was the Chinese ahysx-1/2016 strain, but the homologies of the other IBV strains that were compared were much lower, at the range of 45.6–46.1%.

### Recombination Analysis

Recombination analysis of aligned full-length gammacoronavirus sequences was performed to assess if the analyzed Polish TCoV strain is a recombinant. Our analysis spotted only one recombination event that meets previously set conditions as it was supported by 7 different methods, with a very good global KA  $P$ -value of 3.29E–173 (Table 2). This recombination occurred between the Polish IBV gCoV/Ck/Poland/G195/2012 strain (major parent) and Gf/F/2011 (minor parent), with the breaking point at the nucleotide position 20,099 and the end point at the nucleotide position 23,799. We confirmed this recombination event via Simplot analysis (<https://bio.tools/simplot>; Figure 3).

## DISCUSSION

We report the identification of a gammacoronavirus in turkey flock suffering from acute enteritis. Molecular features of this coronavirus, the probable genesis of its origin, and also the course of the induced disease seem to be interesting. Generally, the overall length and structure of the Polish TCoV genome are similar to those of Polish IBV strains or French TCoV/GfCoV. Phylogenetic analysis based on both the full-length genome and the whole S gene suggested that gCoV/Tk/Poland/G160/2016 is related to TCoV and GfCoV and not IBV strains. It branched in the cluster of European TCoV/GfCoV in the phylogenetic tree constructed on the whole-genome sequences analyzed in this study. In turn, the phylogenetic tree based on the whole S gene showed that gCoV/Tk/Poland/G160/2016 clustered closely with GfCoV and North American TCoV strains and separately from European TCoV. The recombination event was detected within the genome of the Polish TCoV strain, with predicted recombination points in 1b just before S and at the S end, clearly indicating that the S gene is the mobile fragment inserted into its genome. A member represented by Polish IBV of GI-19 lineage gCoV/Ck/Poland/G195/2012 was identified as the major potential parent and a member represented by GfCoV as the minor probable parent. Similar recombination positions were already previously reported by Ducatez et al., 2015, Brown et al., 2016, and Wang et al., 2020. Wang et al. (2020) stated that the IBV strain ahysx-1/2016 is a recombinant of Chinese IBV Ck/CH/LLN/131040 and North American TCoV strains. In turn, Brown et al., 2016 suggested that French TCoV 080385d and GfCoV/2011 strains are recombinants of European IBV ITA/90254/2005 and

**Figure 3.** Bootscan analysis of the recombination event based on pairwise distance, modeled with a window size of 200 and step size of 20.

different donors of the S gene, unknown in the case of the TCoV 080385d strain and related to North American TCoV in the case of GfCoV/2011. A thorough analysis of these recombinations indicates some common features. First of all, major parents—donors of the genome backbone of resultant recombinants—were IBV locally circulating in these regions. But interestingly, all 3 parental IBV strains (Italian ITA/90254/2005, Chinese Ck/CH/LLN/131040, and Polish gCoV/Ck/Poland/G195/2012) belonged to the GI-19 lineage (Brown et al., 2016; Wang et al., 2020). This observation may suggest the exceptional lability of the S gene of this IBV lineage, which has the property of being easily switched with an analog equivalent from other donors. Moreover, suchlike easy transfer property seems to have the S gene related to North American TCoV. A pool of such genes seems to circulate widely—in North and South America, Europe, and Asia (Jackwood et al., 2010; Moura-Alvarez et al., 2014; Ducatez et al., 2015; Wang et al., 2020). These S genes acquired from the recombination event are a driving force for changes of pathogenicity, host specificity, and tissue tropism, later on, undergoing genetic changes, which give viruses a further better adaptation for the new host. Full adaptation requires the virus to be “attenuated” so that it does not kill the host too quickly and provides a permanently persistent transmission chain. The examples of well-adapted pathogens are low pathogenic avian influenza viruses in waterfowl (Olsen et al., 2006). In this aspect, it seems interesting to track the severity of the disease in the animals in which coronaviruses with the North American S gene have been identified.

The coronavirus with the S gene similar to North American TCoV was the ahysx-1 strain identified in November 2016 in apparently healthy chickens in a commercial chicken farm in Anhui province, China (Wang et al., 2020). It cannot be ruled out that such well-adapted viruses to chickens are similar to the ahysx-1 strain. To date, no report of similar coronaviruses in chickens exists, but it may be due to diagnostic shortcomings that prevent reliable detection of a very diverse S gene of IBV. The ahysx-1-like IBV jumped to the turkeys and mutated. In field cases, such changed TCoV are generally detected in flocks affected by enteric diseases, sometimes accompanied with increased mortality, but it depends on the age of infected birds, concurrent infection, and management practices. On the other hand, some reports pointed out the prevalence of TCoV in healthy flocks. In experimental studies, disease symptoms are milder with moderate growth depression and negligible mortality. The Italy/Elvia/2005 strain similar to North American TCoV was detected in 2005 in diseased quail (*Coturnix coturnix*) flocks reared for restocking purposes (Circella et al., 2007). Affected birds showed depression, severe diarrhea, dehydration, reduced growth, and 5–10 or 70% mortality in adult and young birds, respectively. The presence of gammacoronavirus with North American TCoV-like S in guinea fowl was associated with a condition called fulminating disease as infected birds showed very acute

