CARLA saliva antibody response in the Deer Progeny Test

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Client report

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1. **Client Summary** (Target end-user: DEEResearch Board, DEERSelect Manager, DPT and DEERLink Partner herds)

- CARLA IgA antibody is an immune response that develops to larval parasite ingestion. In lambs it is moderately heritable and genetically correlated with live-weight gain. The test for CARLA IgA was developed for the New Zealand sheep industry as the antibody response has been shown to provide protective immunity to gastrointestinal nematodes.
- From initial studies CARLA had been shown to be potentially a useful predictor of resistance to parasites in farmed deer, and that rising yearling (R1) deer with CARLA levels >2.0 had significantly reduced numbers of adult abomasal parasites.
- The Deer Progeny Test (DPT) presented an opportunity to measure CARLA response in a pedigreed well-phenotyped population and estimate genetic parameters for CARLA response.
- All DPT progeny were measured over three birth years for two periods, pre-winter (CARLA06) and pre-slaughter (10-12 months-of-age; CARLA10). In 2014 sub-groups of progeny were additionally tested to measure the change in CARLA response over time (with increasing age).
- Analyses have demonstrated that CARLA responses were heritable; CARLA06 with low (h^2 = 0.10, non-significant) and CARLA10 with moderate (h^2 = 0.35, significant (p,0.05)) heritability. There was as much within-breed as across-breed variability. Wapiti-crossbred (terminal) and red (maternal) progeny expressed similar responses.
- Estimated breeding values were produced for all 35 DPT sires.
- The CARLA traits were genetically and phenotypically correlated with growth traits at a very low or zero level, except CARLA06, which was moderate-highly correlated with growth rate from weaning to 10-months-of-age.
- Different farms, mobs and years showed different levels of CARLA expression.
- The CARLA saliva test is a tool that the deer industry could utilise moving forward to select animals for resistance/resilience to parasites using CARLA10 trait.
- The industry should be informed of the potential uses of CARLA, with the caveat that there is still little known about how CARLA responses impact on production systems.
- Breeders may wish to incorporate the CARLA traits in to the genetic selection objectives and, if so, there should be facility made available for them to do this in DEERSelect.

2. Science Summary

The CARLA IgA antibody response was measured in all progeny from the DPT 2011-2013 birth cohorts. This CARLA response has been shown to measure protective immunity to gastrointestinal nematodes, being moderately heritable and positively genetically correlated with live-weight in lambs. It has also been shown in rising yearling deer that individuals with CARLA levels >2.0 have significantly lower numbers of adult abomasal parasites, although no significant correlations with live-weight were demonstrated (Mackintosh et al., 2014b).

The DPT presented a unique opportunity to investigate the genetic parameters of the CARLA response in R1 deer, in the presence of potential natural parasite challenge in deer treated with anthelmintic.

CARLA responses were measured at two periods, the start of winter (CARLA06) and at pre-slaughter in spring (CARLA10). Both of these responses were heritable, with the h^2 for CARLA06 being low and non-significant (0.10) and the h^2 for CARLA10 being moderate (0.35) and significant ($p<0.05$). Both traits were either favourably or neutrally genetically (mostly non-significant) and phenotypically (mostly significant; $p<0.05$) correlated with three live-weight traits, live-weights at end-winter and at pre-slaughter (10-12 months) and growth rate from weaning until 10-months of age.

Estimated breeding values (EBV) were produced for all 35 sires used in the DPT. This used the same genetic breed (gBreed) methodology used in previous DPT genetic parameter estimations. There was a good range of sire variation both within-breed and across-breed, contrary to previous research indicating that wapiti-crossbred progeny CARLA response levels were about half that of red deer. This difference may reflect varying management practices of the animals in the different trials.

The percentage of the mob expressing elevated CARLA responses increased with increasing age, but did reduce over winter. There were differences in the percentages of animals expressing elevated CARLA responses between farms, years and mobs.

The measurement of CARLA IgA provides a potential tool to the deer industry that could be used to select for host resistance to parasites in rising yearling deer.

