



Coronavirus associated with an enteric syndrome in a quail farm

Elena Circella, Antonio Camarda, Vito Martella, Giordano Bruni, Antonio Lavazza, Canio Buonavoglia

► To cite this version:

Elena Circella, Antonio Camarda, Vito Martella, Giordano Bruni, Antonio Lavazza, et al.. Coronavirus associated with an enteric syndrome in a quail farm. Avian Pathology, 2007, 36 (03), pp.251-258. <10.1080/03079450701344738>. <hal-00540081>

HAL Id: hal-00540081

<https://hal.science/hal-00540081v1>

Submitted on 26 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



Coronavirus associated with an enteric syndrome in a quail farm

Journal:	<i>Avian Pathology</i>
Manuscript ID:	CAVP-2007-0004.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	22-Feb-2007
Complete List of Authors:	Circella, Elena; Università di Bari, Facoltà di Medicina Veterinaria, Sanità e Benessere degli Animali camarda, antonio; Università di Bari, Facoltà di Medicina Veterinaria, Sanità e Benessere degli Animali martella, vito; Università di Bari, Facoltà di Medicina Veterinaria, Sanità e Benessere degli Animali bruni, giordano; Università di Bari, Facoltà di Medicina Veterinaria, Sanità e Benessere degli Animali Lavazza, Antonio; Istituto Zooprofilattico Sperimentale Lombardia ed Emilia Romagna buonavoglia, canio; Università di Bari, Facoltà di medicina Veterinaria, Sanità e Benessere degli Animali
Keywords:	coronavirus, quail, enteritis, sequence analyses

