Research Note: First evidence of infectious bronchitis virus Middle-East GI-23 lineage (Var2-like) in Germany[1]

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ABSTRACT The infectious bronchitis virus Middle-East GI-23 lineage (Var2-like) was observed on a German broiler farm, for the first time. The animals suffered from respiratory and nephropathogenic disease. Gross lesions observed during necropsy included tracheitis, aerosacculitis, and nephritis. Tracheal swabs were tested positive for infectious bronchitis virus Middle-East GI-23 lineage (Var2-like) by PCR. Furthermore, sequence analysis of the S1 spike protein showed close relationship to the commercially available vaccine TAbic IBVAR206 and polish isolates.

Key words: infectious bronchitis virus, Middle-East GI-23 lineage, broiler, Germany

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INTRODUCTION

Infectious bronchitis (**IB**) is one of the most relevant viral diseases in poultry production. The avian infectious bronchitis virus (**IBV**), belonging to the family *Coronaviridae*, can cause severe economic losses, mainly because of the induced respiratory and urogenital tract pathology (Jackwood, 2012; Bande et al., 2016). Infections of the gastrointestinal tract are described as well (Bande et al., 2016). In layers, reduction of egg-production and egg-shell quality are the major economic consequences of an IBV infection. In broilers, reduced weight-gain and the increased risk for bacterial secondary infections are the main drivers of IBV-induced economic losses.

Avian IBV is a single-stranded, positive-sense RNA virus and classified as *Gammacoronavirus* (International Committee on Taxonomy of Viruses, http://wwwictvonline.org). Five structural proteins are encoded—the spike protein (S), the envelope protein (E), the membrane protein (M), and the nucleocapsid (N). There are several other nonstructural proteins encoded (Cavanagh, 2007). The S protein is responsible for virus binding and host cell entry and is an important inducer of neutralizing antibodies (Wickramasinghe et al., 2014). It is posttranslationally cleaved in the S1 and S2 subunit (Wickramasinghe et al., 2014). The S1 subunit is of main interest regarding genetic comparisons between IBV isolates. S1 nucleotide sequences of different serotypes differ up to 25% (Cavanagh, 2005). There are 3 hypervariable regions described within the S1 subunit sequence (HVR I aa 38-67, HVR II aa 91-141, HVR III aa 274-387) (Cavanagh et al., 1988), in which most nucleotide substitutions were located (Bande et al., 2017; Lin and Chen, 2017). It was shown that comparison of HVR I and whole S1 gene resulted in the same grouping data (Wang and Huang, 2000).

Prevention of infection by proper management and strict biosecurity can be enhanced by proper use of IBV vaccines, as specific postinfection therapies for viral disease are not known (Jordan, 2017). The high-diversity of IBV strains and the ongoing emergence of new strains, because of genetic drift, are big challenges for developing vaccines and vaccination programs. For the continuous improvement of those, the knowledge of circulating field viruses in the specific region is essential. Recently an IBV variant called Israel strain 2 belonging to the GI-23 lineage was detected in Europe (Valastro et al., 2016; Lisowska et al., 2017). The strain was first described in Israel, causing severe respiratory and nephropathogenic lesions (Meir et al., 2004). Here, we describe the first evidence of IBV Israel strain 2 in a German broiler farm.

MATERIAL AND METHODS

Animals

The broiler chickens (ROSS 308) were housed in a commercial broiler farm in Mecklenburg-Western

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[1]The nucleotide sequence data reported in the present study has been submitted to GenBank (http://www.ncbi.nlm.nih.gov) and has been assigned to accession number MN004969.

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Pomerania. The farm consisted of 5 areas, each containing 8 to 10 stables. Stocking density was 136.000 to 170.000 broiler per area. The houses were temperature and light controlled. Straw pellets were used as litter, and feed and water were given *ad libitum*. In January 2019, the chickens were hatched and placed on the farm the same day. In the hatchery, the chickens were vaccinated by spray with a live-attenuated IB QX strain (Poulvac IB QX; Zoetis, Berlin, Germany). At 10 D of age, the chickens got a second IB vaccination with a live-attenuated IB CR 88121 strain (Gallivac IB88 NEO; Merial, Hallbergmoos, Germany) mixed in drinking water. Additionally, birds were vaccinated against Newcastle disease (Avishield ND; Albrecht, Aulendorf, Germany) and Infectious bursal disease (AviPro Precise; Elanco Animal Health, Bad Homburg, Germany) on day 17 via drinking water. At 30 D of age, broilers stunted and showed ruffled feathers. In around 10% of the animals, headshaking and sneezing could be observed. The litter was becoming wet because of polyuria and diarrhea. Sick birds were stunned by manually applied blunt force and euthanized by cervical dislocation. In necropsy, animals showed congestion in the trachea and turbidity and foam in the air sacs. As a result of dehydration, the muscles appeared dark red. Ureters were congested, and kidneys were swollen. Five tracheae were removed and brought to the lab. On transport, samples were stored by 7°C.

