mellitus (20%). The most common symptoms were altered mental status and fever (50% each), followed by paresis and headache. Pneumocephalus was observed in 30% at the moment of diagnosis and it has been associated with a worse prognosis.[5,7,9] The surgical treatments were burr holes (40%) and craniotomy (30%). Two cases presented recurrences (minicraniotomy 1, burr hole 1), forcing an expanded craniotomy. The mortality was 40%.

Hematogenous spread seems to be the pathophysiological mechanism of spontaneous SE. In 40% of cases presented a distant infection with same germ.[1,6–8] Other risk factors for overt infections could be previous CSDH,[4,7] diabetes mellitus and immunosuppression states.[7,9] In our case, we relate the genesis of spontaneous SE to hematogenous spread of an unconfirmed UTI, in association with diabetes mellitus.

The treatment of choice is surgical evacuation associated to culture adjusted intravenous antibiotics during 4–6 weeks.[4,9] The most appropriate surgical approach is still under discussion, as some authors argue that the burr holes are sufficient for evacuation; others suggest that wider exposure as a craniotomy is more effective.[3] Yilmaz et al.[3] presented a lower recurrence rate on craniotomies (10%) vs. burr holes (38%). In cases with difficult pus extraction, as in multiloculated collections,[4] parafalcine location[3] and recurrences, is better to carry out a wide craniotomy. Both surgical techniques must perform thorough washing until clear liquid outlet, take care to remove the adherent material in the cortex for its lesion risk; placement of subdural drainage can be left up to 72 h. In our case, the realization of minicraniotomy does not allow an extensive cavity wash, and the initial treatment with cefotaxime (2 g/8 h) was with low dose; these may favor the recurrence.

In conclusion, the spontaneous SE should be suspected in patients with fever and hypodense subdural collections in the head CT, and the administration of intravenous contrast may to increase its sensitivity. To achieve a favorable outcome must make an early surgery and begin antibiotic therapy. It seems that the craniotomy is associated with a lower relapse rate, however more studies are needed to confirm this fact.

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Luis Mariano Rojas-Medina[a,*], Lucía Esteban-Fernández[b], José Antonio Gutiérrez-Cierco[a], Víctor Rodríguez-Berrocal[a]

[a] *Servicio de Neurocirugía, Hospital Universitario Ramón y Cajal, Madrid, Spain*
[b] *Servicio de Neurología, Hospital Universitario Ramón y Cajal, Madrid, Spain*

* Corresponding author.
E-mail address: luismarianorojas@gmail.com (L.M. Rojas-Medina).

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Autochthonous *Cryptosporidium cuniculus* infection in Spain: First report in a symptomatic paediatric patient from Madrid

Infección autóctona por Cryptosporidium cuniculus *en España: primer informe en un paciente pediátrico sintomático del área de Madrid*

Human cryptosporidiosis, an infection caused by parasitic protozoan of the genus *Cryptosporidium*, is a major cause of gastrointestinal disease worldwide including Spain.[1] Most human cases are attributed to *C. hominis* and *C. parvum*, although infections with zoonotic *C. meleagridis*, *C. canis*, *C. felis*, and a number of rare genotypes are also sporadically documented.[2] Among these, *Cryptosporidium cuniculus* (previously known as the *Cryptosporidium* rabbit genotype) was first identified in an adult female rabbit in 1979[3] and fully re-described and characterized in 2010.[4] Since then, very few cases of human infections by *C. cuniculus* have been identified in UK ($n=37$),[5] Nigeria ($n=5$),[6] Australia ($n=1$),[7] and France ($n=1$).[8]

In May 2015 a 7-year-old female child complaining of gastrointestinal symptoms including acute, non-bloody watery diarrhoea and abdominal pain, was admitted to the outpatient clinic of the University Hospital Puerta de Hierro Majadahonda (Madrid) for routine coproparasitological examination. The patient had a normal immune status and no relevant record of recent travelling abroad. Information regarding contact with pet or wild rabbits was unavailable. A single, concentrated stool sample tested positive for the presence of *Cryptosporidium* oocysts by a commercial immunochromatographic test (Cer Test Biotec S.L., Zaragoza, Spain) and by microscopic examination of a fresh faecal smear stained with the modified Ziehl–Neelsen method.