SCHOLARONE™
Manuscripts

Figure 1

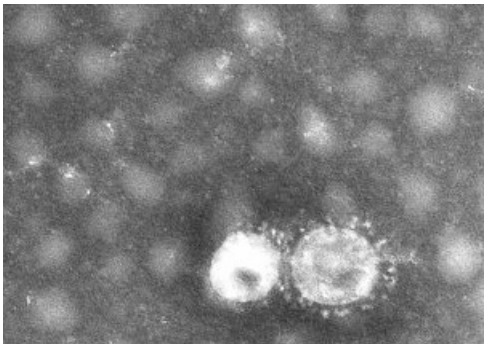


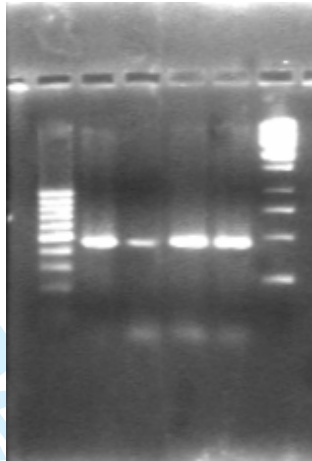
Figure 2

Figure 4

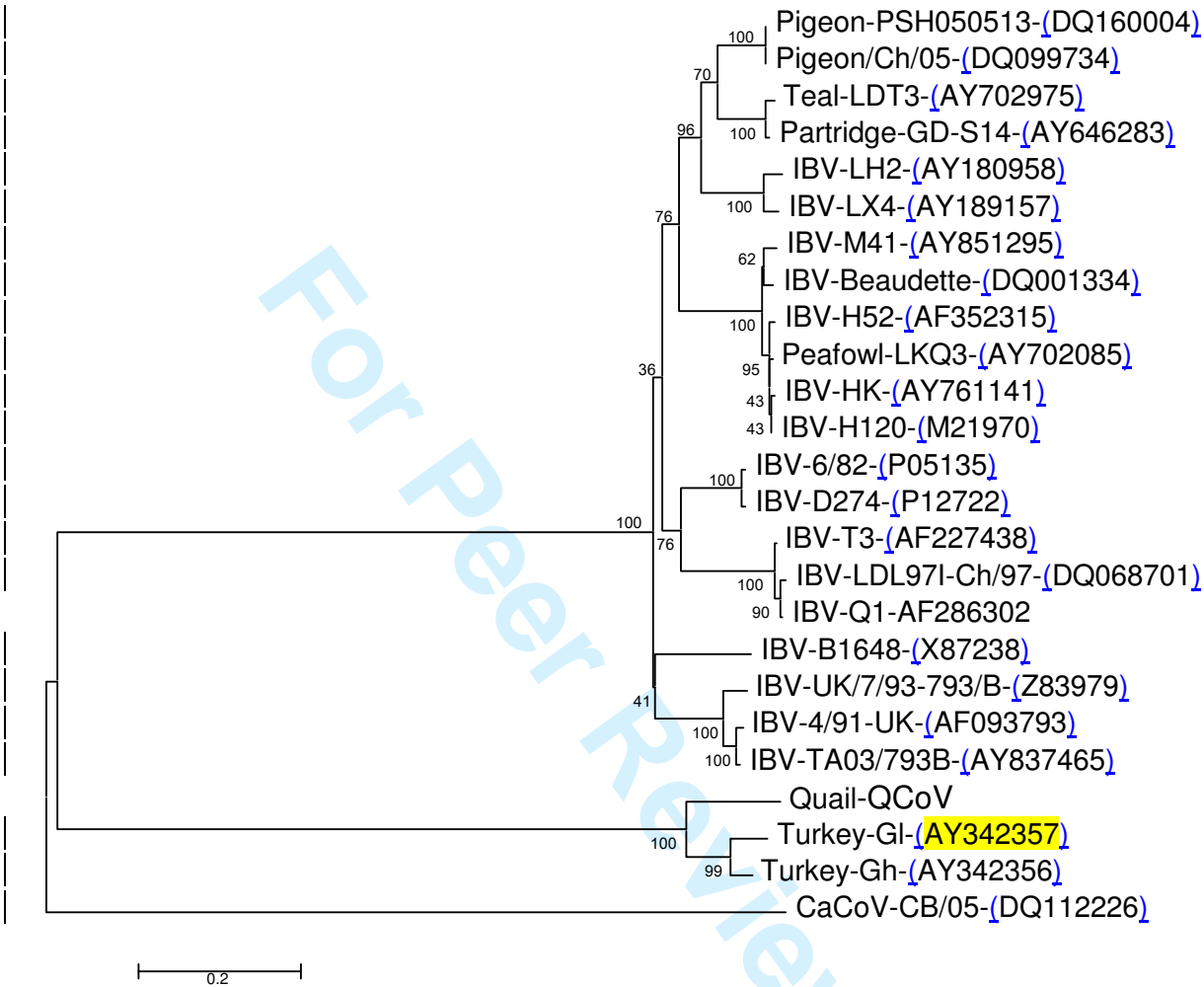


Figure 5

```

      10      20      30      40      50      60      70      80
Quail CoV      MLAPFCYLTIILSLVCNVSPIPTICSNVTVGSGECISCTIPQYTDGKNGGEALDFYSPDVMRPPDGVYTQSGYYEPLFT
TCoV-G1-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.RVNVNASS

      90      100     110     120     130     140     150     160
Quail CoV      GCFNQTNQTDNTCKNGLYKGTGPGNFTIPGDFLKNYDAFGIMFWWGLNTNFDR-SYDPTFNLTWGNFFLNSHNLTGFSKVK
TCoV-G1-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.LTVSAKYPT.RSFQ

      170     180     190     200     210     220     230     240
Quail CoV      SVIFIANGDIFLNGVLMGVYNLNFQTTLTIWLAQCVGTMKAVVLNRNSNALVQFSAGNVVAFEPCTGNTIINKLRCSYQQF
TCoV-G1-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      NFSTGFYDIDTFVPTSNTIYLPYPDLKNTGQQVYELFVALKGDVLYNRSCVDPKFSFFKLKCNNSYSWDRAKLCILG
TCoV-G1-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      SEIYLPGYVVYTNRHSYAG--TIPNYPTCSSKGLSLQNIYNNIG--FNRFCVTKLLETSDISSITQYTCVYVVSVEGFD
TCoV-G1-AY342357 .....V...N....TATFICWHYTQIIQLVLP....D.....R.....
TCoV-Gh-AY342356 .....V...N....TATFICRHYTQIIQLVLP....E.....R.....
IBV-Beaudette-DQ001334 .FV.KESNFM.GSY.PSCNFRLETINNGLWFNS..VSIA.GPLQGCKQSVFSGRATCCYAY.YGGPSL.KG.Y.G.LDL

      410     420     430     440     450     460     470     480
Quail CoV      YTDGIVGGVKYTYVGIDFDFGTSMYSVESLPTQTIYTEQCHNYNIYNIRGVGQILNVTGKDNTT--LVDGGLAITSGNGL
TCoV-G1-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.EPPVITQHNNYN.TLNT.VD...GRT.Q.F.T...DSAVSYNY.A.A...LDTS.S

      490     500     510     520     530     540     550     560
Quail CoV      LAFRNNG----SIYGVQPCHTVSTQVVIVNGSFVGLFIPASCELASEFALGNHTEYVDGGCLISNKTSIRKRSTGGSVY
TCoV-G1-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..ILTSRNETGSQLEENQFYIKITN.-----T...FRR..IT

