Short Communications

Enterotoxaemia-like syndrome and *Clostridium perfringens* in veal calves

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SEVERAL enteritis/enterotoxaemia syndromes in mammals and birds are the consequence of an uncontrolled overgrowth of *Clostridium perfringens* invading the small intestine from the caecum and the colon and producing different exotoxins. In suckling beef calves the α, or CPA, and β2, or CPB2, major toxins act in synergy to produce intestinal necrohaemorrhagic lesions. The CPA toxin subsequently transfers into the bloodstream and reaches the brain, causing sudden death (Manteca and Daube 1994, Songer 1996, Manteca and others 2002, Smedley and others 2004, Uzal and Songer 2008, Cooper and Songer 2009, Popoff and Bouvet 2009, van Asten and others 2010).

Different alleles of the *cpb2* gene have been identified and grouped into the consensus (*cpb2*^{con}) and the atypical (*cpb2*^{aty}) variants. While the atypical variant is more common in healthy individuals, the consensus variant can become more common in diseased animals. In newborn piglets with necrotic enteritis, 90 per cent of *C perfringens* isolates harbour the *cpb2*^{con} gene. A similar observation has recently been reported in suckling beef calves with enterotoxaemia, but not in other animal species, or in human beings (Gibert and others 1997, Bueschel and others 2003, Fisher and others 2005, Jost and others 2005, Lebrun and others 2007, van Asten and others 2010).

Cases of enterotoxaemia-like syndrome with sudden death and lesions of haemorrhagic enteritis of the small intestine are also frequent in the veal calf industry, especially in calves belonging to beef cattle breeds (Manteca and others 2001). The present study took place on a Belgian farm with a production of approximately 120,000 veal calves per year, of which 75 per cent belonged to the Belgian blue breed. The aim was to isolate and toxin-type *C perfringens* from such cases, with special reference to the identification of the *cpb2* gene variant(s) present.

Eighteen Belgian blue veal calves without any clinical signs observed before death, but with gaseous dilation, haemorrhagic lesions, and liquid and haemorrhagic contents in the small intestine at postmortem examination performed within a few hours after death, were sampled; these animals are thereafter referred to as the case calves. For each animal, a small intestinal loop with lesions was removed, stored in a jar in anaerobic conditions (Anaerocult; Merck) and transported to the laboratory to perform bacteriological analysis, as described by Lebrun and others (2007).

TABLE 1: PCR results on 77 *Clostridium perfringens* isolates from calves affected with an enterotoxaemia-like syndrome (cases) and on 15 *C perfringens* isolates from control calves for the *cpb2* gene family and for the *cpb2*^{con} and *cpb2*^{aty} gene variants

Calves	Lesions	*C perfringens* count (cfu)	Calf	Number of colonies*	*cpb2*+ colonies	*cpb2* variant
Case	Typical	≥10^6	1	10	–	–
			2	8	–	–
			3	12	–	–
			4	12	ND	ND
			5	12	ND	ND
		10^3-10^5	6	12	1	Consensus
			7	4	–	–
			8	12	6	Atypical (4) Consensus (2)
	Suspicious	≥10^6	9	2	–	–
		10^3-10^5	10	10	10	Atypical
			11	7	2	Consensus
Control	Absent	10^1-10^3	12	3	–	–
			13	4	–	–
			14	3	–	–
			15	5	–	–

* All colonies tested positive with the PCR targeting the *cpa* gene coding for the α toxin and negative with the PCRs targeting the *cpb*, *etx*, *iap* and *cpe* genes coding for the β, ε and ι major toxins and for the enterotoxin
ND Not done (no colony was tested due to enterococcal overgrowth of the plates)

