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Coronavirus associated with an enteric syndrome on a quail farm

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An enteric syndrome was observed in quail (*Coturnix coturnix*) semi-intensively reared for restocking in Apulia (southern Italy). The birds showed depression, severe diarrhoea, dehydration and reduced growth. Mortality occurred particularly in young birds. At necropsy the prominent lesion was enteritis. A coronavirus was detected by electron microscopy and reverse transcriptase-polymerase chain reaction in the faeces and in the intestinal content of the dead quails. The virus could not be cultivated in chicken embryos. By sequence analyses of a fragment (409 nucleotides) of region 1b of the polymerase gene, the quail coronavirus displayed ≤93% nucleotide identity to avian coronaviruses (group 3 coronaviruses)—whereas by analysis of the S1 portion of the spike protein-encoding gene, the quail coronavirus displayed 16% to 18% amino acid identity with infectious bronchitis virus, and 79% to 81% identity with turkey coronavirus. Altogether, the findings suggest the existence of a novel coronavirus genetically related to turkey coronavirus.

Introduction

Coronaviruses (family *Coronaviridae*) belong to the order *Nidovirales*, and contain a positive-stranded RNA genome that ranges from 27 to 31 kb in size (Cavanagh, 1997). Members of the *Coronaviridae* infect a wide range of hosts, including mammals and avians, and have been classified into at least three groups on the basis of antigenic relationships, genome organization and sequence similarity (Gonzales *et al*., 2003).

Coronavirus (CoV) infections are of high relevance in the poultry industry, CoV-associated diseases having been known for many years. Infectious bronchitis virus (IBV) is a highly contagious disease of chickens that can affect a variety of tissues and organs, and IBV strains with various tissue tropisms have been identified (Cavanagh & Naqi, 1997).

Turkey CoV (TCoV) has a marked enteric tropism (Nagaraja & Pomeroy, 1997). Infections by TCoV are common in the USA, Canada and Australia (Nagaraja & Pomeroy, 1997) and are responsible for severe economic losses due to high mortality in young birds and decreased reproductive performances in adults (Guy *et al*., 2000; Guy 2003). TCoV-related diseases, characterized by low growth, enteritis and mortality, have recently also been observed in turkey flocks in Europe (Cavanagh *et al*., 2001). TCoV has been identified in Italy also, in turkeys affected by enteritis (Moreno *et al*., 2002).

Pheasant CoV (PhCoV) infection has been associated with respiratory and urinary syndromes (Spackman & Cameron, 1983; Gough *et al*., 1996; Pennycott, 2000). In Italy, PhCoV has been detected in pheasant with kidney lesions (De Marco *et al*., 1999). In addition, PhCoV may be associated with a drop in egg production in pheasants, similar to IBV infection in layer hens (Gough *et al*., 1998).

CoV infections are not necessarily associated with clinical signs as they have also been detected in the gut of a numbers of asymptomatic birds, including domestic peafowl, teal (Liu *et al*., 2005), graylag geese, mallard duck and feral pigeon (Jonassen *et al*., 2005).

In 2005, an enteric syndrome was observed in quail (*Coturnix coturnix*) reared in a farm in southern Italy, and a CoV strain was detected in the intestinal contents. In order to investigate the genetic features and the origin of the virus, the quail virus was characterized molecularly by analysis of the genes encoding the polymerase and the S protein.

Materials and Methods

Clinical and necropsy findings. An enteric syndrome was observed in a semi-intensive farm of European quail (*C. coturnix*) raised for restocking nature reserves and hunting purposes. Approximately 90 pairs of breeders were present in the farm. Mechanical incubation of the eggs was routinely adopted. After hatching, young birds were kept in cages until 20 to 25 days of age. Afterwards, the animals were transferred into an external, covered aviary. The syndrome appeared in spring 2005 and was first observed in 3-week-old birds, which were still housed in cages. The disease became more severe after the quails were transferred to the external aviary. The birds appeared weak and depressed, and presented severe diarrhoea, dehydration and a drop in feed consumption. Mortality ranged initially from 10% to 15% and subsequently increased to 70%, notably in the birds housed in the
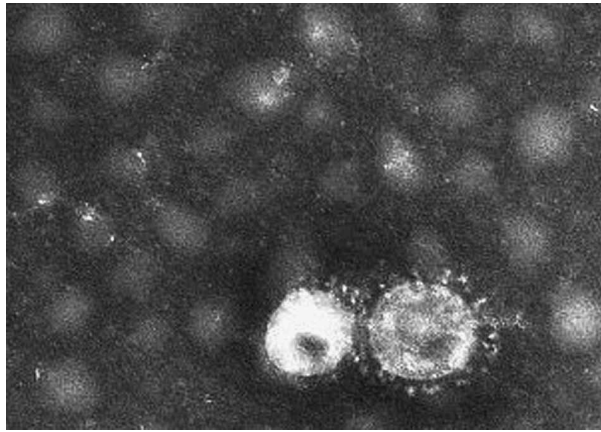
Figure 1.   *Electron micrograph of QCoV in the intestinal content of quails.*

external aviary. The recovered birds appeared under-sized and poorly feathered. Successively enteric signs were also observed in breeder quails, but in a milder form and with lower mortality (5% to 10%).

The carcasses of 10 animals were sent to our department for necropsy and laboratory investigations. The animals appeared dehydrated and underweight. Feathers were broken and rarefied. At necropsy, the most prominent gross lesion was enteritis, with the gut distended and filled with clear watery content. No additional gross lesions were observed in the examined animals. Histopathological examination revealed severe inflammatory reaction of the intestinal mucosa.

