Evaluating anti-CarLA salivary IgA response in a deer progeny test

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Abstract

Farmed red deer (*Cervus elaphus spp*) are susceptible to infection by nematode parasites. A salivary antibody (IgA) that is naturally produced in response to exposure to a nematode parasite carbohydrate larval surface antigen (CarLA) can provide a protection against gastro-intestinal nematodes in sheep and rising-yearling farmed red deer. The concentrations of this CarLA-IgA were measured in saliva samples collected at 6 and 10 months of age, in 1531 rising-yearling progeny of 36 sires across three farms during a three-year progeny test. The CarLA-IgA concentrations differed across sires, farms and years, with 6-month levels being much lower than 10-month levels. Heritability estimates were low (h^2=0.10) for 6 months, but moderate and significant (h^2=0.37, P<0.05) for 10 months. These two traits were highly genetically correlated (r_g=0.86) and moderately phenotypically correlated (r_p=0.27). There were low (r_p 0.08-0.13) but significant (P<0.05) favourable phenotypic correlations with spring and pre-slaughter live weight, and with growth rate from 3 to 10 months of age and 6 to 10 months of age. Sire variation and heritability of 10-month response indicates that it may have utility as a selection criterion for improved ability of farmed deer to cope with nematode challenge.

Keywords: red deer; nematode parasite; carbohydrate larval antigen; immune; *Cervus elaphus*

Introduction

Pasture-based farmed red deer and red deer-wapiti crossbreds (*Cervus elaphus spp.*) in New Zealand are susceptible to clinical infection by parasitic nematodes (Mason 1994), especially as rising-yearlings (R1). The key nematodes are lungworm (*Dictocaulus eckerti*) and gastrointestinal nematodes (GIN): primarily, but not exclusively, abomasal parasites of the *Ostertagia* type (Mackintosh et al. 2014). Lungworm are the most pathogenic with deaths recorded on 5% of farms compared with 3% for GIN (Castillo-Alcala et al. 2007). Anthelmintic resistance is a risk, and New Zealand deer farmers are struggling to find fully effective anthelmintics (Pomroy et al. 2018), frequently utilising off-label preparations that incur a 91-day withholding period (WHP) for meat. Faecal-egg counting (FEC) has been shown to have limited effectiveness for quantifying GIN burden in R1 deer, especially after winter (Mackintosh et al. 2014). These factors all make management of parasites challenging and concerning for producers, presenting risks to animal welfare, productivity and market access, as well as threatening long-term sustainability of pastoral deer production.

One potential tool, which has been used in sheep to measure the immune response to parasitic nematode challenge, is the measurement of naturally-occurring IgA in the saliva that has been produced in response to a Carbohydrate Larval Antigen (CarLA) (Shaw et al. 2012). The antibody is produced in response to exposure to CarLA that has been presented to the animal from natural consumption of nematode parasites. The antigen is present in the complex glycolipid sheath which protects most infective third-stage (L3) common nematode parasites from the environment outside animal hosts. The sheath is normally shed by the parasite soon after entry to the host (Harrison et al. 2003a, b). In farmed sheep in New Zealand, the antibody responses have been shown to be correlated with resistance to challenges by mixed species of parasitic nematodes. The antibody concentration is very labile and reflective of the challenge environment (Shaw et al. 2012). The commercial CARLA® Saliva Test is currently available to farmers. Shaw et al. (2013) showed antibody levels increased over time for lambs grazing naturally infected pastures, and that the levels were moderately heritable and favourably genetically correlated with live weight. Mackintosh et al. (2014) used the CARLA® Saliva Test (CARLA) to demonstrate that for R1 deer concentrations peaked in late autumn and mid-spring, levels were lower for wapiti-crossbreds than for red deer, and that R1 deer with responses ≥2 units/ml had lower adult abomasal nematode burdens than did those with responses <2 units/ml.

