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## CASE REPORT

# Coronavirus-associated enteritis in a quail farm

Elena Circella<sup>1</sup>, Vito Martella<sup>1</sup>, Giordano Bruni<sup>1</sup>,  
Eleonora Lorusso<sup>1</sup>, Antonio Lavazza<sup>2</sup>, Antonio Camarda<sup>1</sup>

<sup>1</sup>Dipartimento di Sanità e Benessere degli Animali. Università di Bari, Italy

<sup>2</sup>Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna. Brescia, Italy

*Corresponding author:* Dr. Elena Circella. Dipartimento di Sanità e Benessere degli Animali, Sez. Patologia Aviaria. Facoltà di Medicina Veterinaria. S.P. per Casamassima Km 3, 70010 Valenzano (BA), Italy - Tel. +39 080 4679910 – Fax: +39 080 4679910 - Email: e.circella@veterinaria.uniba.it

### ABSTRACT

An enteric syndrome observed in semi-intensively reared quails is described. The affected birds showed depression, severe diarrhoea and dehydration. The mortality occurred particularly in young birds. At necropsy, the prominent lesion was catarrhal enteritis. Laboratory investigations demonstrated the presence of *coronavirus* in the gut of dead animals. No additional pathogens were detected. To our knowledge, this is the first evidence for the presence of CoVs in quail with enteritis.

*Key words:* Coronavirus, Quail, Enteritis.

### RIASSUNTO

#### ENTERITE DA CORONAVIRUS IN QUAGLIE D'ALLEVAMENTO

*Gli Autori descrivono una sindrome enterica osservata in un allevamento di quaglie. Gli animali colpiti, che apparivano depressi, presentavano diarrea profusa e disidratazione. La mortalità interessava soprattutto i giovani. All'esame necroscopico, si osservava costantemente enterite catarrale. Le indagini di laboratorio hanno evidenziato la presenza di coronavirus nell'intestino dei soggetti esaminati, in assenza di altri patogeni specifici. Questo lavoro costituisce la prima segnalazione della presenza di CoV nella quaglia associato ad enterite.*

Parole chiave: Coronavirus, Quaglia, Enterite.

### Introduction

Coronaviruses are classified into three major groups on the basis of antigenic and genetic differences (Cavanagh, 2005). A fourth group is formed by coronavirus associated with severe acute respiratory syndrome (SARS) (Kim *et al.*, 2006). All avian coronaviruses (CoV) are classi-

fied in group III. Infection by CoV is of high relevance in poultry. CoV-induced diseases have been known since several years. Until recently, only a few species of birds, such as chicken, turkey and pheasant, were believed to be sensitive to CoVs. However, in the last years, CoVs have been detected in the gut of a number of avian species, including guinea fowl, graylag geese, mallard and

feral pigeon (Jonassen *et al.*, 2005), domestic peafowl and teal (Liu *et al.*, 2005). In Japanese quail (*Coturnix japonica*) Pascucci *et al.* (1983) reported the isolation of coronavirus-like strains from birds with a respiratory syndrome.

In this report we describe the detection of CoVs in quails affected by enteric syndrome.

### Material and methods

The outbreak occurred in a semi-intensive farm from the Apulia region in the spring 2005. In the farm a total of 90 pairs of quail breeders were raised in cage. Mechanical incubation of the eggs was adopted routinely. After hatching, birds were reared in cages, where they remained until about 20-25 days of age, before to be transferred to an external aviary. The enteric syndrome was observed at 3 weeks of age in quails kept in cages, although the disease appeared more severe in the birds kept in the external aviary. The affected birds displayed weakness, depression and presented severe diarrhoea, dehydration and drop in feed consumption. Initially, mortality ranged from 10 to 15% and subsequently increased to 70%, notably in the birds of the external aviary. The quails recovered from the disease appeared under-grown and poorly feathered. Afterwards, the syndrome was also observed in breeding birds, that exhibited attenuated symptoms and lower mortality (about 10%).

The carcasses of 10 animals were sent to the Faculty of Veterinary Medicine, University of Bari, for necropsy and for laboratory investigations. Liver, spleen, kidney, heart blood and gut samples were collected. Bacteriological cultures were performed in aerobic and anaerobic conditions on enrichment media (Blood agar - OXOID) and selective media (MacConkey Agar - OXOID), with incubation at 37° for 24 hours. Parasitological investigations were carried out by preparing fresh smears of the gut content and by flotation. Either pooled samples of intestinal content or individual samples were collected from the animals for virological