RNA Isolation and Polymerase Chain Reaction

Tracheae were sampled with a sterile swab. The swabs were pooled and vortexed in 1 mL sterile Natrium Hypochlorite 0.9% (Carl Roth, Karlsruhe, Germany). RNA was isolated by column purification using the QIAamp cador pathogen Mini Kit (Qiagen, Hilden, Germany) following manufactures' instruction. The real-time reverse transcription polymerase chain reaction was carried out in a CFX96 touch cycler (Bio-Rad Laboratories, Hercules, CA) by using the Kylt IB-aCo Kit for the detection of Avian Coronaviruses and the Kylt IBV-Variant O2 Kit for the detection of IBV Middle-East GI-23 lineage (Var2-like) (both from AniCon Labor, Hoeltinghausen, Germany).

Sequence Analysis

The PCR product of the variant O2 real-time reverse transcription polymerase chain reaction was sequenced by the AniCon Labor. The 479 base pair (**bp**) fragment is coding for the S1 spike protein (bp 112–590, aa 38–196; GenBank: MN004969) and contains the HVR I and II. The sequence was compared with a prototype strain sequence of IBV GI-23 lineage (according to Valastro et al. (Valastro et al., 2016)) to polish isolates (Lisowska et al., 2017) and to the TAbic IBVAR206 vaccine strain using the same nucleotide segment (bp 112–590) for analysis. A bovine coronavirus sequence serves as outgroup. All sequences were downloaded from GenBank (http://www.ncbi.nlm.nih.gov). Using MEGA 7 software, the evolutionary history was inferred using the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993; Kumar et al., 2016). The tree with the highest log likelihood (−1364.42) is shown. Initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 10 nucleotide sequences. Codon positions included were first + second + third + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 472 positions in the final data set.

Additionally, nucleotide and amino acid differences between the isolate described here, the TAbic IBVAR206 vaccine strain, and the polish isolates were analyzed further.

RESULTS

Both PCR reactions show positive results, with a threshold cycle value of 21,6 for Avian Coronaviruses and 16,2 for IBV Middle-East GI-23 lineage, respectively.

The isolate described here clusters in the Middle-East G-23 lineage and shows close relationship to the commercially available vaccine TAbic IBVAR206 (Phibro, Teaneck, NJ) and to recently published Polish IBV GI-23 isolates (Lisowska et al., 2017) (Figure 1).

Comparing the nucleotide sequence of the German isolate, the polish isolates, and the vaccine strain show substitutions in 10 positions (Table 1). On bp 174, 276, and 277, the German isolate show unique nucleotide substitutions, which could not be observed in the other sequences. The substitution on position 277 resulted in

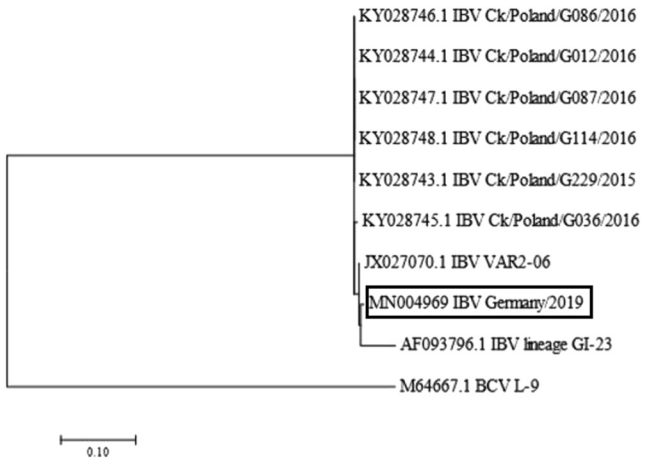

Figure 1. Phylogenetic tree of the German isolate, a prototype sequences of infectious bronchitis virus (IBV) GI-23 lineage, the TAbic IBVAR206 vaccine strain, and polish GI-23 strain sequences. A bovine coronavirus sequence serves as outgroup. The analysis is based on parts of the S1 protein, containing the HVR I and II (bp 112–590).

an amino acid substitution on position 93 (HVR II) from alanine to threonine (Table 1).

DISCUSSION

Surveillance studies in Europe mainly found IBV genotypes 793B, QX-like, Massachusetts, and D274 being the most prevalent ones (Worthington et al., 2008; Krapež, et al., 2011; Pohjola et al., 2014). The genotype Israel strain 2, detected here, was first described in Israel (Meir et al., 2004). In 2015, this virus strain was detected in broilers and layers in Poland (Lisowska et al., 2017). This was the first isolation in Europe. To our knowledge, we here describe the first isolation of the Middle-East GI-23 lineage (Var2-like) IBV genotype in Germany.

The symptoms observed in the case report are consistent with those described in former outbreaks in Egypt, Turkey, and Poland (Yilmaz et al., 2016; Zanaty et al., 2016; Lisowska et al., 2017). Birds showed respiratory as well as nephropathogenic symptoms. The peculiarity of symptoms was mild, and losses were low compared with other outbreaks, and this can be because of the vaccination scheme, consisting of 2 live-attenuated vaccines used. A combination of 2 live-attenuated vaccines from the genotype QX and Massachusetts and D274 combined with H120, respectively, was shown to have 50 and 70% protection (Bru et al., 2017).