```

Table 1. Comparison (amino acid identities, %) based on the S1 subunit of the Quail-CoV with representatives of Group 3 avian coronaviruses. Accession numbers are indicated in parentheses.

	Quail-CoV	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)
Quail-CoV	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-GI-(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-(AY761141)	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

Cavp-2007-0004.R1 FURTHER edited and styled by Dave 16mar07

Coronavirus associated with an enteric syndrome on a quail farm

Elena Circella^{1*}, Antonio Camarda¹, Vito Martella¹, Giordano Bruni¹, Antonio Lavazza² and Canio Buonavoglia¹.

¹ *Dipartimento di Sanità e Benessere degli Animali, Facoltà di Medicina Veterinaria, Università degli Studi di Bari, S.P. Casamassima Km 3, 70010 Valenzano, Bari, Italy, and* ² *Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna, Sezione di Brescia, Italy.*

Running title: Coronavirus in quails

*To whom correspondence should be sent. E-mail e.circella@veterinaria.uniba.it

Received 8 January 2007

Cavp-2007-0004.R1

Coronavirus associated with an enteric syndrome on a quail farm

Elena Circella^{1*}, Antonio Camarda¹, Vito Martella¹, Giordano Bruni¹, Antonio Lavazza² and Canio Buonavoglia¹.

Abstract

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. In the faeces and in the intestinal content of the dead quails a *coronavirus* was detected by electron microscopy and reverse transcriptase–polymerase chain reaction. 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 $\leq 93\%$ 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 16–18% amino acid identity with infectious bronchitis virus, and 79–81% 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.

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 and Naqi, 1997).

Turkey CoV (TCoV) has a marked enteric tropism (Nagaraja and Pomeroy, 1997). Infections by TCoV are common in USA, Canada and Australia (Nagaraja and 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). Recently, TCoV-related diseases, characterised by low growth, enteritis and mortality, have been also observed in turkeys 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 (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 drop in egg production in pheasants, similar to IBV infection in layer hens (Gough *et al.*, 1998).

Coronavirus infections are not necessarily associated with clinical signs as they have been also 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 characterised 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 (*Coturnix 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 20-25 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. Initially mortality ranged from 10 to 15% and subsequently increased to 70%, notably in the birds housed in the 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 (5-10%).

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 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 hours.

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 SPF embryonated chicken eggs. Three pools of gut samples were homogenized and diluted 1:10 in Eagle's minimal essential medium (E-MEM), 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 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 (EM). 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 4000×g and again for 10 min at 9300×g for clarification. The supernatant was then ultracentrifuged (Beckman Airfuge) for 15 min at 82000×g. After

negative staining with 2% sodium phosphotungstate (pH 6.8), samples were examined using a Philips CM10 electron microscope at 19000-39000x. Embryo amniotic and allantoic fluids were also tested by EM.

Detection of coronavirus RNA by RT-PCR. Viral RNA was extracted from 100 µl of homogenate made from 3 pools of gut and from 5 individual gut samples, using the GeneAmp RNA PCR Core Kit (Applied Biosystems, Monza). Samples of liver, spleen, cardiac blood, 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' GGGTTGGGACTATCCTAAGTGTGA3' (nucleotides, nt, 14180-14203 on the genome of strain Beaudette CK, accession AJ311317) and IN-4 (antisense) 5'TAACACACAACICCATCATCA3' (nt 14632-14612) targeting an highly conserved region (1b) of the coronavirus polymerase complex and designed to detect all members within the *Coronavirus* genus (Ksiaszek *et al.* 2003). The product of the RT-PCR was 452 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). RT-PCR thermal cycling parameters were as follows: 50°C for 60 minutes and 94°C for 2 minutes for reverse transcription and denaturation of the reverse transcriptase, respectively; 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 analyzed by agarose gel electrophoresis and visualized after staining with ethidium bromide.