Six of the case calves (33 per cent) had high *C perfringens* counts (>10^6 cfu/ml of intestinal content) and five (28 per cent) had intermediate counts (between 10^3 and 10^6 cfu/ml of intestinal content) (Table 1). No *C perfringens* growth (<10 cfu/ml of intestinal content) was observed from the intestinal contents of the other seven case calves (39 per cent). Of the 11 case calves with intermediate or high cfu, 77 *C perfringens* isolates were collected from the intestinal contents of nine animals, but the plates were overgrown by enterococci for the remaining two (calves 4 and 5) (Table 1). Fifteen isolates of *C perfringens* were also collected at the slaughterhouse of the farm from the small intestinal contents of four six-month-old healthy veal calves with <10^3 cfu/ml of intestinal content (the control calves and isolates) (Table 1).

All 92 *C perfringens* isolates tested positive with a PCR targeting the *cpa* gene and negative with PCRs targeting the *cpb*, *etx*, *iap* and *cpe* genes coding for the β, ε and ι major toxins and for the enterotoxin, respectively (Daube and others 1994, Braun and others 2000, Albini and others 2008). Nineteen of the case isolates (25 per cent) from four calves (44 per cent) and none of the control isolates tested positive with the PCR targeting the *cpb2* gene family (Van Asten and others 2008). The relative frequency of *cpb2*-positive isolates varied from one isolate out of 12 (calf 6) up to all 10 isolates from calf 10 (Table 1). Five isolates from calves 6, 11 and 8 tested positive with three PCRs targeting the *cpb2*^{con} gene (Herholz and others 1999, Jost and others 2005, Van Asten and others 2008), while the remaining 14 isolates from calves 10 and 8 tested positive with one of two PCRs targeting the *cpb2*^{aty} gene (Jost and others 2005) (Table 1). The second *cpb2*^{aty} gene PCR (Van Asten and others 2008) did not give any results, even with the control strains, despite several changes of experimental conditions (data not shown). The sequencing of all amplified fragments confirmed the presence of the *cpb2*^{con} gene and of the *cpb2*^{aty} gene.

According to these results, neither CPB2 toxin nor the CBP2^{con} variant appears to have been involved in the enterotoxaemia-like syn-

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drome in these veal calves. This is in contrast with results obtained during a study in suckling beef calves in which two-thirds of *C perfringens* isolates (28 of 41) from 14 case calves were *cpb2*-positive and all 28 harboured the *cpb2*^{con} gene (Lebrun and others 2007). This suggests that *C perfringens* may not have even been associated with the enterotoxaemia-like syndrome in the veal calves in the present study since only one-third (six of 18) of the case calves had high counts of *C perfringens* (>10^6 cfu/ml of intestinal content). In contrast, in a study by Manteca and others (2001), 79 per cent (62 of 79) of the suckling beef calves had high counts of *C perfringens*.

However, rapid upregulation of toxin production by *C perfringens* has been reported upon contact with host enterocytes (Vidal and others 2009, McClane 2010). Therefore, high counts of *C perfringens* are not necessary to cause an enterotoxaemia-like syndrome if a subpopulation of *cpb2*-positive *C perfringens* rapidly produces large amounts of the CPB^{con} toxin, which is 10 times as toxic as the CPB2^{aty} variant. It is therefore possible that the *cpb2*-negative case calves harboured a *cpb2*^{con}-positive *C perfringens* population below the detection level of the authors' methodology. This might also explain the higher proportion of *cpb2*^{aty}-positive than *cpb2*^{con}-positive isolates in each of the *cpb2*-positive calves (Table 1).

Interestingly, all 19 *cpb2*-positive colonies were isolated from the four calves with 10^3 to 10^5 cfu of *C perfringens* per ml of intestinal content and not from the calves with more than 10^6 cfu of *C perfringens* (Table 1). Although there is no straightforward explanation, it is possible to speculate that the intensive use of antibiotics in the veal calf industry directly or indirectly impaired the *C perfringens* growth and/or rendered the plasmid carrying the *cpb2* genes more unstable in vitro. A larger study in more farms is required.

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