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Association of NetF-positive type A *Clostridium perfringens* with necrotizing enteritis in neonatal foals

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A role for type A *Clostridium perfringens* in necrotizing enterocolitis of neonatal foals has long been suspected but incompletely characterized. The supernatant of an isolate made from a foal that died from this disease was highly cytotoxic for an equine ovarian (EO) cell line. Partial genome sequencing of an isolate revealed three novel putative toxin genes encoding proteins related to the pore-forming Leukocidin/Hemolysin Superfamily; these were designated *netE, netF*, and *netG. netE* and *netF* were located on one large conjugative plasmid, and netG was located with a cpe enterotoxin gene on a second large conjugative plasmid. Mutation and complementation showed that only *netF* was associated with the cytotoxicity. There was a highly significant association ($p<0.0002$) between the presence of netF with type A strains isolated from cases of foal necrotizing enterocolitis. netE and netF were found in all cytotoxic isolates, as was cpe, but netG was less consistently present. Pulsed-field gel electrophoresis showed that *netF*-positive isolates belonged to a clonal lineage that included canine-origin netF strains; some canine and equine *netF*-positive isolates were genetically indistinguishable. Equine antisera to recombinant Net proteins showed that only antiserum to rNetF had high supernatant cytotoxin neutralizing activity. The genomes of two *C. perfringens* strains recovered from a case of foal necrotizing enteritis (JP55) and canine haemorrhagic gastroenteritis (JP838), respectively, were completely sequenced using Single Molecule, Real-Time (SMRT) technology-PacBio and Illumina Hiseq2000. The JP55 and JP838 genomes include a single 3.34 Mb and 3.53 Mb circular chromosome, respectively, and both genomes additionally consist of five circular plasmids. Comparison of these two *C. perfringens* chromosomes with three fully sequenced reference chromosome sequences identified regions (~69 kb) shared between the two isolates, including regions forming a mosaic of plasmid-integrated segments, suggesting that these elements were acquired early in a clonal lineage of *netF*-positive *C. perfringens* strains. In addition, plasmid annotation revealed that both *netF*-positive *C. perfringens* strains, JP55 and JP838, harbour three tcp-conjugative plasmids in common, including a *NetF/NetE* toxins-encoding plasmid, a CPE/CPB2 toxins-encoding plasmid and a putative bacteriocin-encoding plasmid. We also found that JP55 and JP838 strains share unique virulence genes on conserved pathogenicity loci on the large *tcp*-conjugative plasmids. The identification of this novel NetF necrotizing toxin is an important advance in understanding the virulence of type A *C. perfringens* in necrotizing enteritis of neonatal foals.

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Novel viruses determined using fecal virome analysis in the feces of foals with diarrea

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Within the first six months of life, up to 20% of foals have episodes of diarrhea caused by infectious agents. However, there is a limited understanding of the relative prevalence of common agents and the mechanism of coinfections. In equine veterinary medicine, coinfections and the consequent interactions contributing to intensify gastrointestinal disease have not been deeply characterized. In this study we performed viral metagenomic analysis of fecal samples from foals suffering diarrhea. Two pools of three samples from different individuals were prepared and sequenced on a HiSeq 2500 platform (Illumina). We detected the presence Rotavirus A together with novel and highly divergent viruses belonging to *Picornaviridae* and *Astroviridae* families. An almost complete Kobuvirus genome was obtained. It showed to be phylogenetically related with bovine, ferret and ovine strains but more distantly related to strains infecting pigs and dogs. To our knowledge, this is the first report of Kobuviruses detected in horses. On the other side, a partial sequence from *Astroviridae* family was also detected. This was highly divergent and would also represent the first report of this virus in feces from foals suffering diarrhea. Together these results underline that many novel viruses affecting foals are yet to be discovered. This study aims to

	Samples	Viruses found
Pool 1	Foals with diarrhea, younger than 4 months	Group A Rotavirus Kobuvirus Astrovirus-like
Pool 2	(3 individual samples/pool)	Group A Rotavirus Picornavirus Astrovirus (Mamastrovirus)

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expand the knowledge of viral species present in feces of foals suffering diarrhea using Next Generation Sequencing. This will be useful to further explore the role of new infectious agents in this illness in foals.

