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Virulent feline calicivirus disease in a shelter in italy: A case description

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ABSTRACT

Feline calicivirus (FCV) is a common pathogen of cats that is particularly widespread in multi-cat environments such as shelters and catteries. FCV infections are usually associated with acute, mild and self-limiting upper respiratory tract disease characterized by oral vesicles/ulcers. Recently, virulent systemic disease (VSD) associated with FCV infection has been reported in the USA and Europe. This paper describes a case of VSD affecting one adult, FIV infected cat ("Oscar") living in a shelter located in Northern Italy; the clinical, post-mortem and laboratory findings indicate that this is the first case of suspected FCV-VSD in this country. Similar to a previous report (Meyer et al., 2011), the disease affected only one cat, while others remained asymptomatic, despite their direct contact with "Oscar". Phylogenetic analysis identified unique features in the "Oscar" FCV isolate. The FIV infection of the patient might have favoured the generation of the virulent FCV strains in this cat.

1. Introduction

Feline calicivirus (FCV) is a highly prevalent pathogen of cats, with a widespread distribution in the feline population. FCV infections are associated with a range of clinical syndromes. They can be clinically inapparent or be seen as relatively mild oral and upper respiratory tract disease, with or without acute polyarthritis. Less commonly, sudden death, ulcerative dermatitis, limping, abortion, jaundice and severe pneumonia can occur (Greene, 2006). FCV has also been implicated in the pathogenesis of the feline chronic gingivo-stomatitis complex (FCGS), but its role remains uncertain (Knowles et al., 1991).

FCV is a RNA virus with strong genetic and antigenic variability. The genomic adaptability and strain variability is believed to generate viral strains of variable virulence that may be responsible for the wide range of clinical manifestations (Pedersen et al., 2000).

Vaccination is widely practiced and provides moderate protection against acute disease but does not prevent FCV infection and both vaccinated and unvaccinated cats can become persistently infected carriers (Wardley, 1976; Wardley et al., 1974).

More recently, virulent systemic disease (VSD) associated with FCV infection has been reported in the USA and Europe (Pedersen et al., 2000; Schorr-Evans et al., 2003; Hurley et al., 2004; Coyne et al., 2006; Reynolds et al., 2009). Outbreaks have been associated with a high mortality rate (up to 50%); besides upper respiratory tract disease, affected cats showed a range of clinical signs of variable severity, including fever, cutaneous oedema, ulcerative dermatitis, anorexia and jaundice. VSD, initially termed haemorrhagic-like fever, shares some features with rabbit haemorrhagic disease caused by another calicivirus, the Rabbit Haemorrhagic Disease Virus (RHDV). FCV-VSD strains are described as highly contagious, with marked tropism for endothelial and epithelial cells of the skin and parenchymal organs which explains the clinical manifestation of the disease. Adult cats are often more severely affected than kittens, and vaccination does not prevent VSD.

A recent report of FCV-VSD in a cat in Germany which was not associated with an epizootic course, demonstrated that FCV-VSD can also occur as a disease of individual animals (Meyer et al., 2011). This has also been indicated by earlier clinical reports of FCV infected cats which mentioned jaundice and sudden death (Love and Baker, 1972; Ellis, 1981).

This paper describes a systemic caliciviral disease affecting an adult cat living in a shelter in Northern Italy. Clinical, laboratory and post-mortem findings indicate that this is the first reported case of FCV-VSD in this country.

2. Materials and methods

2.1. Case description

A 10-years-old neutered male Domestic Shorthair cat, named "Oscar", which had been living in a rescue shelter in a residential

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area of Bologna (Emilia-Romagna region, Northern Italy) for two months, was presented to and treated by one of the authors (MSC), for a 7-day period of disease. No other cats living in the rescue shelter which practices strict annual vaccination with Feligen CRP (Virbac), developed similar clinical signs while "Oscar" was ill or afterwards. Clinical signs worsened during the following days and the cat died 7 days after initial presentation despite intensive care treatment.

2.2. Sample collection

Venous blood was collected from "Oscar" upon admission for a minimum database and FIV-FeLV testing (SNAP® FIV/FeLV Combo Test, IDEXX, Italy). Blood samples were taken by jugular venipuncture using a vacuum collection system (Sarstedt®) with tubes containing K_3EDTA or clot activator. Samples were immediately analysed or stored at −20 °C. Complete blood count (CBC) and biochemistry were performed, using an automated blood cell counter (Cell-Dyn 3500R) and a biochemistry analyzer (Olympus AU 400), respectively. Dry cotton swabs were rolled over the caudal pharynx and were then placed in 1.0 ml of sterile 0.01 M phosphate buffered saline and stored at −80 °C for virus culture or RNA isolation. In addition, oropharyngeal samples were collected from three other healthy cats, aged between one and two years (cat #3, cat #6, cat #7) that had lived in direct contact with "Oscar". These specimens were submitted for a virological examination at the Department of Veterinary Medical Sciences, University of Bologna (DIMEVET).

2.3. Post mortem, histopathological and immunohistological examination

The carcase was submitted to DIMEVET for a full post mortem examination. Tissue samples (tongue, tonsils, heart, lungs, liver, intestine, bone marrow, spleen, kidney, brain) were fixed in 4% buffered formalin and paraffin wax-embedded. Sections (3–5 μm) were prepared and stained with haematoxylin and eosin (HE) for light microscopical examination.