Bacteriological cultures. Samples of the liver, spleen, cardiac blood, kidney and gut were collected. Bacteriological cultures were performed on enrichment (Blood agar; OXOID) and selective media (MacConkey Agar; OXOID), with incubation in aerobic and anaerobic conditions at 37°C for 24 h.

Parasitological investigations. Fresh smears were prepared from the intestinal mucosa and the gut content for direct microscopic examination. Samples were also observed after flotation in saturated NaCl flotation fluid solution with 650 g/l glucose.

Virus isolation. Virus isolation was attempted using specific pathogen free embryonated chicken eggs. Three pools of gut samples were homogenized and diluted 1:10 in Eagle's minimal essential medium, treated with antibiotics (penicillin 5000 IU/ml, streptomycin 2500 μg/ml) at 37°C for 30 min, and inoculated into the amniotic and allantoic cavities of 10-day-old embryonated eggs. The eggs were incubated at
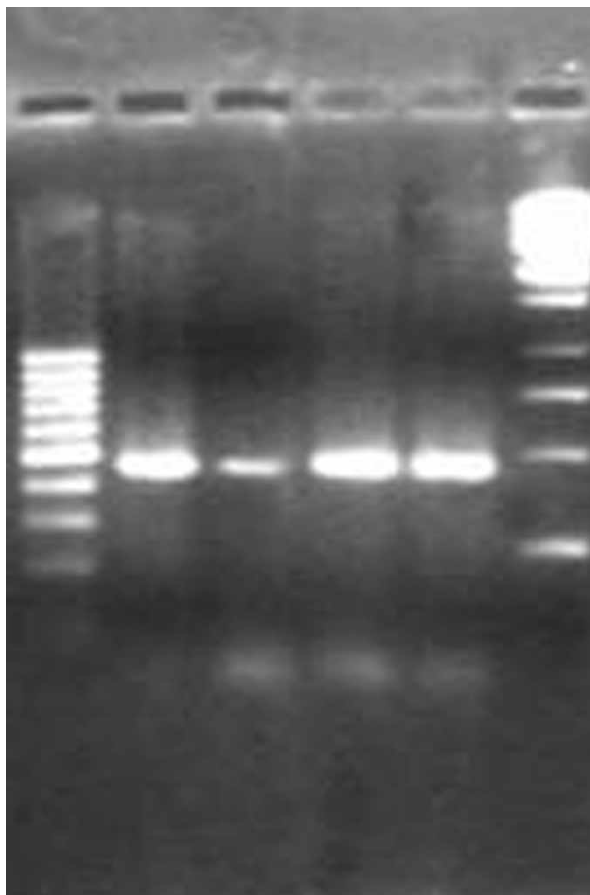

Figure 2.   *Results of the general (gene 1) RT-PCR for coronaviruses (primers IN-2/IN-4). Lane 1 (left), 100 bp DNA ladder (GeneRuler™; Fermentas Gmbh, Germany); lane 2, canine coronavirus; lanes 3 to 5, intestinal contents of quails, pools 1, 2 and 3, respectively; lane 6 (right), 1 kb DNA ladder (Gene Ruler™; Fermentas Gmbh).*
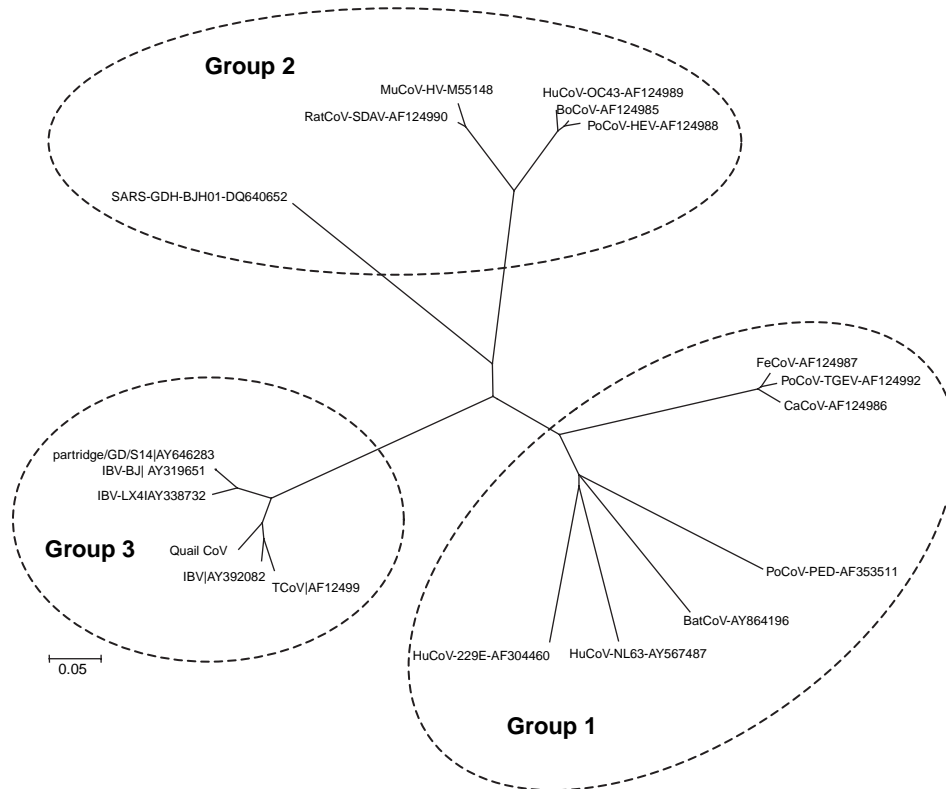
Figure 3.   *Phylogenetic tree based on the polymerase gene 1 fragment (409 nucleotides, corresponding to nucleotides 14 203 to 14 612 of the IBV Beaudette genome). The tree displays the genetic relationships between QCoV and representatives of the three genetic groups of the genus Coronavirus. The accession numbers of the sequences are presented.*

100°F and daily candled to check embryo viability. Embryo allantoic and amniotic fluids were harvested after 5 days of incubation. Three blind passages were performed in the same way.