enteritis: severe prostration, a dramatic decrease in water and feed consumption, and daily mortality of up to 20%. Similar disease symptoms were also reproduced under laboratory conditions (Liais et al., 2014). Subsequent French GfCoV strains identified in field cases of fulmination disease in 2014 had the altered S gene, resulting in a higher affinity to guinea fowl coronavirus receptor binding. This phenomenon most probably reflects the process of the virus’s adaptation to this bird species (Bouwman et al., 2019). The symptoms observed in the Polish turkey flock seem to be similar to those observed in guinea fowl flocks in France infected with GfCoV. The severe course of the disease is usually identified as the first phase of breaking the interspecies barrier and indicates incomplete adaptation of the pathogen to the host. It would be extremely interesting to accurately trace the course of the disease and concomitant changes of the virus genome during several passages through the organisms of various bird species. Unfortunately, the main barrier in these types of studies is the problem with virus propagation in in vitro models.

In conclusion, we identified a recombinant TCoV strain from heavily diseased turkeys. Recombination analysis showed that this virus emerges as a result of the exchange of the S gene of GI-19 IBV lineage, with the S gene related to the North American TCoV and GfCoV lineage. Analysis of earlier, similar recombinations suggests that both the S gene structures may be particularly mobile, willingly switching between different gammacoronavirus genomic backbones. The identified recombinant caused a severe course of the disease, which may imply that it is in the first phase of breaking the barriers between different bird species. Understanding the mechanism of coronavirus jumping and adaptation to the new host is also key in the aspect of the current severe acute respiratory syndrome coronavirus 2 pandemic.

## ACKNOWLEDGMENTS

The authors wish to acknowledge Dr. Siamak Zohari and Karin Ullman (Department of Microbiology, National Veterinary Institute - SVA, Uppsala, Sweden) for their support while conducting next generation sequencing. authors also acknowledge Anna Lisowska and Justyna Opolska for their help in molecular diagnostic tests. This article does not contain any studies with animals performed by any of the authors. Sampling was carried out by a veterinarian, who took different samples as part of his routine work and under the permission of the farm owner. For these reasons, sampling did not require the approval of the ethics committee.

## DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## REFERENCES

- Beach, J. R., and O. W. Schalm. 1936. A filterable virus, distinct from that of laryngotracheitis, the cause of a respiratory disease of chicks. *Poult. Sci* 15:199–206.
- Bouwman, K. M., M. Delpont, F. Broszeit, R. Berger, E. A. W. S. Weerts, M.-N. Lucas, M. Delverdier, S. Belkasmi, A. Papanikolaou, G.-J. Boons, J.-L. Guérin, R. P. de Vries, M. F. Ducatez, and M. H. Verheije. 2019. Guinea fowl coronavirus diversity has phenotypic consequences for glycan and tissue binding. *J Virol* 93:e00067-19.
- Brown, P. A., F. Touzain, F. X. Briand, A. M. Gouilh, C. Courtillon, C. Allee, E. Lemaitre, C. De Boisseson, Y. Blanchard, and N. Etteradossi. 2016. First complete genome sequence of European Turkey coronavirus suggests complex recombination history related with US Turkey and Guinea fowl coronaviruses. *J. Gen. Virol.* 97:110–120.
- Callison, S. A., D. A. Hilt, T. O. Boynton, B. F. Sample, R. Robison, D. E. Swayne, and M. W. Jackwood. 2006. Development and evaluation of a real-time Taqman RT-PCR assay for the detection of infectious bronchitis virus from infected chickens. *J. Virol. Methods* 138:60–65.
- Circella, E., A. Camarda, V. Martella, G. Bruni, A. Lavazza, and C. Buonavoglia. 2007. Coronavirus associated with an enteric syndrome on a quail farm. *Avian Pathol.* 36:251–258.
- Cui, J., F. Li, and Z.-L. Shi. 2019. Origin and evolution of pathogenic coronaviruses. *Nat. Rev. Microbiol.* 17:181–192.
- Day, J. M., E. Spackman, and M. Pantin-Jackwood. 2007. A multiplex RT-PCR test for the differential identification of Turkey astrovirus type 1, Turkey astrovirus type 2, chicken astrovirus, avian nephritis virus, and avian rotavirus. *Avian Dis.* 51:681–684.
- de Wit, J. J., J. K. A. Cook, and H. M. J. F. van der Heijden. 2011. Infectious bronchitis virus variants: a review of the history, current situation and control measures. *Avian Pathol.* 40:223–235.
- Domanska-Blicharz, K., A. Lisowska, and J. Sajewicz-Krukowska. 2020. Molecular epidemiology of infectious bronchitis virus in Poland from 1980 to 2017. *Infect. Genet. Evol.* 80:104177.
- Ducatez, M. F., E. Liais, G. Croville, and J.-L. Guérin. 2015. Full genome sequence of Guinea fowl coronavirus associated with fulminating disease. *Virus Gen.* 50:514–517.
- Guy, J. S. 2013. Turkey Coronavirus Enteritis. Pages 376–381 in *Diseases of Poultry*. D. E. Swayne, ed. A John Wiley & Sons, Inc., Ames, Iowa.
- ICTV. 2020. International Committee on Taxonomy of viruses. Master species list 2019 v1. Accessed Nov. 2020. <https://talk.ictvonline.org/taxonomy/>.
- Jackwood, M. W. 2012. Review of infectious bronchitis virus around the world. *Avian Dis.* 56:634–641.
- Jackwood, M. W., T. O. Boynton, D. A. Hilt, E. T. McKinley, J. C. Kissinger, A. H. Paterson, J. Robertson, C. Lemke, A. W. McCall, S. M. Williams, J. W. Jackwood, and L. A. Byrd. 2010. Emergence of a group 3 coronavirus through recombination. *Virol* 398:98–108.
- Jackwood, M. W., D. Hall, and A. Handel. 2012. Molecular evolution and emergence of avian gammacoronaviruses. *Infect. Genet. Evol.* 12:1305–1311.
- Jordan, B. 2017. Vaccination against infectious bronchitis virus: a continuous challenge. *Vet. Microbiol.* 206:137–143.
- Liais, E., G. Croville, J. Mariette, M. Delverdier, M. N. Lucas, C. Klopp, J. Lluch, C. Donnadiou, J. S. Guy, L. Corrand, M. F. Ducatez, and J. L. Guerin. 2014. Novel avian coronavirus and fulminating disease in Guinea fowl, France. *Emerg. Infect. Dis.* 20:105–108.
- Martin, D. P., B. Murrell, M. Golden, A. Khoosal, and B. Muhire. 2015. RDP4: detection and analysis of recombination patterns in virus genomes. *Virus Evol.* 1:vev003.
- Maurel, S., D. Toquin, F. X. Briand, M. Queguiner, C. Allee, J. Bertin, L. Ravillion, C. Retaux, V. Turblin, H. Morvan, and A. J. Etteradossi. 2011. First full-length sequences of the S gene of European isolates reveal further diversity among Turkey coronaviruses. *Avian Pathol.* 40:179–189.
- Moura-Alvarez, J., L. F. Nunez, C. S. Astolfi-Ferreira, T. Knobl, J. L. Chacon, A. M. Moreno, R. C. Jones, and A. J. Ferreira. 2014. Detection of enteric pathogens in Turkey flocks affected with severe enteritis, in Brazil. *Trop. Anim. Health Prod.* 46:1051–1058.
- Olsen, B., V. J. Munster, A. Wallensten, J. Waldenstrom, A. D. M. E. Osterhaus, and R. A. M. Fouchier. 2006. Global patterns of influenza A virus in wild birds. *Science* 312:384–388.
- Schalk, A. F. 1931. An apparent new respiratory disease of baby chicks. *J. Am. Vet. Med. Assoc.* 78:413–422.
- Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30:2725–2729.
- Tang, Y., M. M. Ismail, and Y. M. Saif. 2005. Development of antigen-capture enzyme-linked immunosorbent assay and RT-PCR for detection of Turkey astroviruses. *Avian Dis.* 49:182–188.
- Wang, Y., X. Cui, X. Chen, S. Yang, Y. Ling, Q. Song, S. Zhu, L. Sun, C. Li, Y. Li, X. Deng, E. Delwart, and W. Zhang. 2020. A recombinant infectious bronchitis virus from a chicken with a spike gene closely related to that of a Turkey coronavirus. *Arch. Virol.* 165:703–707.
- Wickramasinghe, I. N. A., R. P. de Vries, E. A. W. S. Weerts, S. J. van Beurden, W. Peng, R. McBride, M. Ducatez, J. Guy, P. Brown, N. Etteradossi, A. Grone, J. C. Paulson, and M. H. Verheije. 2015. Novel receptor specificity of avian gammacoronaviruses that cause enteritis. *J. Virol.* 89:8783–8792.
- Zsak, L., K. O. Strother, and J. M. Day. 2009. Development of a polymerase chain reaction procedure for detection of chicken and Turkey parvovirus. *Avian Dis.* 53:83–88.