A new aliquot of the faecal material was sent to the National Centre for Microbiology at Majadahonda (Madrid) for genotyping analyses. Total DNA was extracted and purified using the QIAamp® DNA stool mini test kit (Qiagen, Hilden, Germany). Identification and molecular characterization of the obtained isolate was conducted by multilocus genotyping (involving PCR-based protocols and DNA sequence analyses) of the small-subunit ribosomal RNA (*SSU* rRNA)[9] and the 60-kDa glycoprotein (*GP60*)[10] genes of *Cryptosporidium*. Both *SSU* rRNA-PCR and *GP60*-PCR assays generated the expected amplicons of ~587 bp and ~870 bp, respectively. Sequence analysis of the corresponding PCR products confirmed the presence of *C. cuniculus*, allowing the assignment of this isolate to the sub-genotype VbA34 of the parasite. As expected, phylogenetic analysis at the *GP60* locus between the sequence from this study and reference sequences previously deposited in GenBank placed our isolate into a well-defined allelic group (family Vb), while other representative sub-genotypes of *C. cuniculus* clustered together in a separate allelic group (family Va) (Fig. 1). The

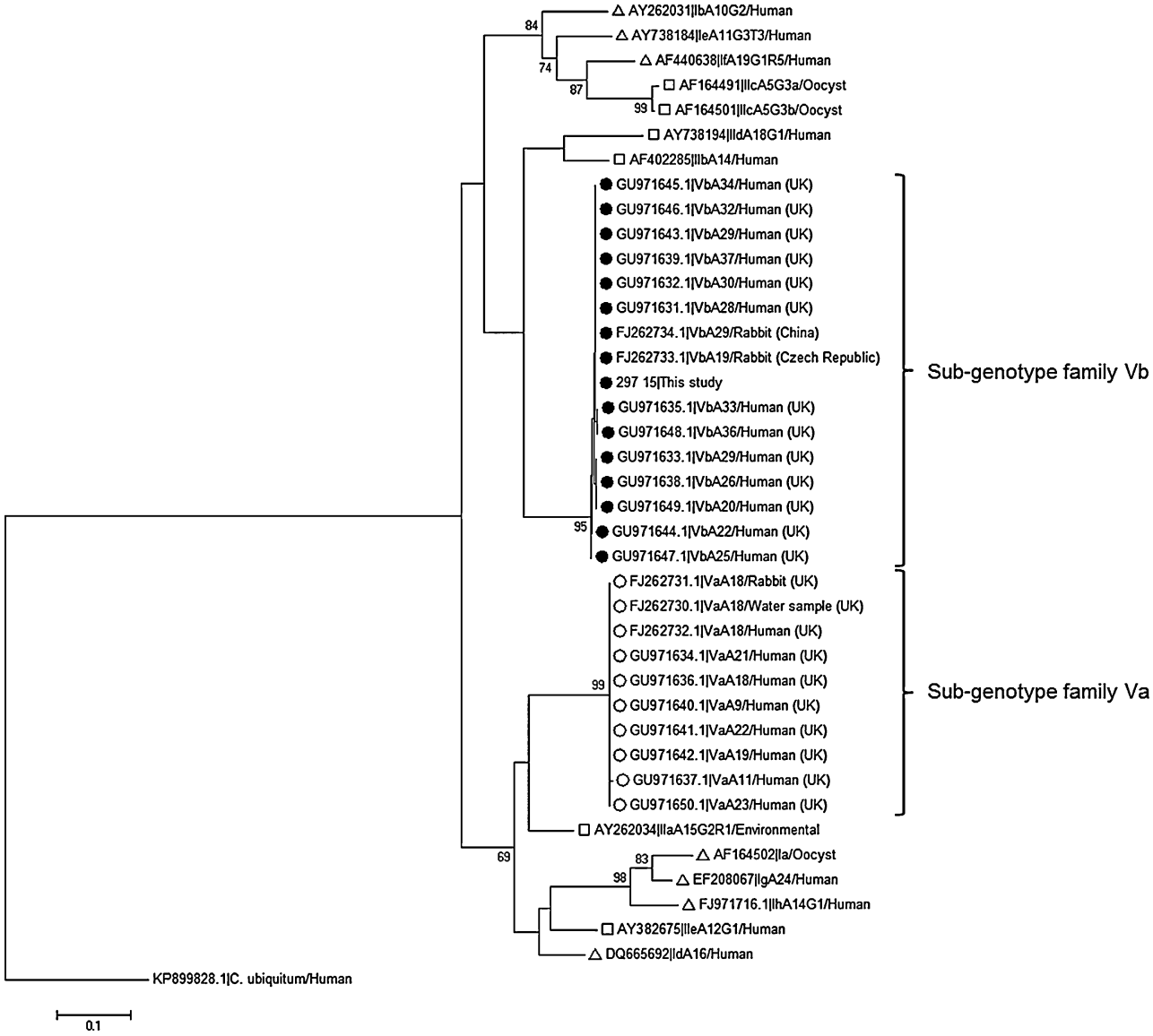

Fig. 1. Phylogenetic relationships between *Cryptosporidium cuniculus* and *Cryptosporidium* spp. sub-genotypes at the *GP60* locus inferred by a neighbour-joining analysis of the nucleotide sequence covering a 445-bp region (positions 1 to 445 of GenBank accession number GU971645, allele VbA34) of the gene. Empty and filled circles indicate representative *C. cuniculus* sequences belonging to the sub-genotype families Va and Vb, respectively. Empty triangles and squares indicate representative sequences of the most frequent sub-genotypes of *C. hominis* and *C. parvum*, respectively. Bootstrapping values over 50% from 1000 replicates are indicated at the branch points. The evolutionary distances were computed using the Kimura 2-parameter method. The rate variation among sites was modelled with a gamma distribution (shape parameter = 2). *C. ubiquitum* was used as outgroup taxa.

nucleotide sequence data obtained in this study at the *SSU* rRNA and the *GP60* loci have been deposited in GenBank under the accession numbers **KU129015** and **KU129016**, respectively.