Beside severe clinical symptoms and high mortality rate, reduced weight gain and other mild symptoms as wet litter can have high economic and animal welfare importance, as well. Feed cost dominates the direct costs of broiler production. Even, slightly reduced weight gain can have an enormous impact on feed conversion rate and thereby makes it uneconomic (Gocsik et al., 2014; Onsongo et al., 2018). Wet litter can cause severe cases of pododermatitis, which is an important indicator for animal welfare in Germany and which is monitored in the slaughter houses by the veterinary authorities (Granquist et al., 2019). Therefore, it seems to be very important to find sufficient alternatives for vaccination to improve the protection level. Recently, a new IB-VAR206 vaccine was proven infield to show a good efficacy to protect broilers from clinical illness after exposure to field virus (Elhady et al., 2018). Until now, this vaccine has not been approved in Europe and can thereby only be imported, having a special authorization from the responsible veterinary authorities.

The phylogenetic analysis shows the current isolate clustering in the GI-23 lineage, with the nearest phylogenetic relation toward the vaccine IBV VAR206 and the Polish isolates.

The small geographical distance between Poland and Mecklenburg-Western Pomerania allows the hypothesis of a virus entry from Poland. As the GI-23 strains show a high tendency for recombination, these could have

Table 1. Nucleotide and amino acid substitutions in HVR I and II of the S1 protein (bp 112–590) comparing the German (MN004969), 7 Polish (KY028743.1-48.1), and the vaccine strain (TAbic IBVAR206; JX0270701).

	aa 38				aa 57				aa 58			
GenBank accession number	bp 112	bp 113	bp 114	aa	bp 169	bp 170	bp 171	aa	bp 172	bp 173	bp 174	aa
KY028747.1	A	C	T	Thr	A	G	C	Ser	A	A	C	Asn
KY028748.1	A	C	T	Thr	A	G	C	Ser	A	A	C	Asn
KY028746.1	A	C	T	Thr	A	G	C	Ser	A	A	C	Asn
KY028744.1	A	C	T	Thr	A	G	C	Ser	A	A	C	Asn
KY028743.1	A	C	T	Thr	A	G	C	Ser	A	A	C	Asn
JX027070.1	A	C	T	Thr	A	G	C	Ser	A	A	C	Asn
KY028745.1	A	A	T	Asn	A	G	A	Arg	A	A	C	Asn
MN004969	A	C	T	Thr	A	G	C	Ser	A	A	T	Asn

	aa 61				aa 63				aa 92			
GenBank accession number	bp 181	bp 182	bp 183	aa	bp 187	bp 188	bp 189	aa	bp 274	bp 275	bp 276	aa
KY028747.1	T	C	A	Ser	C	A	G	Gln	T	C	A	Ser
KY028748.1	T	C	A	Ser	C	A	G	Gln	T	C	A	Ser
KY028746.1	T	C	A	Ser	C	A	G	Gln	T	C	A	Ser
KY028744.1	T	C	A	Ser	C	A	G	Gln	T	C	A	Ser
KY028743.1	T	C	A	Ser	C	A	G	Gln	T	C	A	Ser
JX027070.1	C	C	A	Pro	C	A	A	Gln	T	C	A	Ser
KY028745.1	T	C	A	Ser	C	A	G	Gln	T	C	A	Ser
MN004969	C	C	A	Pro	C	A	A	Gln	T	C	T	Ser

	aa 93				aa 94				aa 122			
GenBank accession number	bp 277	bp 278	bp 279	aa	bp 280	bp 281	bp 282	aa	bp 364	bp 365	bp 366	aa
KY028747.1	G	T	C	Val	A	G	C	Ser	C	C	T	Pro
KY028748.1	G	T	C	Val	A	G	C	Ser	C	C	T	Pro
KY028746.1	G	T	C	Val	A	G	C	Ser	C	C	T	Pro
KY028744.1	G	T	C	Val	A	G	M	?	C	C	T	Pro
KY028743.1	G	T	C	Val	A	G	C	Ser	C	C	T	Pro
JX027070.1	G	C	C	Ala	A	G	C	Ser	Y	C	T	?
KY028745.1	G	T	C	Val	A	G	C	Ser	C	C	T	Pro
MN004969	A	C	C	Thr	A	G	C	Ser	T	C	T	Ser

occurred between polish GI-23 IBV strains and German field or vaccine strains (Zanaty et al., 2016). This hypothesis has to be proved in future studies.

This report shows that even in cases, where a vaccination scheme is offering a broad cross-protection, the introduction of new IBV variants can lead to clinical cases of IB. In Germany, it could be necessary to adopt the vaccination scheme, using the commercial IB-VAR2 vaccine, in areas where the IBV genotype Israel strain 2 will become more prevalent.

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