General Technical

Preliminary findings on the use of PCR assay for MCF in red deer

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Abstract

Malignant catarrhal fever (MCF) is believed to be caused by Ovine herpesvirus-2 (OHV-2), a sheep associated gamma herpes virus. Recently a polymerase chain reaction (PCR) test has been developed to detect genetic material of OHV-2. This study was undertaken to assess the PCR as an epidemiological tool. Ten male and ten female newly weaned deer were chosen at random from each of a group of red deer and a group three-quarter red, one-quarter Père David's (¼ PD) deer. A 10 ml EDTA tube of blood was taken from each calf by jugular venepuncture on April 17, July 17 and November 4, 1996 and MCF-PCR tests were carried out on these samples. In April 1997, eight red males and eight ¼ PD males were slaughtered and MCF-PCR tests were carried out on samples of blood, spleen and mesenteric lymph node.

In April 1996, when the weaners were approximately 4 months old, 45% were PCR positive. Three months later the number of positives had declined to 17.5% and by November, when they were 11 months old, it was 0 %. However, by the following April the percentage of PCR positive blood samples was 44 %. At slaughter, three of the eight red deer had PCR positive spleen samples while four of the eight ¼ PD deer had PCR positive lymph node samples.

If it is assumed that the MCF-PCR in this current trial is detecting viable virus, then it suggests that over half the newly weaned deer at Invermay have been exposed to OHV-2 and have become infected. The PCR may accurately indicate exposure, but may not be a true indicator of current infection.

The value of the MCF PCR for epidemiological studies is still being assessed and further trials are planned to validate it and to assist in interpreting the results.

Introduction

Malignant catarrhal fever (MCF) is a fatal disease of farmed deer in New Zealand characterised by acute onset of depression, inappetance, pyrexia and diarrhoea. It usually rapidly progresses over the course of 1 or 2 days to severe bloody diarrhoea and death. Often the first sign the farmer sees is finding the animal dead. Occasionally deer develop a more chronic form of the disease with variable degrees of ocular and nasal discharges, corneal opacity, hypopion, crusting around the naries and anus, erosions of the buccal cavity and there may be haemorrhage into the anterior chamber of the eye. Chronically affected animals tend to lose weight and succumb after 3 to 5 weeks. There is a distinct seasonal pattern of incidence with the peak of cases in July, August and September, and the attack rate is higher in males than in females and higher in adults than in calves (Fennessy, 1988). It appears that

the onset of disease is predisposed to by stressors such as underfeeding, severe weather and exposure.

The disease in New Zealand is believed to be caused by a sheep associated gamma herpes virus (Ovine herpesvirus-2 or OHV-2) and is usually referred to as sheep associated MCF or SA-MCF (Reid, 1991). It is closely related to Alcelaphine herpesvirus-1 (AHV-1), which causes wildebeest associated MCF of cattle in Africa, as shown by antigenic and genomic similarities.

Recently a polymerase chain reaction (PCR) test has been developed to detect genetic material of SA-MCF. (Wiyono *et al*, 1994, Baxter *et al.*, 1993, Lahijani *et al*, 1994, Tham *et al*, 1994).

The PCR for SA-MCF has been shown to be well correlated with clinical cases of MCF in deer which have been confirmed by finding typical histopathological lesions (Tham, 1997, Tomkins *et al*, 1997). The PCR test is based on the amplification of a particular DNA sequence thought to be specific for OHV-2.

The study was undertaken to assess the PCR as an epidemiological tool which could assist the understanding of factors such as exposure rate, time of exposure and latency.

Materials and Methods

In April, 1996, 10 male and 10 female newly weaned deer were chosen at random from each of a group of red deer and a group three-quarter red, one-quarter Père David's (1/4 PD) deer bred on the AgResearch Invermay farm.

A 10 ml EDTA tube of blood was taken from each calf by jugular venepuncture, using a new needle each time, on April 17, July 17 and November 4, 1996. One male red weaner fractured its leg and was euthanased prior to November. The blood samples were kept cool and couriered to the Wallaceville Animal Health Laboratory (WAHL) for PCR testing. Eight red males and eight ¼ PD males were slaughtered on April 8, 1997 at the Otago Venison Deer Slaughter Plant and samples of blood, spleen and mesenteric lymph node were taken carefully to avoid cross-contamination and sent to WAHL for PCR testing. All the other deer were aged 25 months and were healthy at the time of writing.

Buffy coat cells, prepared from the EDTA blood, were processed for DNA extraction as described previously (Tham,1997). The extracted DNA was tested by a nested PCR with SA-MCF specific primers using the method described previously. Briefly, the PCR assay consisted of a standard amplification reaction in a buffer supplemented with deoxynucleoside triphosphates, a set of oligonucleotide primers (556/755) and DNA polymerase. The cycling programme of the reaction included a precycle at 99°C for three minutes and 25 repetitive cycles of denaturation at 94°C for 20 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 30 seconds. An aliquot of the primary amplification product was transferred directly to a new reaction mixture and amplified, using primer pair 555/556, under identical conditions for 25 cycles of secondary amplification reactions and concluded with a final extension at 72°C for five minutes.