A deer industry progeny test (DPT) (Ward et al. 2014), provided an opportunity to evaluate the antibody concentrations in saliva from offspring of multiple industry sires in different farm environments and to assess its suitability as a trait for genetic selection. The hypothesis was that the antibody concentrations would increase with natural exposure to nematodes in R1 deer as in lambs, and that the concentrations exhibit heritable variation.

Materials and methods

Animals

The animals in this trial were enrolled in the DPT over the period from March 2012 to June 2015 (Ward et al. 2014). All procedures involving animals were approved by the Invermay Animal Ethics Committee under the New Zealand Animal Welfare Act 1999. The progeny used were all bred by artificial insemination (AI), from 35 sires (n=

24 red deer and 11 wapiti-crossbred), out of red deer dams, and were born in late November or early December. A total of 1798 were weaned, 950 (all males and wapiti-crossbred females) were finished for slaughter at 11-12 months of age and 762 red deer females submitted for first mating as rising-two-year-olds (R2) at 15 months-of-age. The final, analysed dataset included 1531 individuals. Animals were run in either a single mixed-sex mob, or up to two mobs per sex, depending on farms and years (for more details on animals see Ward et al. (2014; 2019)).

Farms and animal management

The three farms were described in detail by Ward et al. (2014). Briefly, Haldon Station is a partly irrigated high-country sheep, beef, and deer station farm in the Mackenzie Basin, Invermay is an Otago hill-country sheep and deer research farm, and Whiterock Station is a Canterbury high-country beef and deer farm. Haldon and Whiterock actively integrate non-deer species (i.e., they graze or co-graze the same pastures) but Invermay did not integrate other livestock with the deer. Haldon and Whiterock Stations calved their hinds in hill blocks. After weaning, the calves were grazed on improved specialist pastures, including lucerne at Haldon and clover, chicory and plantain mixes at Whiterock. At Invermay the deer were grazed on improved ryegrass-dominant pastures before and after weaning. During the winter period R1 deer were on pasture and lucerne baleage at Haldon, brassica crops at Invermay and Whiterock, Whiterock supplemented with pea-vine and Invermay fed lucerne hay, both in racks. Winter feeding at Invermay was June-September, Haldon and Whiterock Jun-Oct. In 2014, both Whiterock and Haldon Stations experienced a summer drought during lactation and after weaning, restricting pasture availability, quality and reducing sward height and mass. Anthelmintic regimes differed between farms and years. Whiterock Station used an injectable moxidectin and oral benzimidazole at weaning then six- and 12-weeks after weaning, as did Invermay for the 2013 cohort. The 2011 Invermay birth cohort received pour-on moxidectin six-weekly after weaning until mid-winter, when injectable moxidectin was used, and Haldon alternated between oral benzimidazole and pour-on moxidectin, again six weekly after weaning. In spring, only the replacement (red deer) females were drenched. Any parasite challenges were natural and anthelmintic treatments were instigated to prevent any adverse effects on growth of the animals through parasitism.

Trait measurement

Live weights (LWT) were collected, as described by Ward et al. (2019), approximately every three months, from weaning at three-months of age at the start of March until just prior to transport to slaughter (i.e., pre-slaughter LWT) for all males and female wapiti-crossbreds, or pre-mating at 15-months for R2 red deer females. Growth rates (ADG (g/day)) were calculated between various LWT measurements. Carcass traits were collected after-slaughter at a licensed deer slaughter plant (DSP) (Alliance Group Limited DSP, Makarewa, Southland). Hot carcass weight (HCW) was measured after slaughter with the animal skinned, gutted, head removed and carcass trimmed by the meat inspector, hot dressing-out percentage was HCW/pre-slaughter LWT, and 24 h pH was the average of three pH measures along the striploin (*longissimus dorsi*) 24 hours after slaughter and after chilling at 4°C prior to carcass breakdown. Reproduction traits were determined by rectal ultrasonography (White et al. 1989) in R2 hinds in June at 18 months of age following joining with multiple stags for natural mating from late February until around May 10th.