Immunohistology for the demonstration of FCV antigen was carried out on sections from the lung, liver, kidney, intestine and tongue, using a rabbit anti-peptide antibody directed against a conserved epitope (antigenic site 4) of the FCV capsid following a previously published protocol (Coyne et al., 2006). Briefly, the peroxidase anti-peroxidase method was applied after blocking of endogenous peroxidase, antigen retrieval with citrate buffer (pH 6.0) at 96 °C and incubation with the primary antibody at 4 °C for 15–18 h and using diaminobenzidine for visualisation. A formalin-fixed and paraffin-embedded cell pellet of FCV strain F9-infected feline embryo cells and sections from a tongue ulcer with marked FCV antigen expression from previous diagnostic case were used as positive controls. Consecutive sections from the tested organs of the present case, in which the primary antibody was replaced by an irrelevant isotype-matched antibody served as negative controls.

2.4. Virological investigations

2.4.1. Virus isolation

Virus culture was performed for FCV and Feline Herpesvirus (FHV) isolation from the oropharyngeal swabs from cat #3, cat #6, cat #7 and several tissue specimens, collected at the time of necropsy from the cat "Oscar", such as tongue, liver, lungs, intestine, brain, kidney, bladder and bone marrow.

Specimens were cultured on a confluent monolayer of Crandell-Reese Feline Kidney (CrFK) cells at 37 °C in a 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM; Gibco®, Life Technologies Corporation, Grand Island, NY, USA) supplemented with 1% antibiotic-antimycotic solution (10000 units/mL of penicillin, 10000 μg/mL of streptomycin, and 25 μg/mL of Fungizone® (amphotericin B; Gibco®, Life Technologies Corporation), 10% fetal calf serum (FCS), 2 mmol/L of L-glutamine, 1 mmol/L of sodium pyruvate and 7.5% sodium bicarbonate. For virus isolation, CRFK monolayers were incubated for 2 h with a 0.1 ml aliquot of supernatant derived from oropharyngeal swabs to which antibiotic-antimycotic solution (0.02 mg/ml) had been added. The inoculum was then removed and replaced by culture medium with 2% FCS.

Tissue samples were grinded with a sterile pestle in a mortar with sterile sand to a 10% suspension in DMEM supplemented with antibiotic-antimycotic solution. After cold centrifugation at 3000*g* for 15 min, the supernatants were filtered through a 0.45-μm filter, and 0.1 ml of each was added to a well of a 24-well tissue culture plate. The culture plates were incubated at 37 °C in a 5% CO_2 humid atmosphere.

Infection was confirmed by the presence of the characteristic cytopathic effects within 12–48 h post infection; three blank cell passages were carried out for each sample (Povey and Johnson, 1971), followed by FCV-FHV specific molecular techniques described below.

2.4.2. Molecular diagnostic assay

The third cell passage supernatant was used to detect FHV DNA and FCV RNA by molecular techniques.

The FHV polymerase chain reaction (PCR) was performed as previously described (Vögtlin et al., 2002) on DNA extracted from the freeze-thawed cell lysate of each specimen using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

To detect FCV RNA and to quantify viral loads, a two step specific real-time reverse transcriptase PCR (RT-PCR) was performed as previously described (Helps et al., 2002).

Total RNA was isolated from 140 μl of freeze-thawed cell lysate, using a QIAamp Viral RNA Kit (Qiagen) according to the manufacturer's instructions.

Complementary DNA (cDNA) was generated, using the Multiscribe Reverse Transcriptase (Applied Biosystem®, Life Technologies Corporation, Grand Island, NY, USA) and random hexamers in a final volume of 20 μl. The cycling parameters were the following: 10 min at 25 °C, 15 min at 45 °C and 2 min at 95 °C. Quantitative PCR was performed, using a Rotor Gene 3000 (Corbett Research, Sydney, Australia) and Sybr Green technology. Reactions were carried out in a final volume of 25 μl with 12.5 μl of Sybr Premix Ex Taq (Takara Bio Inc., Otsu, Japan), 100 nM of forward and reverse primers (qFCV_ for 5′-TAA TTC GGT GTT TGA TTT GGC CTG GGC T-3′; qFCV_rev 5′ CAT ATG CGG CTC TGA TGG CTT GAA AC TG 3′), 9.5 μl of water and 2 μl of cDNA. Samples were subjected to the following thermal cycling conditions: 10 min at 95 °C, followed by 40 cycles of 10 s at 95 °C, 10 s at 60 °C and 20 s at 72 °C. Immediately following the PCR a melting curve was performed, raising the incubation temperature from 72 °C to 95 °C in 0.1 °C increments, with a hold of 10 s at each increment. In order to generate a standard curve, FCV RNA was amplified by RT-PCR and cloned into a pCR 4-TOPO Vector (TOPO TA Cloning Kit, Life Technologies Corporation). The plasmid was used as standard in serial 10-fold dilutions to precisely quantify the viral load. A limit of detection of 2–4 copies of viral cDNA (detected in 95% of real time RT-PCR runs) was achieved.

2.4.3. Capsid gene amplification and sequencing

To investigate the relationships of the FCV isolates obtained in this study, the ORF2 encoding the precursor of the major structural capsid protein was amplified and sequenced from each sample.