This paediatric case represents the first report of cryptosporidiosis by *C. cuniculus* in Spain. To date *C. cuniculus* has been almost exclusively found in rabbits and humans. This fact is hardly surprising when considering that this *Cryptosporidium* species is genetically closely related to *C. hominis*, from which it differs in only 0.27% of base pairs. However, deep morphological and biological differences have been demonstrated between both species.[4] In UK *C. cuniculus* was first identified as a human pathogen of zoonotic origin during a waterborne outbreak in July 2008, being the third most commonly identified *Cryptosporidium* species in clinical patients during the period 2007–2008. Contrasting with the typical distribution patterns of *C. hominis* and *C. parvum*, *C. cuniculus* cases were detected in individuals of all ages with a seasonal peak in late summer and autumn.[5]

Taken advantage of the inherent genetic heterogeneity of the *GP60* gene two different families (Va and Vb) have been described within *C. cuniculus*, each containing at least seven and 12 different sub-genotypes, respectively.[4,5] Sub-genotypes within these allele families were assigned on the basis of the number of TCA serine codons present in the microsatellite region of the gene. In this regard, the *C. cuniculus* isolate obtained in this study was characterized as sub-genotype VbA34, sharing 100% homology with the sequence of a human isolate from UK (GenBank accession number GU971645). Although the actual origin of the *C. cuniculus* infection in our paediatric patient could not be investigated, direct contact with pet or wild rabbits seems the most likely source of infective oocysts. Therefore, good personal hygiene practices, especially

hand washing, are highly advisable when handling rabbits or their faecal material.

Conflict of interest

The authors declare no conflict of interest.

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Rocío Martínez-Ruiz [a], Aida de Lucio [b], Isabel Fuentes [b], David Carmena [b,*]

[a] *Microbiology and Clinical Parasitology Service, University Hospital Puerta de Hierro Majadahonda, Madrid, Spain*
[b] *Parasitology Service, National Centre for Microbiology, Health Institute Carlos III, Madrid, Spain*

* Corresponding author.
E-mail addresses: dacarmena@isciii.es, d.carmena71@gmail.com (D. Carmena).