Electron microscopy. Samples of intestinal content were examined by negative staining electron microscopy. The samples were diluted 1:10 in distilled water, vortexed and centrifuged for 20 min at 4000g and again for 10 min at 9300g for clarification. The supernatant was then ultracentrifuged (Beckman Airfuge) for 15 min at 82 000g. After negative staining with 2% sodium phosphotungstate (pH 6.8), samples were examined using a Philips CM10 electron microscope at 19 000x to 39 000x magnification. Embryo amniotic and allantoic fluids were also tested by electron microscopy.

Detection of coronavirus RNA by reverse transcriptase-polymerase chain reaction. Viral RNA was extracted from 100 µl homogenate obtained from three pools of gut and from five individual gut samples, using the GeneAmp RNA PCR Core Kit (Applera Italia, Monza, Italy). Samples of the liver, spleen, cardiac blood and kidney were also examined. Allantoic and amniotic fluids were also tested. Amplification was carried out by reverse transcriptase-polymerase chain reaction (RT-PCR) using primers IN-2 (sense) 5′-GGGTTGGGACTATCCTAAGTGTGA-3′ (nucleotides 14 180 to 14 203 on the genome of strain Beaudette CK, accession AJ311317) and IN-4 (antisense) 5′-TAACACACAACICCAT CATCA-3′ (nucleotides 14 632 to 14 612), targeting a highly conserved region (region 1b) of the coronavirus polymerase complex and designed to detect all members within the *Coronavirus* genus (Ksiazek et al., 2003). The product of the RT-PCR was 452 base pairs (bp), of which 409 bp were derived from the target viral RNA (i.e. excluding the primers).

RT-PCR was performed in a one-step procedure using Superscript III One step (Invitrogen, UK). The RT-PCR thermal cycling parameters were as follows: 50°C for 60 min and 94°C for 2 min for reverse transcription and for denaturation of the reverse transcriptase, respectively; and 40 cycles of 94°C for 1 min, 55°C for 1 min and 68°C for 2 min, with a final extension of 68°C for 10 min. The RT-PCR products (450 bp in length) were analysed by agarose gel electrophoresis and visualized after staining with ethidium bromide.

In order to amplify the S gene, primers S-cor (sense) 5′-TGAAAACTGAACAAAAGACAGACT-3′ (nucleotides 20 302 to 20 325) and AS-cor (antisense) 5′-CCAAACATACCAAGGCCACTT-3′ (nucleotides 23 658 to 23 638) were used (Lin et al., 2004).

Sequence and phylogenetic analyses. After purification on Ultrafree DA Columns (Amicon Millipore, Bedford, Massachusetts, USA), the amplicons underwent sequence analysis with the ABI-PRISM 377 (Perkin-Elmer, Applied Biosystems Division). The sequences were assembled and analysed using the Bioedit software package (Department of Microbiology, North Carolina State University, USA) (Hall, 1999) and the BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and FASTA (http://www.ebi.ac.uk/fasta33) web-based programs. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3 (Arizona State University, USA) (Kumar et al. 2004). The phylogenetic tree was elaborated with both parsimony and distance methods, supplying a statistical support with bootstrapping over 100 replicates. For phylogenetic analyses of the polymerase gene, representatives of the various coronavirus groups (groups 1, 2 and 3) were selected. In order to investigate the relationships between the quail CoV (QCoV) and group III avian CoVs, sequences of the S gene of chicken, turkey, pigeon, teal, partridge and parrot CoVs were retrieved from the databases.

Accession number. The partial sequence of region 1b of open reading frame 1 is available in Genbank under accession number EF446156. The sequence of the S1 subunit of the gene coding for the spike protein S gene is available in Genbank under accession number EF446155.

Results and Discussion

Bacterial and parasite pathogens were not detected in any of the organs of the examined quails. However, CoV-like viral particles were observed in the gut samples of the animals by electron microscopy (Figure 1). In addition, CoV RNA was detected using the broadly

Table 1. *Comparison of amino acid identities (%) based on the S1 subunit of QCoV with representatives of group 3 avian coronaviruses*

