First report of feline morbillivirus in Europe

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Riassunto
In questa comunicazione viene evidenziata per la prima volta in Europa la presenza di morbillivirus felino. L’RNA virale è stato rilevato nelle urine di un gatto nefropatico di 15 anni, in cui la ricerca degli agenti eziologici più comunemente associati a patologia renale risultava negativa. Il virus non è stato evidenziato nei campioni di sangue e feci prelevati dallo stesso gatto.

Keywords
Cat, Europe, Feline morbillivirus, Nephropathy.

Summary
Feline morbillivirus was detected in urine samples of a 15 year old cat suffering from severe nephropathy. Viral RNA was not detected in blood and faecal samples and also the most common pathogens associated to cat kidney failure were not found. This report describes the first evidence of feline morbillivirus in Europe.

Parole chiave
Europa, Gatto, Morbillivirus felino, Nefropatia.

Prima segnalazione di morbillivirus felino in Europa

On June 29, 2015 a 15-year-old domestic shorthair neutered male stray cat of 2.3 Kg of weight was referred to the Veterinary Teaching Hospital of the University of Teramo, Teramo (Abruzzo, Central Italy) as it was found seemingly injured - probably hit by a car - by the Veterinary Public Services of the same municipality. At first sight, the cat was dyspnoeic with abdominal breathing pattern. During clinical examination, the cat showed very poor nutritional status (BCS-1), dehydration (8%), dyspnoea (48 bpm), and hypothermia (37.0 °C). The animal was depressed but alert, and the appetite was good. Thoracic auscultation resulted in dampening of lung and heart sounds ventrally. Thoracic radiography showed the presence of interlobar fissure lines blurring the cardiac and diaphragmatic borders consistent with pleural effusion. Analysis of pleural fluid obtained by thoracentesis revealed chyloous effusion. Haemato-biochemical profile indicated lymphopenia (0.3 x 10^9 lymphocytes/L, reference range 12-18 x 10^9 lymphocytes/L), mild hypoproteinemia (5.26 g/100 ml, reference range 5.5-8), hypoalbuminemia (1.79 g/dL, reference range 2.6-3.8), mild increase in enzyme activity of asparate aminotransferase (AST) (93 U/l, reference range 10-40), fructosamine (176 μmol/l, reference range 210-360), increased basal bile acid (12.6 μmol/l, reference range < 6), mild-low trygliceridemia (36 mg/100 ml, reference range 40-130), and mild-low magnesemia (1.36 mg/100 ml, reference range 1.60-3.60). Urinalysis revealed specific gravity of 1015, proteinuria (+++) with a Urine Protein to Creatinine Ratio (UPCR) of 4.3 (reference range > 0.4). Microscopic examination of the sediment was unremarkable. Serum total T4 was in the lower half of the normal range. Abdominal ultrasound revealed an irregular wall thickening and biliary intrahepatic stasis consistent with hepatobiliary disease. Renal echogenicity was altered, with an increased cortical echogenicity and reduced cortico-medullary definition compatible with chronic inflammatory renal disease. During hospitalization, the cat showed polyuria and polydipsia (PU/PD) and occasional vomiting. The cat was treated with fluid (Ringer Lactate, 5 ml/kg/h) for the first 24 hours and antiemetic therapy with maropitant (1 mg/kg/sid sc) for 3 days. Virological examinations were conducted at the Virology Unit of the Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise (IZSAM), Teramo, Italy. Viral nucleic acids were purified (High Pure Viral Nucleic Acid Kit, Roche
Life Science, Roche Diagnostics S.p.A. Monza, Italy) from EDTA-blood, urine, pleural fluid, and faecal samples collected on June the 29th. EDTA-blood samples were tested for feline infectious peritonitis virus (FIPV, Dual IPC-TaqVet™, LSI, Lissieu, France), feline immunodeficiency virus (FIV, gag protein gene-genesig® Advanced Kit, Rowhams, UK), feline leukemia virus (FeLV, U3 region LTR- genesig® Advanced Kit), Leishmania spp. (Rodgers et al. 1990), while pleural fluid was tested for FIPV. Furthermore, purified RNAs from urine, EDTA-blood and faecal samples were tested for the presence of morbillivirus by RT-PCR (Woo et al. 2012) targeting a portion of the L protein-encoding gene of morbilliviruses. DNA purified from urine was also tested for the presence of Leptospira spp. (Stoddard et al. 2009). As for the RT-PCR for morbilliviruses, the QIAGEN® OneStep RT-PCR kit (Hilden, Germany) was used according to manufacturer’s instructions with primers LPW12490, 5’- CAGAGACTTAATGAAATTTATGG-3’ and LPW12941, 5’- CCACCCTACGGGTACCTT-3’ adopting the following thermal protocol: reverse transcription, 30 minutes at 50°C; initial PCR activation step, 15 minutes at 95°C; 3-step cycling consisting of denaturation, 30 seconds at 94°C; annealing, 30 seconds at 54°C; extension, 1 minute at 54°C (for 40 cycles); final extension 72°C for 10 minutes. Blood and pleural fluid samples tested negative for FIPV. Similarly the blood sample was negative for FIV and FeLV. Leishmania spp. and Leptospira spp. were not detected nor bacteria grew when urine samples were cultured. The RT-PCR targeting the L protein-encoding gene of morbilliviruses amplified a 172 bp amplicon from the urine sample whereas blood and faecal samples did not show any amplification. A second urine sample which was collected on July the 14th also tested positive for morbillivirus. One amplicon was used for direct sequencing in both directions using RT-PCR primers. The obtained sequence was deposited in GenBank under the accession number (KT306750).