Antibody concentration was measured after collecting saliva samples on cotton dental swabs held in forceps, as described for sheep by Shaw et al. (2012) and deer by Mackintosh et al. (2014). Animals were either free-standing in groups of five to eight in small pens or restrained in a hydraulic or mechanical crush during ultrasonic eye-muscle scanning (Ward et al. 2010). Samples were collected at the start of winter at six months of age (CARLA 6) and in spring at 10 months of age (CARLA 10) then stored frozen at –20°C until analysis in annual farm batches as per the method of Shaw et al. (2012).

Salivary antibody assay

Saliva samples were extracted from the cotton swabs by centrifugation at 2000 × g for 10 minutes, then stored frozen at –20°C. The CarLA-IgA ELISA was essentially the same as that described by Merlin et al. (2017), except that saliva samples were diluted to 1/20 in sample dilution buffer, then diluted in the assay to 1/33 and 1/50. Rabbit anti-sheep IgA conjugated with horseradish peroxidase (Bethyl Laboratories Inc., USA) diluted at 1/5000 with sample dilution buffer was used to detect CarLA specific deer IgA. Samples with very high CarLA-IgA responses (>40 units/ml) were re-assayed at 1/100 or 1/200 dilution. The intra-assay coefficient of variation (CV = (SD/mean) × 100) was 7.6% while the inter-assay CV was 21.0.

Statistical analysis

All zero antibody concentrations were assigned a value of 0.1 units/ml, which was the smallest reliable response measurable by the assay. Prior to analysis, CarLA-IgA data were transformed to natural logarithms. Statistical analyses were as described by Ward et al. (2019). Briefly, all traits were analysed using ASREML (Gilmour et al. 2009) using a first univariate, then a bivariate model. All models fitted Animal as a random effect with fixed effects of herd by year, sex, weaning mob, and breed type covariate (Ward et al. 2019). All twins and any animals with contradictory mob information or missing trait data were excluded from analysis. Significance testing assumed normal distribution of data after accounting for fixed effects in the model; CARLA 10 was normally distributed CARLA 6 was not: it was biased as many individuals expressed zero response.

Results

Collection of the saliva samples was straightforward

Table 1 Percentage and number (in parentheses) of antibody concentrations measured from saliva samples of singleton rising-yearling red deer and wapiti-crossbreds from three progeny test farms, across three birth years (2011-2013) at about 6 (CARLA 6) and 10-months of age (CARLA 10), for five response classifications. Responses classified as none detected (<0.10), trace (0.10-0.49), low (0.50-0.99), medium (1.00-4.99), high (≥5.00).

CARLA trait	Birth Year	Farm	None	Trace	Low	Medium	High	Total n
CARLA 6	2011	Invermay	30 (72)	40 (94)	14 (34)	12 (29)	3 (8)	237
	2011	Whiterock	85 (212)	13 (31)	2 (4)	0.4 (1)	0 (0)	248
	2012	Haldon	29 (73)	61 (156)	9 (22)	2 (5)	0 (0)	256
	2012	Whiterock	4 (10)	31 (88)	37 (106)	24 (69)	4 (11)	284
	2013	Haldon	53 (138)	3 (7)	19 (50)	20 (51)	5 (12)	258
	2013	Invermay	90 (222)	2 (4)	3 (8)	5 (12)	1 (2)	248
CARLA 10	2011	Invermay	0 (0)	10 (23)	8 (20)	50 (120)	32 (75)	238
	2011	Whiterock	30 (73)	50 (121)	13 (31)	7 (18)	0.4 (1)	244
	2012	Haldon	0 (0)	0.4 (1)	4 (11)	57 (146)	38 (97)	255
	2012	Whiterock	1 (4)	11 (30)	26 (71)	53 (145)	9 (24)	274
	2013	Haldon	6 (14)	1 (2)	6 (14)	40 (102)	48 (122)	254
	2013	Invermay	4 (11)	1 (3)	11 (28)	42 (104)	41 (100)	246

Table 2 Genetic and phenotypic correlations (±SE) between CarLA-IgA response traits (CARLA) at 6- and 10-months of age, in rising-yearling red deer and wapiti-crossbreds, and production traits of live weight (LWT), growth rate (ADG) (3, 6, and 10 months-of-age), pre-slaughter live weight and carcass traits at 10-12 months-of-age, and rising-two-year-old female live weight and reproduction traits (at 15 and 18 months of age). Bold type and * indicates significant correlation at P<0.05.