	QCoV	Turkey (AY342357)	Turkey (AY342356)	IBV (AY180958)	Teal (AY702975)	Partridge (AY646283)	IBV (X87238)	IBV-Beaudette (DQ001334)	IBV (DQ068701)	IBV (P05135)	IBV (AY189157)	IBV (P12722)	IBV (AY851295)	IBV (AY761141)	IBV (AF093793)	IBV (AF286302)	IBV (AF227438)	IBV-H120 (M21970)	IBV-H52 (AF352315)	Peafowl (AY702085)	IBV-793B (AY837465)	IBV-793/B (Z83979)	Pigeon-PSH050513 (DQ160004)	Pigeon (DQ099734)
QCoV	ID	81	79	17	17	16	16	17	15	17	17	17	18	17	17	15	15	17	17	17	17	17	16	16
Turkey-Gl (AY342357)	81	ID	92	17	18	18	17	17	16	18	17	17	18	18	17	16	16	17	18	18	17	17	17	17
Turkey-Gh (AY342356)	79	92	ID	17	17	17	17	18	16	18	17	18	18	18	17	16	16	18	18	18	17	17	17	17
IBV-LH2 (AY180958)	17	17	17	ID	84	85	76	76	75	76	95	76	75	75	77	75	75	75	75	75	77	77	79	79
Teal (AY702975)	17	18	17	84	ID	98	72	79	73	75	84	75	78	79	75	73	73	79	78	79	75	74	86	86
Partridge (AY646283)	16	18	17	85	98	ID	73	80	74	76	85	76	79	79	75	74	74	79	79	79	75	74	87	87
IBV-B1648 (X87238)	16	17	17	76	72	73	ID	75	76	79	75	79	74	74	77	76	76	74	74	75	77	77	73	73
IBV-Beau (DQ001334)	17	17	18	76	79	80	75	ID	73	77	76	77	97	97	74	74	74	96	97	97	74	74	78	78
IBV (DQ068701)	15	16	16	75	73	74	76	73	ID	80	75	79	73	73	75	98	98	73	73	73	75	74	74	74
IBV (P05135)	17	18	18	76	75	76	79	77	80	ID	76	99	77	77	78	80	81	77	77	77	78	78	75	75
IBV (AY189157)	17	17	17	95	84	85	75	76	75	76	ID	75	75	75	77	75	75	75	75	75	78	77	78	78
IBV-D274 (P12722)	17	17	18	76	75	76	79	77	79	99	75	ID	77	77	78	80	80	77	77	77	78	78	76	76
IBV-M41 (AY851295)	18	18	18	75	78	79	74	97	73	77	75	77	ID	96	73	73	74	96	96	96	74	74	77	77
IBV (AY7611419)	17	18	18	75	79	79	74	97	73	77	75	77	96	ID	74	73	74	99	99	99	74	74	77	77
IBV-4/91 (AF093793)	17	17	17	77	75	75	77	74	75	78	77	78	73	74	ID	76	76	74	74	74	99	94	76	76
IBV (AF286302)	15	16	16	75	73	74	76	74	98	80	75	80	73	73	76	ID	99	74	73	73	76	75	74	74
IBV (AF227438)	15	16	16	75	73	74	76	74	98	81	75	80	74	74	76	99	ID	74	74	74	76	75	74	74
IBV-H120 (M21970)	17	17	18	75	79	79	74	96	73	77	75	77	96	99	74	74	74	ID	98	99	75	75	77	77
IBV-H52 (AF352315)	17	18	18	75	78	79	74	97	73	77	75	77	96	99	74	73	74	98	ID	99	74	74	77	77
Peafowl (AY702085)	17	18	18	75	79	79	75	97	73	77	75	77	96	99	74	73	74	99	99	ID	74	74	78	78
IBV-793B (AY837465)	17	17	17	77	75	75	77	74	75	78	78	78	74	74	99	76	76	75	74	74	ID	95	76	76
IBV-793/B (Z83979)	17	17	17	77	74	74	77	74	74	78	77	78	74	74	94	75	75	75	74	74	95	ID	74	74
Pigeon-PSH050513 (DQ160004)	16	17	17	79	86	87	73	78	74	75	78	76	77	77	76	74	74	77	77	78	76	74	ID	100
Pigeon/Ch/05 (DQ099734)	16	17	17	79	86	87	73	78	74	75	78	76	77	77	76	74	74	77	77	78	76	74	100	ID

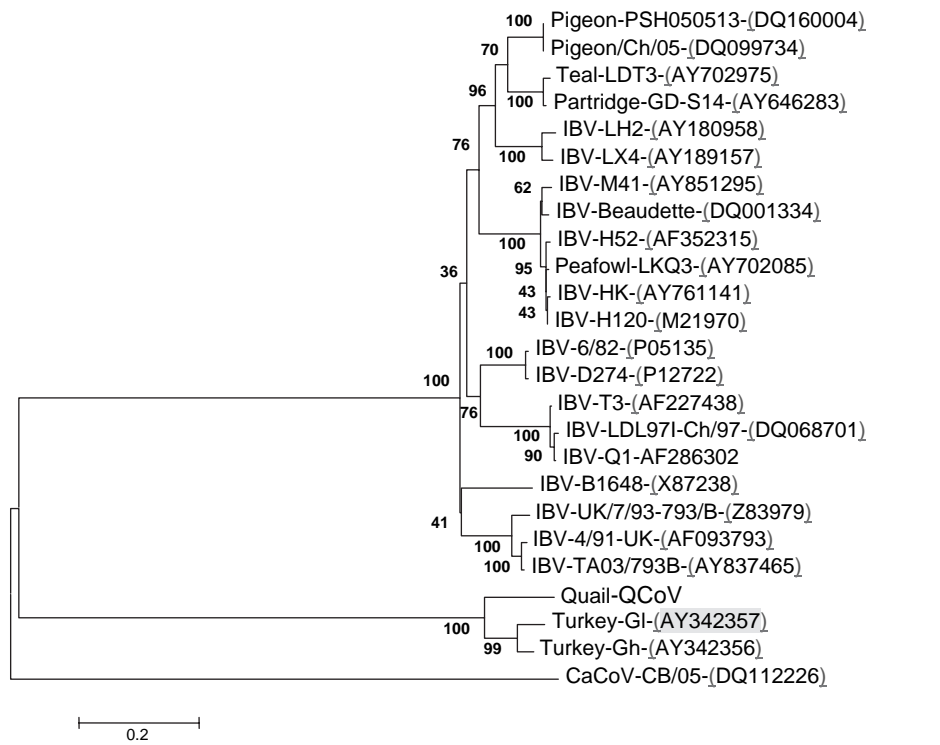Accession numbers indicated in parentheses.