In order to amplify the S gene, primers S-cor (sense) 5'TGAAACTGAACAAAAGACAGACT3' (nt 20302-20325) and AS-cor (antisense) 5'CCAAACATACCAAGGCCACTT3' (nt 23658-23638) were used (Lin *et al.* 2004).

Sequence and phylogenetic analyses. After purification on Ultrafree DA Columns (Amicon Millipore, Bedford, USA), the amplicons underwent sequence analysis with ABI-PRISM 377 (Perkin-Elmer, Applied Biosystems Division). The sequences were assembled and analyzed using the Bioedit software package (Department of Microbiology, North Carolina State University, USA) (27 Hall, 1999) and 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 2.1 (Arizona State University, USA) (34). 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, 1, 2 and 3, were selected. In order to investigate the relationships between the 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 numbers of the polymerase and S gene sequences are indicated in Figures 3 and 4.

Accession number. The partial sequences of the region 1b of ORF1 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, coronaviruses-like viral particles were observed in the gut samples of the animals by electron microscopy (Figure 1). In addition, coronavirus RNA was detected using the broadly-reactive primers IN-2 and IN-4, which are able to recognise 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 liver, spleen, cardiac blood and kidney were RT-PCR negative.

Coronavirus-like particles have been already observed by electron microscopy in nasal and tracheal swabs of quails exhibiting a respiratory syndrome (Pascucci *et al.* 1983) but the viruses were not further characterised. Accordingly, the findings of the present study provide the first convincing genomic evidence for the presence of CoV in quails (quail coronavirus, QCoV). We determined 409 nt of region 1b of the polymerase gene, spanning nt 14203 to 14612 (in comparison with the genome of IBV Beaudette). By BLAST and FASTA analysis, the fragment displayed $\leq 93\%$ nt 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 (Ksiasek *et al.* 2003; Stephensen *et al.* 1999), 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 and turkey CoVs 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 (Figure 4) provided interesting clues to understand the nature of the virus. The S1 of QCoV showed low amino acid (aa) identity to all the IBV strains available in the databases. The aa sequence identity with IBVs was 16-18%, suggesting that QCoV is not an IBV variant. In contrast, the S1 of QCoV displayed 79-81% aa identity with TCoV strains. IBV strains within the same serotype usually share > 95% aa identity (Cavanagh *et al.*, 2001) while IBV strains of other serotypes may share 75-85% aa 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. 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% aa 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, that 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, that 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 an host-range restriction between chickens and turkeys, although TCoV growth in chicken eggs has been reported, although with titres 10^4 -fold lower (Adams & Hofstad, 1971). The QCoV strain had 79-81% aa identity with TCoVs, and only 16-18% aa 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.

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' UTR region, while these two avian CoVs are dramatically different in the S gene (Lin *et al.*, 2004; Breslin *et al.* 1999b). 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 *et al.*, 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 as 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 though without causing disease (Lister *et al.* 1985). Also, analysis of the genome of CoVs detected in graylag goose and feral pigeons has revealed the presence of additional open reading frames (ORFs) downstream 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 coronaviruses (human coronavirus HCoV-OC43, bovine coronavirus, porcine hemagglutinating encephalomyelitis virus and canine respiratory coronavirus) suggests that these viruses with different host

specificities have diverged fairly recently (Vijgen *et al.*, 2006; Erles *et al.*, 2003). Also, drastic shift of tissue tropisms in feline, porcine, murine and canine coronaviruses (Vennema *et al.*, 1998; Laude *et al.*, 1993; Haspel *et al.*, 1978; Buonavoglia C.) and adaptation to humans of the recently-recognized SARS-associated coronavirus (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 as 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 in 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.

