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***Campylobacter* abortion in sheep:
a study of strain types and vaccine protection.**

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Abstract

The bacteria *Campylobacter fetus* subsp. *fetus* and *C. jejuni* are important causes of abortion in sheep, and the majority of *Campylobacter* sheep abortion in New Zealand is associated with *C. fetus* subsp. *fetus*. Campylovexin[®] (Schering-Plough Animal Health Ltd., Upper Hutt) is a single-strain, killed vaccine available in New Zealand for the prevention of abortion in sheep due to *C. fetus* subsp. *fetus*. The present study was initiated in response to questions regarding the protection offered by this vaccine against other strains of *C. fetus* subsp. *fetus* causing sheep abortion in New Zealand.

Campylobacter isolates from aborted sheep fetuses were collected after culture in veterinary diagnostic laboratories from sheep flocks in the Hawke's Bay region in 1999. This was extended in 2000, when *Campylobacter* sheep abortion isolates cultured in the same way from farms throughout New Zealand were collected. In total, 374 *C. fetus* subsp. *fetus* isolates, 30 *C. jejuni* isolates, and one *C. coli* isolate were collected from 249 farms. These isolates were sub-typed using the genetic technique, pulsed-field gel electrophoresis (PFGE). A total of 26 distinct PFGE types were identified amongst the *C. fetus* subsp. *fetus* sheep abortion isolates, and one type, PFGE type B1, predominated. Of the farms from which *C. fetus* subsp. *fetus* was isolated, PFGE type B1 was found on 19 out of the 25 farms in the Hawke's Bay study (75%) and 132 out of the 200 farms in the national study (66%).

Nine distinct PFGE types were found amongst the 30 *C. jejuni* isolates from the Hawke's Bay and national studies. No single *C. jejuni* PFGE type was identified from sheep abortion most frequently. Investigation of a large sheep abortion outbreak initially attributed to *C. jejuni* revealed that the initial diagnosis was misleading due to contamination of the foetal sample, and that the outbreak was more likely to have been associated with *C. fetus* subsp. *fetus*.

Seven *C. fetus* subsp. *fetus* isolates representative of the genotypes found amongst New Zealand sheep abortion isolates in a 1987 study were lodged with the New Zealand Reference Culture Collection, Medical Section (de Lisle et al. 1987). These isolates were typed in the present study using PFGE, and were found to be similar or

indistinguishable from the PFGE types identified in the Hawke's Bay or national studies. The frequency of isolation of the types also appeared to be similar between 1987 and 2000, as the prevalent type from 1987 was identified as PFGE type B1.

A nationwide call for suspected cases of Campylovexin[®] breakdown resulted in the identification of one flock in which abortions due to *C. fetus* subsp. *fetus* were reported in ewes apparently vaccinated that season. Approximately 40 ewes aborted out of a flock of 1100 ewes, and it was thought that four ewes that had aborted were vaccinated that season. The high stocking density management of this flock meant that there was likely to have been a high challenge to vaccinated ewes from unvaccinated aborting ewes. Investigation of two other apparent Campylovexin[®] breakdown cases revealed that ewes had not been vaccinated that season and were also run at a high stocking density. In addition, the *C. fetus* subsp. *fetus* isolates in these two cases were the same PFGE type as the vaccine strain, PFGE type A1, which suggested that in these cases apparent vaccine breakdown was not due to lack of cross-protection against other strains. Despite the widespread occurrence of distinct strains of *C. fetus* subsp. *fetus*, abortion due to *C. fetus* subsp. *fetus* in ewes vaccinated with Campylovexin[®] appeared to be rare and was not associated with a particular PFGE type.

Serum from 15 ewes vaccinated with Campylovexin[®] was collected and used in Western blotting experiments to investigate antibody binding with the proteins of the various PFGE types of *C. fetus* subsp. *fetus*. Serum from vaccinated ewes contained antibodies that bound to the proteins of the different *C. fetus* subsp. *fetus* PFGE types. This *in vitro* study suggested that sheep vaccinated with Campylovexin[®] develop antibodies that recognise proteins of each of the *C. fetus* subsp. *fetus* strain types.

Vaccine challenge experiments were performed using pregnant guinea pigs as a model. Initially, it was shown using this model that Campylovexin[®] provided a statistically significant level of protection from homologous challenge with the vaccine strain. Then, using an isolate of PFGE type B1, it was demonstrated that Campylovexin[®] also provided a statistically significant level of protection against heterologous challenge.

Despite the range of *C. fetus* subsp. *fetus* PFGE types identified in this study, there was no evidence to suggest that the different strain types compromised Campylovexin[®]

efficacy in protection against *C. fetus* subsp. *fetus* sheep abortion. The protein recognition by serum from ewes vaccinated with Campylovexin[®], the lack of a robust vaccine breakdown case, and the demonstration of cross-protection in the guinea pig model, provide evidence that Campylovexin[®] appears to offer cross-protection against strains of *C. fetus* subsp. *fetus* causing abortion in sheep in New Zealand.

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