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Development of daptomycin resistance during therapy in a patient with methicillin-resistant *Staphylococcus aureus* endocarditis: A case report

Desarrollo de resistencia a daptomicina durante el tratamiento de un paciente con endocarditis por Staphylococcus aureus *resistente a meticilina*

A 69-year-old woman was sent to the emergency room by her cardiologist after a gastrointestinal bleeding episode. She had a history of allergy to amoxicillin-clavulanic acid (rash), type 2 diabetes, hypertension, hypothyroidism, peptic ulcer disease and iron-deficiency anemia. She had a mechanic prosthetic mitral valve placed four years before due to rheumatic mitral stenosis. At that time a tricuspid anuloplasty had been performed. She had atrial fibrillation and severe pulmonary hypertension and was receiving oral anticoagulation, all of which leading to a NYHA class II–III congestive heart failure (CHF) with multiple hospital admissions due to decompensated CHF. Hematocrit was stable and an upper gastrointestinal endoscopy did not show bleeding, ruling out acute gastrointestinal bleed. Elevated creatinine was observed (1.97 mg/dL); acute renal failure was considered to be pre-renal, associated to diuretics. On day 5 after hospitalization she presented fever and a painful hematoma around a previous peripheral intravenous line. Blood cultures were obtained and levofloxacin therapy was started. Blood cultures became positive in less than 24 h, gram-positive cocci in clusters were observed and identified as *Staphylococcus aureus* by MALDI-TOF mass spectrometry. Antimicrobial therapy was switched to daptomycin 9 mg/kg q48 h because her creatinine was rising and her estimated creatinine clearance at that time was 33 mL/min. MIC testing was conducted by VITEK® and confirmed by Microscan® and E-test Biomerieux®. The isolate was resistant to methicillin, erythromycin, levofloxacin and tobramycin, and susceptible to trimethoprim-sulfamethoxazole, clindamycin, vancomycin (MIC < or = 0.5 mg/L), and daptomycin (MIC = 0.25 mg/L). She became afebrile in less than 48 h and blood cultures were serially obtained. As part of the initial workup a transesophageal echocardiogram (TEE), which was negative for endocarditis, and an angio-CT scan were performed, disclosing intramuscular hematoma without venous thrombosis and several pulmonary nodules consistent with septic emboli. Blood culture from day 11 was positive, so linezolid (600 mg BID iv) was added to daptomycin on day 13. Blood cultures from day 13 continued to be positive for MRSA with the same susceptibility profile as the previous isolates except for daptomycin (MIC of 2 mg/L) and vancomycin (MIC of 1 mg/L). This was confirmed by E-test®. Antimicrobial therapy was switched to teicoplanin (8 mg/kg) plus intravenous fosfomycin (4 g TID). On day 15 a repeat TEE showed a 7.5 mm-long filiform vegetation attached to the auricular side of the prosthetic mitral valve. Thickening of mitro-aortic junction was found too. Cardiac surgery consultation was performed but the patient was not considered a surgical candidate given her baseline dyspnea and severe pulmonary hypertension despite a non-disfunctioning prosthetic valve. She remained afebrile during the whole hospitalization but having dyspnea on minimal physical activity. On day 25 she had a cardiac arrest and died.

Genetic relatedness between the isolates was confirmed by pulsed-field gel electrophoresis (PFGE) with *Sma*I digestion using the protocol described by Pérez-Vázquez et al. Sequencing of genes reported to be involved in decreased susceptibility to daptomycin (*walkA*, *rpoB*, *agrA* and *mprF*) showed a G2476A mutation (numbering refers to Genbank entry HM140976) in the *mprF* gene of the resistant isolate, but not in the susceptible isolate. MLST analysis showed that the *S. aureus* isolates from days 5 and 13 belonged to ST125. This mutation would translate into a leucine to phenylalanine change in position 826 of the protein sequence (L826F), close