References

- Adams N. R., Hofstad M. S. (1971). Isolation of transmissible gastroenteritis agent of turkeys in avian embryos. *Avian Disease*, 15, 426-433.
- Beach J. R., Schalm O. W. (1936). A filterable virus, distinct from that of Laryngotracheitis, the cause of a respiratory disease of chicks. *Poultry Science*, 15, 199-206.
- Breslin J.J., Smith L.G., Fuller F.J., Guy J.S. (1999a). Sequence analysis of the matrix/nucleocapsid gene region of turkey coronavirus. *Intervirology*, 42, 22-29.
- Breslin J.J., Smith L.G., Fuller F.J., Guy J.S. (1999b). Sequence analysis of the turkey coronavirus nucleocapsid gene and 3' untranslated region identifies the virus as a close relative of infectious bronchitis virus. *Virus Research*, 65, 187-198.
- Cavanagh D. (1997). Nidovirales: a new order comprising Coronaviridae and Arteriviridae. *Archives of Virology*, 142, 629-633.
- Cavanagh D. (2001). A nomenclature for avian coronavirus isolates and the question of species status. *Avian Pathology*, 30, 109-115.
- Cavanagh D. (2005). Coronaviruses in poultry and other birds. *Avian Pathology*, 34, 439-448.
- Cavanagh D. Naqi S.A. (1997). Infectious bronchitis. In: Calnek B.W., Barnes H.J., Beard C.W., McDougald L.R., Saif Y.M. (Eds.), *Disease of Poultry*, 10th ed. Iowa State University Press, Ames, IA, pp. 511-526.

- Cavanagh D., Mawditt K., Sharma M., Drury S.E., Ainsworth H.L., Britton P., Gough R.E. (2001). Detection of a coronavirus from turkey poult in Europe genetically related to infectious bronchitis virus of chickens. *Avian Pathology*, 30, 365-378.
- Cavanagh D., Mawditt K., Welchman D.d.B., Britton P., Gough R.E. (2002). Coronaviruses from pheasant (*Phasianus colchicus*) are genetically closely related to coronaviruses of domestic fowl (infectious bronchitis virus) and turkeys. *Avian Pathology*, 31, 181-193.
- Clarke J. K., McFerran J. B., Gay F. W. (1972). Use of allantoic cells for the detection of avian infectious bronchitis virus. *Archive der Gesamte Virusforschung* 36, 62-70.
- De Marco M.A., Catelli E., Raffini E., Lavazza A., Delogu M., Terregino C., Frasnelli M., Govoni S. (1999). Isolamento di un coronavirus da fagiani con lesioni renali. *Selezione Veterinaria* 8/9, 677-685
- De Wit J. J. (2000). Detection of infectious bronchitis virus. *Avian Pathology*, 29, 71-93.
- Dea S., Verbeek A. J., Tijssen P. (1990). Antigenic and genomic relationships among turkey and bovine enteric coronaviruses. *Journal of Virology*, 64, 3112-3118.
- Erles K, Toomey C, Brooks HW, Brownlie J. (2003). Detection of a group 2 coronavirus in dogs with canine infectious respiratory disease. *Virology*, 310, 216-23.
- Gonzales J. M., Gomez-Puertas P., Cavanagh D., Gorbalenya A. E., Enjuanes L. (2003). A comparative sequence analysis to revise the current taxonomy of the family Coronaviridae. *Archives of Virology*, 148, 2207-2235.
- Gough R.E., Cox W.J., Cavanagh D. e Mawditt K. (1998). Isolation and identification of infectious bronchitis virus from pheasants in the United Kingdom. In E.F. Kaleta e U. Heffels-Redmann (Eds.), *Proceedings of the International Symposium on Infectious Bronchitis and Pneumovirus Infections in Poultry* (pp. 200-204). Rauischholzhausen, Giessen, Germany.