Figure 4. *Phylogenetic tree based on the S1 subunit of the S protein displaying the genetic relationships of QCoV with reference IBV and TCoV strains, and coronaviruses of pigeon, peafowl, teal and partridge. Accession numbers are presented.*

reactive primers IN-2 and IN-4, which are able to recognize most viruses within the *Coronavirus* genus. An amplicon of the expected size (450 bp) was obtained from the pooled gut samples (Figure 2) and from the gut of three out of five animals examined individually. All tested samples of the liver, spleen, cardiac blood and kidney were RT-PCR negative.

CoV-like particles have been observed already by electron microscopy in nasal and tracheal swabs of quails exhibiting a respiratory syndrome (Pascucci *et al*., 1983) but the viruses were not further characterized. Accordingly, the findings of the present study provide the first convincing genomic evidence for the presence of CoV in quails (QCoV). We determined 409 nucleotides of region 1b of the polymerase gene, spanning nucleotides 14 203 to 14 612 (in comparison with the genome of IBV Beaudette). By BLAST and FASTA analysis, the fragment displayed ≤93% nucleotide identity with avian coronaviruses (group 3). A phylogenetic tree was elaborated using a selection of cognate sequences of coronaviruses retrieved from the databases. In the tree (Figure 3), the QCoV was grouped along with CoVs representative of coronavirus group 3. Due to the different genome region targeted by the diagnostic primers (Stephensen *et al*., 1999; Ksiazek *et al*., 2003), in our phylogeny it was not possible to include the polymerase sequences of CoV from parrot, graylag goose, mallard duck and pigeon; only sequences of chicken CoV and TCoV were used.

In order to evaluate precisely the genetic relationships between the QCoV and other avian CoVs, the sequence of the S1 portion of the S protein gene was determined. S1-based sequence comparison (Table 1) and phylogenetic analysis (Figures 4) provided interesting clues to understand the nature of the virus. The S1 of QCoV showed low amino acid identity to all the IBV strains available in the databases. The amino acid sequence identity with IBVs was 16% to 18%, suggesting that

QCoV is not an IBV variant. In contrast, the S1 of QCoV displayed 79% to 81% amino acid identity with TCoV strains. IBV strains within the same serotype usually share >95% amino acid identity (Cavanagh *et al*., 2001) while IBV strains of other serotypes may share 75% to 85% amino acid identity, or, in some instances, as low as 60% (Cavanagh, 2005). According to these classification criteria, the QCoV strain appears to be a TCoV-like virus.