By nucleotide blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome) it bears the highest nucleotide (nt) identities, 94% and 92%, with feline morbillivirus (FmoPV) strains 761U (JQ411014) and 776U (JQ411015), respectively. Both strains were isolated in Hong Kong. The nt identity with remaining publicly available FmoPV sequences, mainly from Japan, ranges from 86 to 91%. Conversely, the nt identity with extant morbilliviruses including canine distemper virus, peste-des-petit-ruminants virus, dolphin morbillivirus, and measles virus ranges from 70 to 75%. As far as we are aware, although the presence of partial FmPoV sequences deposited from Germany, this is the first report describing FmPoV circulation in Europe. FmPoVs have been so far isolated and reported only in Hong Kong and Japan (Woo et al. 2012, Furuya et al. 2014, Sakaguchi et al. 2014). FmPoVs are phylogenetically related to the other morbilliviruses and thus are considered to belong to the genus Morbillivirus (Woo et al. 2012). Tubule-interstitial nephritis (TIN) is a disorder that involves tubules and the interstitium of the kidney. It is the most common cause of renal failure and one of the leading causes of deaths in domestic cats. The origin of TIN is still unknown. FmPoV has been associated with TIN in cats (Woo et al. 2012) although its patho-biological role and epidemiology have not been determined yet (Sakaguchi et al. 2014). In the case reported here the infected animal shed the virus in its urine for at least 14 days following the first detection by RT-PCR. However we were not able to know whether the virus shedding was continuous or intermittent and, similarly, at the moment we don’t know if the virus shed in the urine is still infectious. After 14 days of hospitalization the cat did not show any improvement in terms of body condition; poliuria and proteinuria were still present even though UPCR improved significantly (0.7). Due to the advanced age of the cat, expected costs and anaesthetic risks, it was not possible to perform kidney biopsy and have a proper histological description of the case. Although no anamnestic data suggesting the infection stage of the infected cat were available, clinical signs, laboratory analyses and the fact that RNA was detected in urine samples might indicate a localized and chronic viral infection of the kidneys. Furthermore, FmPoV was the only known pathogen found in the infected cat and likely it has played a major role in determining the renal failure. Further studies are warranted in order to elucidate either the epidemiology of the virus in Europe or its pathogenesis and association with renal infection. The observed nt identity (94%) between the amplified portion of the L protein-encoding gene of the Italian FmPoV, the homologous sequences of the prototype strain 761U and of the other FmPoVs publicly available, may suggest the presence of an additional FmPoV lineage that is circulating among cats in Europe, divergent from those described in Hong Kong and Japan. Similar or even lower values of nt identities were also observed with other morbilliviruses belonging to the same species (Marcacci et al. 2014). In order to achieve a proper biological and genomic characterization, viral isolation and whole genome sequencing by next generation sequencing straight from the infected urine samples are currently on-going.

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References