	Genetic correlation (r_g)		Phenotypic correlation (r_p)	
	CARLA 6	CARLA 10	CARLA 6	CARLA 10
LWT 3	-0.28 ± 0.31	0.02 ± 0.20	-0.03 ± 0.04	0.04 ± 0.04
LWT 6	0.26 ± 0.33	0.14 ± 0.19	0.02 ± 0.04	0.04 ± 0.03
LWT 10	0.15 ± 0.31	0.06 ± 0.18	0.08 ± 0.04	**0.09 ± 0.03***
LWT pre-slaughter	0.14 ± 0.34	0.22 ± 0.20	0.09 ± 0.04	**0.13 ± 0.04***
ADG 3-6	0.82 ± 0.23	0.23 ± 0.22	0.08 ± 0.03	0.01 ± 0.03
ADG 3-10	0.47 ± 0.27	0.04 ± 0.20	0.12 ± 0.03	**0.08 ± 0.03***
ADG 6-10	-0.02 ± 0.33	-0.08 ± 0.19	0.10 ± 0.04	**0.10 ± 0.03***
Eye-muscle area	-0.50 ± 0.27	0.01 ± 0.18	-0.03 ± 0.03	-0.02 ± 0.03
Hot carcass weight	-0.07 ± 0.35	0.08 ± 0.21	0.05 ± 0.04	**0.10 ± 0.04***
Carcass dress-out	-0.54 ± 0.28	**-0.43 ± 0.16***	-0.13 ± 0.04	**-0.12 ± 0.04***
Primal meat yield	-0.45 ± 0.30	0.13 ± 0.21	-0.05 ± 0.05	0.05 ± 0.04
Striploin 24hr pH	-0.60 ± 0.42	-0.45 ± 0.25	-0.04 ± 0.04	**-0.12 ± 0.04***
LWT 15	-0.05 ± 0.37	**-0.41 ± 0.21***	0.05 ± 0.05	-0.01 ± 0.05
Conception date	0.31 ± 0.52	0.43 ± 0.35	-0.04 ± 0.05	-0.02 ± 0.05
Pregnancy status	0.19 ± 0.47	0.09 ± 0.33	-0.02 ± 0.05	0.06 ± 0.05
CARLA 6	1.00	0.86 ± 0.22	1.00	0.27 ± 0.03
CARLA 10	0.86 ± 0.22	1.00	0.27 ± 0.03	1.00

as most animals were not aversive to the procedure and very few had to be physically restrained for sampling when free-standing in a pen. The restrained individuals generally became non-aversive once the cotton swab was in their mouths. The saliva swabs received in the laboratory contained consistently more saliva than those collected from lambs. The R1 CARLA 6 and CARLA 10 means varied across farms, sampling periods and years (Table 1). Means for CARLA 6 were much lower than CARLA 10 and the extent of "no detectable response" decreased from 48% to 7% across farms and birth years (Table 1). The CARLA 6 non-detected response was highly variable across birth years within farms; the most extreme example was Whiterock Station with 4% and 85% not detected in 2012 and 2011 respectively (Table 1). The spring response at 10 months of age was much more normally distributed and repeatable within farms and birth years, except for Whiterock Station 2011 (Table 1). Extreme values for CARLA 6 or 10 were considerably higher than comparable measurements that had been observed in lambs. There was no significant (P<0.05) difference between the regressions of antibody concentration on breed fraction of Eastern European and English red deer compared to wapiti, with regression coefficients for CARLA 6 –0.33 ± 0.26 and –0.27 ± 0.25, and for CARLA 10 of –0.59 ± 0.33 and 0.04 ± 0.32, for Eastern European and English red deer, relative to wapiti.