Aliquots of the amplified products were analysed by 2% agarose gel electrophoresis and visualised by ultraviolet transillumination after staining with ethidium bromide. The identity of the amplified fragments was confirmed by chemiluminescence Southern blot hybridisation, using diboxigenin-labelled primer 555 as an internal probe (Tham etal.,1994)

Results

A summary of the PCR results are presented in Table 1. In April, when the weaners were approximately 4 months old, 45% were PCR positive. Three months later this had declined to 17.5% and by November, when they were 11 months old, it was 0 %. However, by the following April the percentage of PCR positive blood samples was 44 %. There were no consistent differences between male or female, nor red or ¼ PD deer. At slaughter, although the numbers were small, it was notable that of the eight red deer, three had PCR positive spleen samples only while of the eight ¼ PD deer, four had PCR positive lymph node samples only.

Table 1: MCF PCR results for blood, spleen and lymph node samples

		17/04/96	17/07/96	04/11/96	07/04/97 slaughter		
Group	Tag No	EDTA	EDTA	EDTA	EDTA	Spleen	LN
Red male deer	G517	neg	neg	neg			
	B519	neg	neg	neg	pos	neg	neg
	B524	neg	neg	neg	pos	neg	neg
	B528	neg	neg	neg	neg	pos	neg
	B536	neg	neg	neg	pos	pos	neg
	B550	neg	pos	neg	neg	pos	neg
	Y512	neg	neg	neg	neg	neg	neg
	Y526	pos	pos	neg	neg	neg	neg
	Y558	neg	neg	dead			
	Y563	neg	pos	neg	pos	neg	neg
	% pos	10	30	0	50	37 5	0
Red female deer	G575	pos	neg	neg			
	Y502	pos	neg	neg			
	Y528	neg	neg	neg			
	B513	pos	neg	neg			
	B516	neg	neg	neg			
	B518	neg	neg	neg			
	B521	neg	neg	neg			
	B540	pos	neg	neg			
	B542	pos	neg	neg			
	B543	neg	neg	neg			
	% pos	50	0	0			
PD ¼ male deer	RW501	neg	neg	neg	neg	neg	neg
	RW503	neg	neg	neg	neg	neg	neg
	RW512	neg	neg	neg			
	RW535	neg	neg	neg	pos	neg	pos
	RW555	neg	neg	neg	pos	neg	neg
	RW557	pos	pos	neg	neg	neg	pos
	RW562	pos	neg	neg	pos	neg	pos
	RW569	pos	neg	neg			
	RW571	pos	neg	neg	neg	neg	pos

		17/04/96	17/07/96	04/11/96	07/04/97 slaughter		
Group	**Tag No**	**EDTA**	**EDTA**	**EDTA**	**EDTA**	**Spleen**	**LN**
	RW573	pos	pos	neg	neg	neg	neg
	% pos	50	20	0	37 5	0	50
PD ¼ female deer	RW517	pos	neg	neg			
	RW521	pos	neg	neg			
	RW526	neg	neg	neg			
	RW534	neg	pos	neg			
	RW542	neg	neg	neg			
	RW543	pos	pos	neg			
	RW548	pos	neg	neg			
	RW576	pos	neg	neg			
	RW591	pos	neg	neg			
	RW594	pos	neg	neg			
	% pos	70	20	0			
Overall	% pos	45	18	0	44	19	25

Discussion

The PCR test detects a specific segment of DNA with a genetic sequence believed to be unique for the OHV-2. The probe has been tested against seven other known herpesviruses and shown to be specific for OHV-2. However, there is a remote possibility that there may be another virus which cross-reacts, such as a non-pathogenic strain of herpesvirus similar to OHV-2 .

A positive test means that the probe has bound to at least one copy of the DNA sequence and multiplied it up to enable detection. It is believed that OHV-2 is strongly cell associated and therefore a positive test on a blood sample indicates that at least one cell (probably a T lymphocyte) in the 10 ml sample contained virus. This suggests that virus has infected the deer at some point, multiplied and is present in the lympho-reticular system and circulating lymphocytes. However, the PCR test cannot distinguish between viable and non-viable virus and it is possible that a positive PCR test could result from the detection of nonviable viral DNA in a cell. Thus the PCR may accurately indicate exposure, but may not be a true indicator of current infection.

Herpesviruses, as a group, have a strong tendency to enter the nucleus of target cells and become latent for a period. When the host is stressed the virus may become reactivated, multiply, cause cell death and spread to other cells. In the case of MCF it is not known to what extent latency occurs or what actually triggers disease, but it is believed that the disease results from a massive lympho-proliferation, deregulation of the immunomodulatory function of large granular lymphocytes, while cell destruction could arise from the extension of the cytotoxic properties of "natural killer" lymphocytes to normal tissues, resulting in the characteristic damage to endothelial cells and perivascular cuffing. (Reid *et al* , 1989; Schock and Reid 1996).

If it is assumed that the MCF-PCR in this current trial is detecting viable virus, then it suggests that over half the newly weaned deer at Invermay have been exposed to OHV-2 and have

become infected. Over the following year the decline in PCR positives in the EDTA blood samples suggest that over time the virus infected cells are cleared from the bloodstream or become sequestered in other areas of the lympho-reticular system such as the lymph nodes or spleen. Lymphocytes can live for well over a year and individual cells appear or disappear in the blood circulation. The increase in PCR positives in 16 month old deer could be as a consequence of re-emergence of infected cells, recrudesence of existing infection or recent new infections with OHV-2 or similar virus.

The value of the MCF PCR for epidemiological studies is still being assessed and further trials are planned to validate it and to assist in interpreting the results.

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