Cross-reaction of a Campylobacter fetus subspecies venerealis real-time PCR

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Short Communications

Cross-reaction of a *Campylobacter fetus* subspecies *venerealis* real-time PCR

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Campylobacter fetus is a significant veterinary pathogen, which is divided into two subspecies: *C fetus fetus* and *C fetus venerealis* (Véron and Chatelain 1973). Differentiating between the two subspecies of *C fetus* and other *Campylobacter* species can be challenging due to their fastidious growth requirements and biochemical inactivity (On 1996).

In 2009, a sample of faeces from a New Zealand three-year-old Friesian bull with diarrhoea was submitted to a diagnostic veterinary laboratory for bacteriology culture. *Campylobacter* culture was performed on Skirrow's agar under microaerobic conditions for five days at 37°C. Suspected *Campylobacter* colonies were confirmed by standard assays and a presumptive identification of *C fetus venerealis* was made. A real-time PCR assay for *C fetus venerealis* (McMillen and others 2006) was positive on DNA extracted from the cultured isolate (Table 1). Faecal and preputial samples were collected from the same animal 10 days later. The faecal sample was culture-positive and PCR-positive for *C fetus venerealis*; the preputial sample was culture-negative.

Because isolation of *C fetus venerealis* from the faeces of cattle is unusual, the Investigation and Diagnostic Centre, MAF Biosecurity New Zealand, was asked to repeat the initial testing on the two presumptive *C fetus venerealis* isolates obtained from the bull's faeces. The real-time PCR (McMillen and others 2006) produced positive results for both isolates. However, biochemical test results were found to be inconclusive for *C fetus venerealis* (Table 1).

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The two isolates were subsequently sent to the OIE Reference Laboratory for Campylobacteriosis, the Netherlands, for further characterisation. A multiplex PCR (Hum and others 1997) performed on both isolates produced a negative result with the *C fetus* species-specific target but a positive result with the *C fetus venerealis*-specific target. It is of interest that the real-time PCR described by McMillen and others (2006) is based on the *venerealis*-specific part of the multiplex PCR (Hum and others 1997) and uses the same *C fetus venerealis*-specific gene target, *par*A. A second multiplex PCR (Abril and others 2007) produced negative results with both the *C fetus* species-specific target and the *C fetus venerealis*-specific target (Table 1).

The 16S rRNA gene of both isolates was sequenced and an alignment was made of four species-specific 16S rRNA regions, as described by Gorkiewicz and others (2003). Both isolates from the bull showed 12 nucleotide differences from *C fetus* and had a specific pattern for *Campylobacter hyointestinalis/Campylobacter lanienae*. Amplified fragment length polymorphism (AFLP) analysis was then performed (Duim and others 2001, Wagenaar and others 2001); when the profiles were entered into the OIE Reference Laboratory's AFLP database, the two isolates were found to cluster with *C hyointestinalis* isolates and showed less homology with the *C fetus fetus*, *C fetus venerealis* and *C lanienae* isolates in the database. Finally, the use of a *C fetus/C hyointestinalis*-specific PCR identified both isolates as *C hyointestinalis* (Linton and others 1996). In summary, the data showed that the two isolates from the bull's faeces were most likely *C hyointestinalis*, a species commonly isolated from the gastrointestinal tract of cattle (Atabay and Corry 1998).

The observation that two *C hyointestinalis* isolates produced false-positive results with a *C fetus venerealis*-specific real-time PCR is of concern. BLAST analysis of the primer and probe sequences in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) showed homology only to *C fetus* sequences and not *C hyointestinalis*. McMillen and others (2006) evaluated the specificity of the primers and probe in their PCR against only one isolate of *C hyointestinalis*.

The false-positive results could be the result of the *C fetus venerealis*-specific gene target *par*A, or a close homologue, being present in the *C hyointestinalis* isolates tested. In the scientific literature, there has been uncertainty over the location of the *par*A gene (Willoughby and others 2005); however, recent evidence has demonstrated that *par*A is located on a transferable genomic island (Abril and others 2010, Gorkiewicz and others 2010). This provides a potential explanation of how *par*A could spread among *Campylobacter* species and produce false-positive results with the real-time PCR assay.

The findings presented here provide a stark warning that regardless of how well designed, developed and validated an assay is, due to microbial diversity and evolution there is always the potential for false-positive (and false-negative) results to arise. For assays used commercially to diagnose *C fetus venerealis* this could result in misclassification of some cattle herds, leading to unnecessary control programmes and costs for farmers. Therefore, it is recommended that positive results should be confirmed by additional tests.

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TABLE 1: Summary of key biochemical and PCR test results for *Campylobacter* species isolates from faeces from a bull and reference strains of *Campylobacter fetus fetus* and *Campylobacter fetus venerealis*

Isolates tested	Growth at 25°C	Growth at 42°C	Growth in 1 per cent glycine	Hydrogen sulphide production	Susceptibility to nalidixic acid	Real-time PCR†	Conventional PCR‡		Conventional PCR§	
							C fetus	*C fetus venerealis*	*C fetus*	*C fetus venerealis*
Bull faeces isolates 1 and 2	V	V	–*	V	R	+	–	+	–	–
C fetus venerealis (NCTC10354)	+	–	–	–	R	+	+	+	+	+
C fetus fetus (NCTC10842)	+	+	+	–	R	–	+	–	+	–

* Initial testing of the isolates showed no growth on 1 per cent glycine agar although subsequent testing at Investigation and Diagnostic Centre, New Zealand, produced growth on glycine
† McMillen and others 2006
‡ Hum and others 1997
§ Abril and others 2007
– Negative, + Positive, R Resistant, V Variable

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