Heritability estimates for CARLA 6 were low (h^2= 0.10 ± 0.07), but moderate and significant for CARLA 10 (h^2= 0.37 ± 0.09). The two traits were highly genetically

correlated (r_g =0.86 ± 0.22) and moderately phenotypically correlated (r_p =0.27 ± 0.03) (Table 2), and the phenotypic standard deviations were 0.92 and 1.07 for CARLA 6 and 10, respectively. There were three notable genetic correlations with the other production traits: a high positive correlation between CARLA 6 and growth from weaning to start-winter (ADG 3-6), and moderate negative correlations between CARLA 10 and hot-carcass dress-out percentage, and female 15-month live weight (Table 2.) Many of the genetic correlations had a large standard error often combined with a low genetic correlation (Table 2). Phenotypic correlations all were low. Correlations with the growth-rate traits and with spring or pre-slaughter live weights and carcass weight were mostly positive (Table 2). The three exceptions were negative: CARLA 6 and 10 with hot-carcass dressing-out percentage and CARLA 10 with striploin pH 24-hours after slaughter (Table 2).

Discussion

Heritability of CARLA 10 in R1 deer was moderate and significant during spring (10-11 months of age), but low and non-significant at the start of winter (6 months of age). This study did not agree with the hypothesis of Mackintosh et al. (2014) that late autumn would be the optimal single time point to measure the antibody concentration in R1 deer. In the current study, spring would appear to be more suitable for genetic evaluation purposes as >90% of individuals produced a detectable concentration, compared with 48% producing nothing detectable at the start of winter.

The antibody concentration response in R1 deer increased within a mob with increasing age, as reported by Mackintosh et al. (2014), and has been observed in lambs and cattle (Shaw et al. 2013, Merlin et al. 2017). The response in R1 deer also does appear to require an environmental challenge of nematode parasites to elicit detectable measures, as indicated by Mackintosh et al. (2014). The environmental challenge was variable among farms and years, especially during the autumn. At Whiterock Station in 2012, the 2011 birth cohort produced low responses when weaned and grazed on clean (conserved) pastures, and after spending an extended time grazing winter brassica crop, having only grazed pasture for 10 days prior to CARLA 10 sampling (Table 1). Conversely, the 2012 Whiterock birth cohort produced much higher responses especially at CARLA 6 (Table 1) during a drought, where the deer grazed pasture at low covers that had been grazed previously by other deer. The low number of individuals producing a measurable antibody concentration before winter is likely a combination of the development of general immunity and environmental parasite challenge present, and interactions between these two factors.

The response is present in individuals under differing anthelmintic regimens, meaning that animal health does not need to be compromised to measure CARLA as long as the deer are still ingesting and responding to the L3 larvae. The moderate heritability estimate for CARLA 10 (h^2= 0.37 ± 0.09) was similar and correlation between CARLA traits was the same (r_g = 0.86) as the values observed in lambs (Shaw et al. 2013). In contrast to the observations reported by Mackintosh et al. (2014), this study found no significant effect of breed type (red deer or wapiti) on CARLA 6 or 10.

There was little evidence of unfavourable genetic or phenotypic correlations between the other production traits analysed and CARLA 6 or 10. There were favourable phenotypic correlations with growth and liveweight traits and striploin pH. The unfavourable genetic and phenotypic correlations with carcass dressing percentage are likely of no concern; as the hot carcass weight phenotypic correlation was favourable, the dressing percentage may simply relate to rumen digesta weight and associated inaccuracies of determining ruminant live weight (Wilson et al. 2015).

Summary

In this progeny test, the proportion of R1 deer producing an antibody response to a nematode antigen increased with age, but the response was variable across farms and years, during regular anthelmintic treatment. In pasture-grazed R1deer in spring at 10 or 11 months of age, between 70 and 100% (mean 93%) of animals produced a measurable response, providing suitable variation for genetic evaluation. This spring, CARLA 10 was moderately heritable and suitable to use for quantitative genetic selection, with little evidence of negative impacts on other production traits. The data suggests that stud breeders could use high levels of the CARLA 10 trait to select deer with elevated immune response to ingestion of parasitic nematodes.

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