- Gough R.E., Cox W.J., Winkler C.E., Sharp M.W. e Spackman D. (1996). Isolation and identification of infectious bronchitis virus from pheasants. *Veterinary Record*, 138, 208-209.
- Guan Y, Zheng BJ, He YQ, Liu XL, Zhuang ZX, Cheung CL (2003). Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science*, 302, 276-8.
- Guy J. S. (2000). Turkey coronavirus is more closely related to avian infectious bronchitis virus than to mammalian coronaviruses. *Avian Pathology*, 29, 207-212.
- Guy J. S. (2003). Turkey coronavirus enteritis. In H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. McDougald & D. E. Swayne (Eds.), *Diseases of Poultry*, 11th edn (pp. 300-307). Ames: Iowa State Press.
- Guy J. S., Smith L. G., Breslin J. J., Vaillancourt J. P., Barnes H. J. (2000). High mortality and growth depression experimentally produced in young turkeys by dual infection with enteropathogenic *Escherichia coli* and turkey coronavirus. *Avian Diseases*, 44, 105-113.
- Haspel MV, Lampert PW, Oldstone MB. (1978). Temperature-sensitive mutants of mouse hepatitis virus produce a high incidence of demyelination. *Proceedings of the National Academy of Sciences USA*, 75, 4033-36.
- Ismail M. M., Tang A. Y., Saif Y. M. (2003). Pathogenicity of turkey coronavirus in turkeys and chickens. *Avian Disease*, 47, 515-522.
- Ito N. M. K., Miyaji. C.I., Capellaro C. E. M. P. D. M. (1991). Studies on broiler's IBV and IB-like virus from guinea fowl. In E. F. Kaleta e U. Heffels (Eds.) *II International Symposium on Infectious Bronchitis* (pp. 302-307). Giessen: Justus Leibig University.
- Janassen C.M., Kofstad T., Larsen I.L., Lovland A., Handeland K., Follestad A., Lillehaug A. (2005). Molecular identification and characterization of novel coronaviruses infecting

- greylag geese (*Anser anser*), feral pigeons (*Columbia livia*) and mallards (*Anas platyrhynchos*). *Journal of General Virology*, 86, 1597-1607.
- Ksiazek T.G., Erdman D., Goldsmith C.S., Zaki S.R., Peret T., Emery S., Tong S., Urbani C., Comer J.A., Lim W., Rollin P.E., Dowell S.F., Ling A.E., Humphrey C.D., Shieh W.J., Guarner J., Paddock C.D., Rota P., Fields B., DeRisi J., Yang J.Y., Cox N., Hughes J.M., LeDuc J.W., Bellini W.J., Anderson L.J.; SARS Working Group. (2003). A novel coronavirus associated with Severe Acute Respiratory Syndrome. *New England Journal of Medicine*, 348, 1953-1966.
- Landman W.J.M e Feberwee A. (2004). Aerosol-induced Mycoplasma synoviae arthritis: the synergistic effect of infectious bronchitis virus infection. *Avian Pathology*, 33, 591-598.
- Laude H, Van Reeth K, Pensaert M. (1993). Porcine respiratory coronavirus: molecular features and virus-host interactions. *Veterinary Research*, 24, 125-50.
- Lavazza A., Pascucci S., Gelmetti D. (1990). Rod-shaped virus-like particles in intestinal contents of three avian species. *Veterinary Record*, 126, 581.
- Lin T.L., Loa C.C., Wu C.C. (2004). Complete sequences of 3' end coding region for structural protein genes of turkey coronavirus. *Virus Research*, 106, 61-70.
- Lister S.A., Beer j.V., Gough R.E., Holmes R.G., Jones J.M.W., Orton R.G. (1985). Outbreaks of nephritis in pheasants (*Phasianus colchicus*) with a possible coronavirus aetiology. *Veterinary Record*, 117, 612-613.
- Liu S., Chen J., Chen J., Cong X., Shao Y., Han Z., Feng L., Cai X., Gu S., Liu M. (2005). Isolation of avian infectious bronchitis coronavirus from domestic peafowl (*Pavo cristatus*) and teal (*Anas*). *Journal of General Virology*, 86, 719-725.