We recommend providing this information to the wider NZ deer industry with appropriate caveats on the current knowledge gaps in relation to what the CARLA response means to production outcomes. As yet this is not a tool to reduce the current industry reliance on anthelmintic use, but genetic selection using CARLA could be in the future. There may well be a desire by breeders to adopt the CARLA phenotype and include CARLA EBV in their breeding objectives, and if so, this facility could be made available within DEERSelect using the CARLA10 trait.

3. Introduction

The Deer Progeny Test (DPT) was established to improve the genetic connectedness between deer breeding herds recording on DEERSelect, and to evaluate a range of new traits that many be important or valuable to New Zealand venison production systems (Ward et al., 2014).

Farmed red deer and red deer wapiti-crossbreds are commonly infected with a range of parasites under New Zealand pastoral farming conditions. These include lungworm (Johnson et al. 2001) and gastro-intestinal (GI) nematodes (Andrews 1973, McKenna 2009). These infections, particularly in R1 deer, are a major cost to deer production systems, both in lost production and investment in animal health products, with the estimated deer industry annual anthelmintic spend of ~$10M (Mackintosh et al., 2014b).

In recent years both the efficacy of standard drenching regimes for deer and the challenge of drench resistant populations of gastro-intestinal nematodes have been recognized within the NZ deer industry (Hoskin et. al., 2005; Lawrence, 2011; Mackintosh et. al., 2014a). As such the industry requires a range of new strategies to better manage parasites into the future (Hoskin et al., 2005). A potential long-term strategy to reduce the reliance on anthelmintics may include the selection of resistant or resilient deer (Mackintosh et. al., 2011, 2014b).

The DPT presented an opportunity to evaluate an existing technology from the sheep industry, the CARLA saliva test (Shaw et al., 2012), in a well-phenotyped and pedigree recorded population of R1 venison production animals. This new 'CARLA' trait being evaluated by the DPT is the measurement of a salivary IgA response to a carbohydrate larval surface antigen (CARLA) (Shaw et. al., 2012; 2013).

The CARLA IgA antibody test has been proven to be a suitable tool for measuring protective immunity to GI nematodes in sheep in New Zealand (Shaw et. al., 2012). In lambs positive anti-CARLA responses are moderately heritable with significantly positive genetic correlation with live-weight (Shaw et. al., 2013). This latter study indicated that the response to CARLA may be an important genetic correlate in the growth rate of lambs experiencing a GI nematode larval challenge.

In deer it has been shown that the CARLA antibody response may be a useful predictor of resistance to parasites, and that wapiti crossbred deer had significantly more parasites than red deer and significantly lower CARLA antibody response levels (Mackintosh et al 2011). The CARLA response in deer appears to develop in response to a natural nematode challenge (Mackintosh et. al., 2014b). In that study, which grazed R1 wapiti crossbred and red deer together, it was found that twice as many of the red deer had a CARLA IgA response levels ≥2, than for the wapiti-crossbreds. CARLA levels were shown to peak in late autumn and mid spring and that the average number of adult abomasal nematodes was significantly lower in all R1 deer with CARLA ≥2, than those with CARLA <2. Abomasal parasites are considered the most pathogenic of GI parasites of deer (Hoskin et al., 2005). Mackintosh et al. (2011) did not demonstrate any correlations of CARLA with live weight.

The DPT progeny cohort were sired by 35 different sires, pedigreed and recorded for a range of traits including live-weight. As the DPT was designed as a venison production genetic evaluation, not a parasite challenge trial, any parasite challenge was natural (environmental). To maximise production outcomes all progeny in the DPT were regularly treated with anthelmintic according to each farm's animal health plan, so levels of parasitism (clinical or sub-clinical) would be expected to be low, likely masking any extreme live-weight effects (or death) that may ensue from clinical parasitism.

The DPT was undertaken on three different farms, with different farm managements and systems across three different years. These farms represented quite a wide range of farming environments (Ward et al., 2014b).