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Detection and characterisation of *Clostridium difficile* in Australian Thoroughbred foals

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Diarrhoea is a common disease in young foals that is labour intensive and costly to manage. A large number of infectious agents have been detected in foals with diarrhoea, but the pathogenic importance of many of these agents is poorly understood. *Clostridium difficile* has been associated with severe necrotising enteritis in foals, but has also been isolated from healthy foals. Hypervirulent strains of *C. difficile*, such as ribotypes 027 and 078, commonly associated with human hospital outbreaks of diarrhoea, have also been detected in some animal populations. There are limited data on *C. difficile* disease in Australian horses. In a prospective case control investigation of diarrhoea in foals, faecal samples were collected on five Thoroughbred breeding farms in New South Wales, Australia, from foals with diarrhoea and age-matched control foals (age-matched pair). In addition, faeces were collected from foals with diarrhoea at an equine referral hospital. Foal faeces were tested for the presence of *C. difficile* using a quantitative polymerase chain reaction assay (qPCR) targeting the gene encoding triose phosphate isomerase. Anaerobic culture for *C. difficile* was performed on samples that tested positive by qPCR and isolates were further characterised by ribotyping. In total, 117 age-matched case control pairs and 26 hospitalised foals with diarrhoea were sampled. *C. difficile* was isolated from 3/3 case foals and 2/3 control foals positive by qPCR among the matched foals. *C. difficile* was isolated from 5/6 hospitalised foals with diarrhoea that were qPCR positive. Four different ribotypes were detected, including ribotype 012 and 078. This is the first report of the detection of *C. difficile* ribotype 078 in Australian horses. As this ribotype has been associated with severe disease in humans, this finding may have public health implications for veterinarians and horse owners.

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Equine rotavirus in Argentinean foals: an overview

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Group A equine rotavirus (RVA) associated diarrhea in foals represent a main sanitary problem for the equine industry, worldwide. In fact, equine RVA is considered to be the major cause of dehydrating diarrhea in foals younger than 3 month old. Young foals are highly susceptible to RVA infection and develop malabsorptive watery diarrhea leading to severe dehydration and sometimes death, especially in neonates with failure of passive antibody transference. Our group of research has been studying equine RVA since 1992. The circulating strains were characterized as genotypes G3A and G14 associated to P[12]. The complete genome of the equine RVA strains were described and the interaction among the antigens determining the genotypes were also studied. During these years important improvements in the diagnosis and characterization tools have been made as well as in the preventive strategies to control the disease in horse farms. The aim of the present work is to summarized our knowledge and discuss the future prospects regarding this important disease of foals.

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Comparison of serum amyloid A and fibrinogen, in the laboratory assessment of foals with diarrhea

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Diarrhea in young equines is very common and there are many causative agents and conditions (viral, bacterial, protozoa, parasites, drug or dietary associated, toxins and changes in the intestinal flora) that manifest with clinical signs of watery diarrhea in these foals. It is difficult to differentiate which foals may have an infectious cause of diarrhea and those of a non-infectious nature. It is estimated that a definitive cause of the diarrhea can only be reached in 40% of cases[1]. As part of a blinded controlled study to assess the use of a paste formulation of SB-300 in foals with watery diarrhea, assessments included fecal cultures, serum amyloid A (SAA), fibrinogen and complete cell counts. These assessments were performed upon admission to the trial, at the end of the treatment phase (T=72hours) and at the end of the observation period (T=144hours). SAA has been deemed a major acute phase protein in horses with usefulness in the assessment of inflammation and evaluation of response to therapy.[3] The usefulness of this acute phase protein has not been assessed in horses with diarrhea. This abstract aims to look at the relative and comparative usefulness of the acute phase proteins in assessment of foals with diarrhea and correlations if any to the causes of diarrhea and response to therapy. As this is an ongoing study (finish date November 22nd, 2015) the complete results are not yet available but preliminary findings indicate a marked comparative difference between the acute phase proteins. The full results will be published as part of this abstract presentation.

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