Diagnosis of bovine venereal campylobacteriosis in New Zealand

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Natalia Benquet Sansone

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Abstract

Bovine venereal campylobacteriosis (BVC) is a venereal disease causing infertility in cows due to early embryonic loss. Caused by *Campylobacter fetus* subsp *venerealis* (*Cfv*), which lives in the preputial crypts of older asymptomatic carrier bulls and the vagina of carrier cows. Diagnosis of the disease is difficult as it is a fastidious organism to culture and the serological methods which have been developed to diagnose BVC, exhibit substantial cross-reactivity with *Campylobacter fetus* subsp *fetus* (*Cff*), due to the close genomic and phenotypic relationship of the two subspecies. Definitive differentiation between *Cfv* and *Cff* requires molecular biology techniques.

In New Zealand, *Cfv* was last isolated in 1993 and suspected again in 2001/2002 after the use of a recently-developed IgA ELISA test that claimed to be 98.5% specific for *Cfv*. A nation wide study showed that there was no relationship between the test's results and the reproductive performance of the herds. Most of the positive results obtained were false probably due to cross-reactions with *Cff*. The lack of positive isolations of *Cfv* raised questions about the sensitivity of the isolation methods used.

Five experiments were undertaken. Experiments 1–3 examined the microbiological methods that are used to isolated *Cfv*. Results indicated that the optimal samples and culture conditions were preputial washes or scrapes from bulls, or vaginal washes (20 ml PBS) from cows, enriched in Lander's medium for three days before subculture onto blood agar. All cultures need to be undertaken under microaerophilic conditions. These experiments also addressed the reliability of the IgA ELISA for animals managed according to the husbandry conditions that prevail in New Zealand. Results showed that there was a great deal of within- and between- animal variation in ELISA values and that there was substantial cross reactivity with *Cff*. (ICC ranged from 0.29 to 0.03 depending on the Group and all heifers challenged with *Cff* became positive to IgA ELISA). In consequence, the test appears to be of limited diagnostic value in situations where cattle may be cross contaminated with *Cff*.

Experiment 4 evaluated a PCR method for identification of *Cfv* in preputial washings that had been inoculated with small numbers of the organism,

whilst Experiment 5 used the same method to attempt to identify *Cfv* in preputial washings or scrapings that had been collected from experimentally-infected bulls. Positive results were obtained with as little as 880 *Cfv* cells/ml of preputial wash after samples had been concentrated by centrifugation and filtration (5 µm and 0.8µm pore size filters). The PCR also detected the presence of *Cfv* in two bulls, 48 h after infection.

It was concluded that the IgA ELISA test is unlikely to be suitable for use in New Zealand, due to the risk of cows being contaminated with *Cff* from the sheep with which they are co-managed. Microbiological identification, whether through PCR or culture and isolate, although difficult, remains the definitive diagnostic method. If the presence of the disease in New Zealand is to be confirmed or refuted, careful and methodical collection of samples from animals, whose history suggests the possibility of the condition, will be required.

Abbreviations

AFLP	Amplified Fragment Length Polymerase
BHI	Brain heart infusion
BVC	Bovine venereal campylobacteriosis
Cff	*Campylobacter fetus* subsp. *fetus*
CFU	Colonies forming units
Cfv	*Campylobacter fetus* subsp. *venerealis*
C-G	Cytocine-guanine
CVM	Cervicovaginal mucous
DNA	Deoxyribose Nucleic Acid
EVs	Elisa Values
FA	Fluorescent antibody
gly	glycine
H_2S	Sulphur hydroxide
ICC	Itraclass correlation coeficient
IG	Immunoglobulin
IgA, IgG, IgM	Immunoglobulins A, G and M respectively
$MgCl_2$	Magnesium chloride
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFGE	Pulsed Filed Gel Electrophoresis
SLPs	S. layer protein, Surfase layer proteins
µl	Microlitre
µm or um	micron
VMAT	Vaginal Mucus Agglutination test

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