Attempts to cultivate QCoV in chicken embryos were not successful. IBV grows in the allantoic sac of chicken embryos, triggering typical embryonic lesions (Clarke *et al*., 1972; De Wit, 2000). Likewise, PhCoV can be cultivated in the allantoic cavity of domestic fowl eggs (Lister *et al*., 1985; Gough *et al*., 1996). PhCoV shares about 80% amino acid identity in the S1 protein with IBV (Cavanagh *et al*., 2002), and sequence and structural conservation in the viral glycoprotein may allow PhCoV to replicate in domestic fowl embryos. In a similar fashion, CoVs from peafowl and teal, which are genetically highly related to IBV (Liu *et al*., 2005), can replicate at high titres in chicken embryos, inducing IBV-like embryonic lesions. In contrast, CoVs from graylag geese, mallards and feral pigeons, which are scarcely related to IBV in the replicase and nucleocapsid genes, did not replicate in chicken embryos (Jonassen *et al*., 2005). Also, TCoV usually replicates in the amniotic sac of turkey embryos, suggesting a host-range restriction between chickens and turkeys; although TCoV growth in chicken eggs has been reported, but with titres 10^4-fold lower (Adams & Hofstad, 1971). The QCoV strain had 79% to 81% amino acid identity with TCoVs, and only 16% to 18% amino acid identity with IBVs. It may be hypothesized that this genetic heterogeneity between TCoV-like viruses and IBVs accounted for the failure of QCoV to grow in chicken eggs.
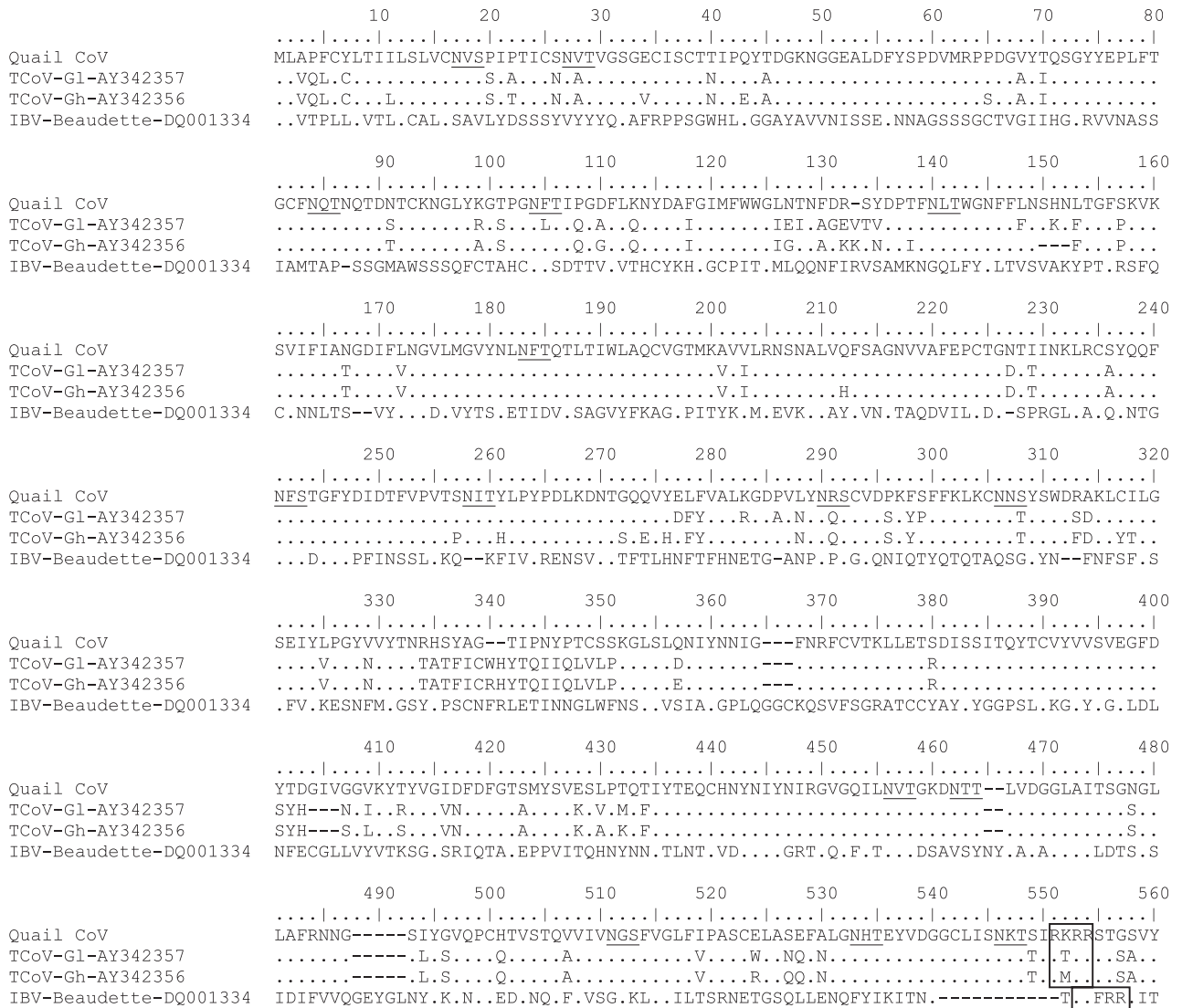
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                          10        20        30        40        50        60        70        80
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Quail CoV          MLAPFCYLTIILSLVCNVSPIPTICSNVTVGSGECISCTTIPQYTDGKNGGEALDFYSPDVMRPPDGVYTQSGYYEPLFT
TCoV-Gl-AY342357   ..VQL.C.............S.A...N.A......N....A..................A.I..........
TCoV-Gh-AY342356   ..VQL.C....L........S.T...N.A.....V.....N..E.A.............S..A.I..........
IBV-Beaudette-DQ001334 ..VTPLL.VTL.CAL.SAVLYDSSSYVYYYQ.AFRPPSGWHL.GGAYAVVNISSE.NNAGSSSGCTVGIIHG.RVVNASS

                          90        100       110       120       130       140       150       160
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Quail CoV          GCFNQTNQTDNTCKNGLYKGTPGNFTIPGDFLKNYDAFGIMFWWGLNTNFDR-SYDPTFNLTWGNFFLNSHNLTGFSKVK
TCoV-Gl-AY342357   ...........S........R.S...L..Q.A..Q....I........IEI.AGEVTV............F..K.F...P...
TCoV-Gh-AY342356   ...........T........A.S......Q.G..Q....I......IG..A.KK.N..I........---F...P...
IBV-Beaudette-DQ001334 IAMTAP-SSGMAWSSSQFCTAHC..SDTTV.VTHCYKH.GCPIT.MLQQNFIRVSAMKNGQLFY.LTVSVAKYPT.RSFQ

                          170       180       190       200       210       220       230       240
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Quail CoV          SVIFIANGDIFLNGVLMGVYNLNFTQTLTIWLAQCVGTMKAVVLRNSNALVQFSAGNVVAFEPCTGNTIINKLRCSYQQF
TCoV-Gl-AY342357   ......T...V.......................V.I.......................D.T......A....
TCoV-Gh-AY342356   ......T...V...............V.I........H...........D.T......A....
IBV-Beaudette-DQ001334 C.NNLTS--VY...D.VYTS.ETIDV.SAGVYFKAG.PITYK.M.EVK..AY.VN.TAQDVIL.D.-SPRGL.A.Q.NTG