investigations. All samples were examined by negative contrast electron microscopy. The samples were inoculated into the allantoic and amniotic cavities of 10-day embryonated hens' eggs after the addition of antibiotics and antimycotics. Eggs were candled daily to monitor embryo liveability. The fluids were harvested after 5 days. Three sequential passages in embryonated hen's egg were made. RNA extracts were prepared from 100 µl of each specimen (or eggs fluids) with the GeneAmp RNA PCR Core Kit (Applied Italia, Monza). The oligonucleotide primers IN-2 (sense) and IN-4 (antisense) were used for detection of coronavirus RNA by reverse transcription (RT) polymerase chain reaction (PCR) (Ksiasek *et al.*, 2003). The primers target a highly conserved region (1b) of the coronavirus polymerase complex. RT and PCR were performed in a one-step procedure using Superscript III One step (Invitrogen, UK). RT-PCR products were analyzed on agarose gel electrophoresis and stained with ethidium bromide. The RT-PCR product bands were visualized by using UV light.

### Results and discussion

The carcasses resulted dehydrated and under-weight. The feathers of the animals were rarefied. At necropsy, catarrhal enteritis was the prominent lesion in all the examined birds. The gut was distended and filled with watery clear content. No additional specific gross lesions were observed. Histopathological examination of the gut confirmed the presence of severe mucosal inflammatory reaction. No specific bacterial and parasitological pathogens were detected in all samples examined. Interestingly, by electron microscopy observation, coronavirus-like viral particles were detected in the gut samples of all the examined animals. The broadly-reactive primers, designed to detect all members within the *Coronavirus* genus, amplified a 405 bp fragment from the pooled samples and from 3 on 5 gut individual samples, thus confirming the electron microscopy observation. Further

investigations about sequence analysis are actually carrying out. To our knowledge, this could be the first evidence for the presence of CoVs in quail.

Virus growth on chicken embryos was not successful and this may have been due to host species barriers between chicken and quail. For instance, CoVs from graylag geese, mallards and feral pigeons were not cultivable in chicken embryos (Jonassen *et al.*, 2005). Turkey CoV was adapted to grow in chicken eggs, although with titres  $10^4$  lower than in turkey embryos (Adams and Hofstad, 1971). Against, CoVs from peafowl and teal were isolated in chicken embryos, replicating at high titres and inducing embryo lesions similar to those of infectious bronchitis virus (IBV), but the viruses were shown to be genetically highly related to IBV (Liu *et al.*, 2005).

It is uncertain whether CoV played a role into the genesis of the enteric syndrome observed in the reared quails. In turkey, CoV is responsible for enteric diseases and may cause high mortality, notably in young birds. In chickens, IBV has a tropism to a broader spectrum of tissues/organs, but often affects the gastroenteric tract. However, infections by CoV are not necessarily associated with specific clinical signs, as CoVs have been also detected in the gut of normal peafowls and teals (Jonassen *et al.*, 2005; Liu *et al.*, 2005). Similarly, Pascucci *et al.* (1983) did not necessarily correlate the coronavirus-like viral strains which they found in quails with the respiratory syndrome observed. Accordingly, it is not sure whether CoV was responsible for the enteric syndrome observed in the outbreak herewith described. However, except for CoV, no other specific pathogens were detected in our investigation. The description of CoV in quail rises several questions, as to whether the quail CoV could be a new members of the *Coronavirus* genus or a CoV from other avian hosts. Epidemiological investigations of the outbreak ruled out the introduction of CoVs in the farm by other avian flocks or farms. CoVs

are usually not considered strictly specie-specific, as they may replicate in heterologous hosts with or without symptoms. Experimental infections of chickens with turkey CoV may result in asymptomatic virus replication in the gut (Ismail *et al.*, 2003). Likewise, IBV-induced disease usually is observed only in chickens. On the contrary, experimental infection of pigs with feline CoV may result in a syndrome similar to the disease induced by TGEV (Woods *et al.*, 1981).

### Conclusions

This is the first description of probable CoV enteric infection in quail. It will be interesting to characterize molecularly the quail CoV to assess whether this is a new CoV or from other avian species. Sequence analysis of viral genome will be useful to clear up this matter. Another important issue is the pathogenic attitude of the quail CoV. The clinical signs and lesions observed in the quails were very similar to those induced by CoVs in turkeys and chickens. Because no other specific pathogen was detected, a pathogenic role of CoV may strongly suggest, although other factors may have been involved in exacerbating the clinical signs. For example, the disease was more severe in the young birds after introduction in the external aviary than in cages. Stress factors such as handling and change of environment may have contributed to increase the severity of the syndrome. Experimental transmission with this pathogen is necessary also in order to address this hypothesis. In addition, it will be paramount to understand the ecology of this novel CoV and to investigate the potential impact in quail breeding.

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