In this study CARLA responses from two periods (pre-winter and pre-slaughter (~ mid-spring)) were analysed to determine if the CARLA response is heritable and if CARLA responses are genetically or phenotypically correlated with live-weight or weight gains. The CARLA IgA responses were modelled to understand the level of CARLA response produced for different breeds and sires, on different farms in different years. A random subset of animals were also regularly sampled throughout 2014 (2013 cohorts) to provide a perspective on the timing on CARLA response throughout the growth of R1 venison animals.

3.1 Objectives

The following report addresses research objective 4.1 Deer Progeny Test for the agreed milestone DPT 4.1.23: *Complete genetic analysis of CARLA phenotypes and report to DEEResearch and DSRG*.

4. Materials and Methods

4.1 DPT and DPT farms and farm management

A general overview of the DPT is in Ward et al., (2014a, 2014b, 2015); the specifics that relate to this study are as follows. The progeny were born over 3 years on three DPT farms: Whiterock Station (2011, 2012), Invermay (2011, 2013) and Haldon Station (2012, 2013) and slaughtered in 2012, 2013 and 2014. All three farms are geographically distinct; they operate within different climates and use different farm systems and animal health plans.

Three years of progeny were required to provide sufficient scale to undertake a genetic analysis on all traits recorded in the DPT. Over the 3 years, 35 sires of two types; maternal (red deer type; n = 24) and terminal (wapiti-crossbred type; n = 11) were used to generate progeny via artificial insemination (AI) of 1581 maternal (red type) dams (total 2417 inseminations). In total, there were 1647 progeny weaned and 950 slaughtered (Table 1). Maternal female progeny were retained to record maternal (e.g. reproduction) traits. Pedigrees and gBreed were determined using the Genomnz^{TM} DNA-microsatellite marker panel from blood (2011 born) or ear tissue punch (2012 & 2013 born).

Of the 35 different sires represented across the three years in this study, two (link) sires were common across all years: one Terminal sire 8334_42/08 (terminal link), and one maternal sire 8110_Czar/06 (maternal link). Another sire, 8109_144/05 (maternal), was used in two years (2011, 2012). Terminal sires had fewer offspring than maternal sires, as maternal females were retained for breeding (to record maternal traits), while all maternal males and all terminal progeny were slaughtered at between 10 and 12 months of age to record growth and meat traits. Each year the sires (2011 n=14, 2012 n=13, 2013 n=13) were used evenly across each farm, so the proportion of progeny per sire were similar for each sire on each farm.

Live-weight traits were measured on the progeny at approximately 20, 27, 38, 45, and 50 weeks of age, from weaning (~ 14 weeks of age) until all males and the wapiti crossbred females were slaughtered at around eleven months of age. Live-weights were recorded non-fasted (i.e. off-paddock or full) to the nearest 0.5kg on each farm, with all animals in the same mob weighed on the same day and in association with other trait recording.

Table 1. Number of Deer Progeny Test progeny by sire type and sex at weaning and slaughter across all three years.

	Maternal sire progeny			Terminal sire progeny			
	Males	Females	Total	Males	Females	Total	Grand Total
Weaning	700	656	1356	158	133	291	1647
Slaughter	673	0	673	151	126	277	950

CARLA data from 2011 and 2012 has previously been reported by Ward et al. (2014a) but used different analysis methods.

4.2 Farms and grazing management

Whiterock Station is situated in high country in Mid-Canterbury up to about 900m above sea level (asl), and runs deer and cattle. Deer calving is on high blocks of improved tussock country, where animals usually stay until weaning. In 2011 the offspring were weaned on to lower flat paddocks of saved pasture. Progeny grazed these pastures until early June when they were put on to a kale crop. On the kale crop they were supplemented with barley, baleage (lucerne, pasture and red cover) and pea vine in racks. They were not removed from the kale until approximately 10 days prior to the 10-month CARLA sample being collected. In 2012 Whiterock suffered a drought, and progeny weaned at lower weights than in 2011. The 2012 progeny were wintered similarly to 2011 progeny, but for less time on kale crop. They were then slaughtered later (Early Nov vs. Mid Oct), so had longer on pasture to develop the CARLA 10 response.