                          250       260       270       280       290       300       310       320
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Quail CoV          NFSTGFYDIDTFVPVTSNITYLPYPDLKDNTGQQVYELFVALKGDPVLYNRSCVDPKFSFFKLKCNNSYSWDRAKLCILG
TCoV-Gl-AY342357   ...................................DFY...R..A.N..Q....S.YP........T....SD......
TCoV-Gh-AY342356   ................P...H.........S.E.H.FY........N..Q....S.Y.........T....FD..YT..
IBV-Beaudette-DQ001334 ...D...PFINSSL.KQ--KFIV.RENSV..TFTLHNFTFHNETG-ANP.P.G.QNIQTYQTQTAQSG.YN--FNFSF.S

                          330       340       350       360       370       380       390       400
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Quail CoV          SEIYLPGYVVVYTNRHSYAG--TIPNYPTCSSKGLSLQNIYNNIG---FNRFCVTKLLETSDISSITQYTCVYVVSVEGFD
TCoV-Gl-AY342357   ....V...N....TATFICWHYTQIIQLVLP.....D.......---.......R............
TCoV-Gh-AY342356   ....V...N....TATFICRHYTQIIQLVLP.....E.......---.......R............
IBV-Beaudette-DQ001334 .FV.KESNFM.GSY.PSCNFRLETINNGLWFNS..VSIA.GPLQGGCKQSVFSGRATCCYAY.YGGPSL.KG.Y.G.LDL

                          410       420       430       440       450       460       470       480
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Quail CoV          YTDGIVGGVKYTYVGIDFDFGTSMYSVESLPTQTIYTEQCHNYNIYNIRGVGQILNVTGKDNTT--LVDGGLAITSGNGL
TCoV-Gl-AY342357   SYH---N.I..R...VN.....A....K.V.M.F..........................--..........S..
TCoV-Gh-AY342356   SYH---S.L..S...VN.....A....K.A.K.F..........................--..........S..
IBV-Beaudette-DQ001334 NFECGLLVYVTKSG.SRIQTA.EPPVITQHNYNN.TLNT.VD....GRT.Q.F.T...DSAVSYNY.A.A....LDTS.S

                          490       500       510       520       530       540       550       560
                   ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Quail CoV          LAFRNNG-----SIYGVQPCHTVSTQVVIVNGSFVGLFIPASCELASEFALGNHTEYVDGGCLISNKTSIRKRRSTGSVY
TCoV-Gl-AY342357   .............-----.L.S....Q.....A..........V...W..NQ.N...................T..T...SA..
TCoV-Gh-AY342356   .............-----.L.S....Q.....A..........V....R..QQ.N..............T..M...SA..
IBV-Beaudette-DQ001334 IDIFVVQGEYGLNY.K.N..ED.NQ.F.VSG.KL..ILTSRNETGSQLLENQFYIKITN.-----------T..FRR.IT
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Figure 5. *Amino acid alignment of the S1 subunit of QCoV in comparison with TCoV and IBV Beaudette. The putative cleavage site RRFRR of the IBV strain and the RXRR stretch on TCoV and QCoV are boxed. Potential N-glycosylation sites NXS/T are underlined.*

Sequence analysis has revealed that TCoV and IBV are genetically closely related in the polymerase, in the M and N genes and in the 3′ untranslated region, while these two avian CoVs are dramatically different in the S gene (Breslin *et al.*, 1999b; Lin *et al.*, 2004). These findings show that TCoV and IBV share close evolutionary relationships (Lin *et al.*, 2004).

Differences in the S glycoprotein-encoding gene, in the genome organization and in the biological properties are important criteria adopted to define taxonomically the various avian CoVs (Cavanagh, 2001). Early in the study of avian CoVs, the issue was raised whether IBV, TCoV and PhCoV should be considered as distinct species or as members of the same species adapted to different avian hosts. On the basis of the great differences in the S protein and of their different tropism, IBV and TCoV have been considered two distinct viruses (Cavanagh, 2005). Although being related to IBV in the S protein, PhCoV has been defined as a separate species because of its unique biological features, although a genetic heterogeneity among the PhCoV strains is observed (Cavanagh *et al.*, 2002).

Indeed, experimental infection of 3-week-old specific pathogen free chickens with PhCoV elicits sero-conversion, although without causing disease (Lister *et al.*, 1985). Also, analysis of the genome of CoVs detected in graylag goose and feral pigeons revealed the presence of additional open reading frames downstream of the nucleocapsid protein gene, and those viruses have been proposed as novel coronavirus species (Jonassen *et al.*, 2005). However, the criteria often used for the classification of CoVs (biological properties, serologic and genetic relationships) must be interpreted and applied with caution. Close genetic and antigenic relatedness among the group 2 CoVs (human HCoV-OC43, bovine CoV, porcine haemagglutinating encephalomyelitis virus and canine respiratory CoV) suggests that these viruses with different host specificities have diverged fairly recently (Erles *et al.*, 2003; Vijgen *et al.*, 2006). Also, a drastic shift of tissue tropisms in feline, porcine, murine and canine CoVs (Vennema *et al.*, 1998; Laude *et al.*, 1993; Haspel *et al.*, 1978; Buonavoglia *et al.*, 2006) and adaptation to humans of the recently recognized Severe Acute Respiratory Syndrome (SARS)-associated CoV (SARS-CoV) (Guan *et al.*, 2003) has been related to only minimal genome mutations and/or deletions.