- Martina B. E. E., Haagmans B. L., Kuiken T., Fouchier R. A. M., Rimmelzwaan G. F., Van Amerongen G., Peiris J. S. M., Lim W., Osterhaus A. D. M. E. (2003). Virology: SARS virus infection of cats and ferrets. *Nature*, 425, 915.
- Matthijs M.G.R., van Eck J.H.H., Landman W.J.M. e Stegeman J.A. (2003). Ability of Massachusetts-type infectious bronchitis virus to increase colibacillosis susceptibility in commercial broilers: a comparison between vaccine and virulent field virus. *Avian Pathology*, 32, 473-481
- Moreno Martin A., Vinco L.J., Cordioli P., Lavazza A. (2002). Diagnosis of turkey viral enteric diseases by electron microscopy and identification of coronavirus in a case of turkey enteritis. *4th Internatinal Symposium on Turkey Diseases*, Berlin, 15th - 18th May 2002 / organized and published by Prof. Dr. H.M. Hafez . - Giessen : DVG Service, p 114-119.
- Nagaraja K. V., Pomeroy B. S. (1997). Coronaviral enteritis of turkeys (bluecomb disease). In: Calnek B. W., Barnes H. J., Beard C. W. McDougald L. R., Saif Y. M. (Eds.), *Diseases of Poultry* 10th ed. Iowa State University Press, Ames, IA, pp. 686-692.
- Pascucci S., Misciattelli M.E., Giovannetti L., Pacchioni G. (1983). Isolamento di un coronavirus simile da quaglie giapponesi (*Coturnix coturnix japonica*) con syndrome respiratoria: I° Descrizione della malattia ed isolamento del virus. *La Clinica Veterinaria*, 106, 33-34.
- Pennycott T.W. (2000). Causes of mortality and culling in adult pheasants. *Veterinary Record*, 146, 273-278.
- Siddel S., Wege H, Ter Meulen V. (1983). The biology of coronaviruses. *Journal of General Virology*, 64, 761-776.
- Spackman D. e Cameron I.R.D. (1983). Isolation of infectious bronchitis virus from pheasants. *Veterinary Record*, 113, 354-355.

- Stephensen C.B, Casebolt D.B., Gangopadhyay N.N. (1999). Phylogenetic analysis of a highly conserved region of the polymerase gene from 11 coronaviruses and development of a consensus polymerase chain reaction assay. *Virus Research* 60, 181-189.
- Vennema H, Poland A, Foley J, Pedersen NC. (1998). Feline infectious peritonitis viruses arise by mutation from endemic feline enteric coronaviruses. *Virology*, 243, 150–7.
- Verbeek A., Dea S., Tijssen P. (1991). Genomic relationship between turkey and bovine enteric coronaviruses identified by hibrydization with BCoV and TCoV specific cDNA probes. *Archives of Virology*, 121, 199-211.
- Verbeek A., Tijssen P. (1991). Sequence analysis of the turkey enteric coronavirus nucleocapsid and membrane protein genes: a close genomic relationship with bovine coronavirus. *Journal of General Virology*, 72, 1659-1666.
- Vijgen L, Keyaerts E, Lemey P, Maes P, Van Reeth K, Nauwynck H, Pensaert M, Van Ranst M. (2006). Evolutionary history of the closely related group 2 coronaviruses: porcine hemagglutinating encephalomyelitis virus, bovine coronavirus, and human coronavirus OC43. *Journal of Virology*, 80, 7270-7274.
- Wege H., Siddell St., ter Meulen V. (1982). The biology and pathogenesis of coronavirus. *Current Topics in Microbiology and Immunology*, 99, 165-200.
- Williams A. K., Wang L., Sneed L. W., Collisson E. W. (1992). Comparative analyses of the nucleocapsid genes of several strains of infectious bronchitis virus and other coronaviruses. *Virus Research*, 25, 213-222.
- Woods R.D., Cheville N.F., Gallagher J.E. (1981). Lesions in the small intestine in newborn pigs inoculated with porcine, feline and canine coronaviruses. *American Journal of Veterinary Research*, 42, 1163-1169.

Figure legends

Figure 1. *Electron micrograph of the quail CoV in intestinal content of quails.*

Figure 2. *Results of the general (gene 1) RT-PCR for coronaviruses (primer IN-2/IN-4). Lane 1, 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, 1 kb DNA ladder (Gene Ruler™, Fermentas GmbH, Germany).*

Figure 3. *Phylogenetic tree based on the polymerase gene 1 fragment (409 nt, corresponding to nt 14203 to 14612 of the IBV Beaudette genome). The tree displays the genetic relationships between the quail CoV and representatives of the three genetic groups of the genus Coronavirus. The accession numbers of the sequences are given in the figure.*

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

Figure 5. *Amino acid alignment of the S1 of the quail CoV in comparison to turkey coronaviruses (TCoV) and IBV Beaudette. The putative cleavage site RRFRR of the IBV strain and the RXRR stretch on turkey and quail CoV are boxed. Potential N-glycosylation sites NXS/T are underlined.*

Figure 3

