Short Communication

NetF-positive *Clostridium perfringens* in neonatal foal necrotising enteritis in Kentucky

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TYPE A *Clostridium perfringens* has been associated with necrotising enteritis in 1–14-day-old foals in Kentucky (Donahue and Williams 2002, Timoney and others 2005) but the pathogenesis of the disease is not well understood, in part because few affected foals die and thus the availability of postmortem samples is rare. An autogenous bacterin-toxoid vaccine utilising a *C. perfringens* Type A strain (UK MF 05/00) that carries genes for the α toxin (CPA), β2 toxin (CPB2) and enterotoxin (CPE), further enriched with recombinant CPB2 toxin, is used to immunise mares on Kentucky breeding farms with histories of foal diarrhoea (Timoney and others 2005). Recently, a novel pore-forming toxin NetF has been strongly associated with foal necrotising enteritis and canine haemorrhagic gastroenteritis (Mehdizadeh Gohari and others 2015). Here, the authors report for the first time the identification of *netF*-positive type A *C. perfringens* in foals with enteritis and enterocolitis in Kentucky and the presence of NetF in the bacterin-toxoid vaccine (UK MF 05/00) used to immunise mares in Kentucky (Timoney and others 2005).

Isolates of *C. perfringens* including UK MF 05/00 from foals in Kentucky from 2000 to 2011, with severe enteritis (Table 1), were screened by multiplex PCR for the presence of *netF* and *cpe*, a gene with which *netF* is strongly linked (Mehdizadeh Gohari and others 2015). These isolates had been stored frozen since initial culture. Their identity as *C. perfringens* was confirmed in the same multiplex PCR by the presence of the *cpa* gene. DNA was extracted using InstaGene Matrix (Bio-Rad Laboratories, Mississauga, Canada) according to the manufacturer's protocol. Multiplex PCR used primers for the cytotoxic genes *cpa*, *cpe* and *netF*, for amplification and other conditions as described (Mehdizadeh Gohari and others 2015; Finley, A., Medizadeh Gohari, I., Parreira, V. R., Abrahams, M., Staempfli, H. R., & Prescott, J. F. (2016) Prevalence of *netF*-positive *Clostridium perfringens* in foals in southwestern Ontario. *Canadian Journal of Veterinary Research* (submitted for publication).

TABLE 1: Kentucky foals with enteric disease from which *netF*-positive *Clostridium perfringens* were isolated

Foal identification	Year	Age	Disease
UK MF 05/00	2001	1 day	Acute necrotising colitis
ST9020	2004	7 days	Enteritis
245084	2006	14 days	Enteritis
82649	2011	7 days	Enteritis
2575	2011	3 days	Acute necrotising colitis
420568	2008	3 days	Enteritis

Of the isolates obtained from 23 individual foals, 6 (26 per cent) were positive for both *cpe* and *netF* (Table 1). These were from foals aged 1–14 days (median 3–7 days) (Table 1). Isolate KY MF 05/00 was from a foal that died with lesions of acute necrotising colitis.

Sera from 18 mares on seven Kentucky farms that had been immunised with the autogenous bacterin-toxoid vaccine made from KY MF 05/00 (Timoney and others 2005) were tested by ELISA for antibodies to recombinant NetF (Mehdizadeh Gohari and others 2015). Sera collected before and after vaccination and booster were all tested at the same time. The dilution cut-off value was defined as the lowest reading exceeding 1.5 times the background reading for each plate. The median serum titre before immunisation for 18 mares was 3,200 (range 800–25,400) and post immunisation for the same mares was 12,800 (range 3200–50,800). Five mares seroconverted (≥two-dilution increase), the titres of six mares increased by one dilution and eight mares showed no change in titre. The 18 sera used in the ELISA were selected because their prevaccination antibody titres to CPA and CPB2 toxin were elevated suggesting previous natural exposure to these toxins and possibly also to NetF. This was confirmed in this study by the high titres (12,800–25,400) of NetF-specific antibody detected in the prevaccination sera of five mares. It is probable the amount of NetF in KY MF 05/00 bacterin toxoid vaccine was low, as was the case for CPB2 toxin before enrichment of the bacterin-toxoid. This would explain the failure of six mares to make detectable responses to the NetF.

This study confirms the presence of *netF*-positive *C. perfringens* in foals with necrotising enteritis in Kentucky and expands the known geographical distribution of this infection (Mehdizadeh Gohari and others 2015). It also confirms, based on the serological response to the KY MF 05/00 bacterin-toxoid vaccine used for mare prepartum immunisation, that the vaccine contains NetF, albeit in amounts insufficient to induce detectable levels of antibody in all mares. Enrichment of the bacterin with recombinant NetF should improve antibody response and enhance lactogenic immunity. Further work is required to document the prevalence of infections by NetF+ *C. perfringens* in foals in other parts of the world, and to evaluate the efficacy of prepartum immunisation with vaccines containing NetF.

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