In spite of the relatively high sequence conservation in the S1 protein between QCoV and TCoV, it is unclear whether QCoV is a variant of TCoV or whether it may be considered a new avian CoV species. Epidemiological

investigations of the outbreak ruled out the possibility that the virus was transmitted to the quails from other flocks as poultry farms were not present in the same area. In Italy, intensive farms of turkeys are concentrated in northern Italy while farms are rare in central and southern Italy. In Apulia the presence of commercial turkeys is occasional, and it appears unlikely that the quails were infected by a TCoV strain originating from turkeys. Evaluation of the biological properties of QCoV will be useful to address this point. However, it is intriguing to observe that the QCoV was associated with an enteric syndrome, mimicking the patterns of TCoV infection in turkeys. TCoV is responsible for severe enteric diseases of turkeys that may result in high mortality, notably in young animals (Cavanagh et al., 2001). At this moment, it is not clear whether QCoV played a role in the genesis of the enteric syndrome.

Experimental infections will help to confirm the pathogenicity of this novel virus in quails. Therefore, virus isolation will be of extreme importance to evaluate the pathogenic attitude in vivo. Also, it will be interesting to assess the pathogenic attitude of QCoV in other avian hosts, in particular turkeys, considering the genetic relatedness observed in the S1 gene between QCoV and TCoV. To address this point and to investigate more precisely the origin of TCoV-like viruses, it will be important to determine the sequence of the genes encoding the other structural proteins, and to draw a map of the complete genome organization of the QCoV strain.

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Non-English Abstracts

Coronavirus associated with an enteric syndrome on a quail farm

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Coronavirus associé à un syndrome entérique dans un élevage de cailles

Un syndrome entérique a été observé chez des cailles (*Coturnix coturnix*) élevées de façon semi intensive pour le repeuplement en Apulie (Italie du Sud). Les oiseaux présentaient une dépression, une diarrhée importante, une déshydratation et un retard de croissance. La mortalité est apparue particulièrement chez lez jeunes sujets. A l'autopsie, la lésion la plus importante était une entérite. Dans les fèces et dans le contenu intestinal des cailles mortes un *coronavirus* a été détecté en microscopie électronique et par les réactions de transcription inverse et de polymérisation en chaîne. Le virus n'a pas pu être cultivé sur embryons de poulet. Les analyses de séquence d'un fragment (409 nucléotides) de la région 1b du gène de la polymérase, ont montré que le coronavirus de la caille présentait ≤ 93% d'identité nucléotidique avec les coronavirus aviaires (coronavirus du groupe 3), alors que l'analyse d'une portion du gène codant la protéine de spicule S1 du coronavirus de la caille présentait 16–18% d'identité en acides aminés avec le virus de la bronchite infectieuse, et 79–81% d'identité avec le coronavirus de la dinde. L'ensemble de ces résultats suggère l'existence d'un nouveau coronavirus génétiquement proche du coronavirus de la dinde.

Coronavirus im Zusammenhang mit einem enteritischen Syndrom auf einer Wachtelfarm

Bei Wachteln (*Coturnix coturnix*), die in Apulien (Süditalien) zum Zweck der Auswilderung in einer semi-intensiven Haltung aufgezogen wurden, wurde ein enteritisches Syndrom beobachtet. Die Tiere zeigten eine Störung des Allgemeinbefindens, Diarrhoe, Dehydratation und Wachstumsverzögerung. Todesfälle traten besonders bei Jungtieren auf. Bei der Sektion war Enteritis die herausragende pathologisch-anatomische Veränderung. In Fäzes und Darminhalt der toten Wachteln wurde mittels Elektronenmikroskopie und Reverse Transkriptase-Polymerasekettenreaktion ein Coronavirus nachgewiesen. In Hühnerembryonen konnte das Virus nicht angezüchtet werden. Durch Sequenzanalyse eines Fragments der 1b-Region des Polymerase-Gens wurde eine Nukleotid-Übereinstimmung des Wachtel-Coronavirus von ≤93% mit den aviären Coronaviren (Coronaviren der Gruppe 3) festgestellt. Bei der Analyse des S1-Teils des für das Spikeprotein kodierenden Gens zeigte das Wachtel-Coronavirus eine 16–18%ige Aminosäurenidentität mit dem Virus der infektiösen Bronchitis des Huhnes und eine 79–81%ige Übereinstimmung mit dem Puten-Coronavirus. Zusammengefasst lassen die Befunde auf die Existenz eines neuen Coronavirus, das genetisch mit dem Puten-Coronavirus verwandt ist, schließen.

Coronavirus asociado a un síndrome entérico en una granja de codornices

Se observó un síndrome entérico en codornices (*Coturnix coturnix*) criadas de forma semi-intesiva para reposición en Apulia (sur de Italia). Las aves mostraban depresión, diarrea grave, deshidratación y retraso en el crecimiento. La mortalidad ocurría mayoritariamente en aves jóvenes. A la necropsia la lesión más destacada fue la enteritis. Se detectó un coronavirus en heces y contenido intestinal de codornices muertas mediante microscopía electrónica y transcripción reversa-reacción en cadena de la polimerasa. El virus no pudo cultivarse en embrión de pollo. El coronavirus de la codorniz mostró ≤ 93% de similitud nucleotídica con otros coronavirus aviares (coronavirus del grupo 3) en el análisis de secuencias de un fragmento (409 nucleótidos) de la región 1b del gen de la polimerasa, mientras que en el análisis de la porción S1 del gen que codifica para la proteína de la espícula el coronavirus de la codorniz mostró una similitud aminoacídica del 16–18% con el virus de la bronquitis infecciosa aviar, y del 79–81% con el coronavirus del pavo. En conjunto, estos resultados sugieren la existencia de un nuevo coronavirus genéticamente relacionado al coronavirus del pavo.