Invermay is a low-elevation hill country property in coastal Otago, running deer and sheep. In 2011 there was very little grazing of sheep on the deer farm, but in 2013 it was common practice. In 2011 animals were on pasture all the way through until slaughter, with lucerne hay (in racks) and barley supplementation through winter, almost up until slaughter in late October. In 2013 the DPT progeny were grazed on pasture except for six weeks from mid-May when they were grazed on a swede crop. The 2013 Invermay progeny were slaughtered in mid-November, so had longer than in 2011 to develop CARLA10 response in 2013.

Haldon Station is a Mackenzie Basin high country property that experiences both very dry and very cold conditions. There is access to both spray (centre-pivot) and flood (border-dyke) irrigation on some paddocks. Calving is on extensive hill blocks with partially improved tussock country and a lot of briar rose scrub. Later in lactation hinds and calves are allowed onto lucerne paddocks prior to weaning. Post-weaning progeny are on lucerne paddocks, and pastures under irrigation. Wintering is hard feeding on lucerne baleage and barley on the ground in sacrifice (zero grazing) paddocks. Haldon animals were slaughtered in the first week of December, so had the longest time to develop a CARLA 10 response. Haldon grazes cattle, merino and cross-bred sheep and deer in the same irrigated paddocks (sometimes grazing together).

All progeny were in mobs which represented both sire (breed) types, but each cohort differed on each farm. Post-weaning Whiterock ran all animals together in one mob 2011, per sex mobs 2012; Haldon ran sexed mobs in 2012, one mob in 2013 and Invermay two mobs per sex 2011 and one mob per sex 2013.

4.3 Anthelmintic regimes

All animals were weaned at around 90 days of age. Parasite challenge was a natural (environmental) challenge only. These animals were not intended to accumulate parasite burdens that may impact their growth, as venison traits and growth were the primary trial focus. Animal health programmes were in place on each farm to maximise production and ensure good overall animal health. There was no intent for progeny to become parasitized such that it might impact on growth.

Whiterock Station used a drench regime of oral oxfendazole (high mineral), plus injectable moxidectin at weaning, 6 weeks post weaning, and 12 weeks post weaning, as did Invermay for the 2013 cohort. For the 2011 cohort Invermay used pour-on moxidectin up until mid-winter, at that time progeny received an injectable moxidectin. Haldon Station alternated oral oxfendazole and pour-on moxidectin. Replacement (maternal) females were the only animals drenched in spring, on all three farms.

4.4 Trait measurement

Saliva samples for CARLA measurement were collected at two sampling times, pre-winter and pre-slaughter, with animal yarded in deer handling yards. Individuals were sampled in small pens in groups of 4-6 animals, or while the animals were in individual hydraulic crush restraint for ultrasound eye muscle scanning (Ward et al., 2010).

During 2014 (DPT2013 birth cohorts), at Haldon and Invermay samples were taken at all weighing occasions post-weaning of a random sub-sample of approximately 20 DPT progeny. This was done to observe the changes in CARLA on each farm overtime, providing a potential indication of seasonal parasite challenge and/or changing immune response status.

Individual animals were identified with low-frequency electronic identification ear tags (EID) and visual management tags. Prior to collection of the CARLA saliva swab, the EID of the animal was scanned using an HR3 (Gallagher AMS, Hamilton NZ) handheld EID reading wand, which transmitted the EID to the TSi Smart Scale (Gallagher AMS, Hamilton NZ). Sample tubes were labelled using automated printing and a ‘smartcable’ connected to a RW420 printer (Zebra Technologies, China).

Immediately prior to scheduled anthelmintic treatment(s) the saliva sample was collected by inserting a cotton dental swab held in forceps in to the mouth of the animals and moving it around the mouth until it became saturated (approximately 10 seconds), as described by Mackintosh (et. al., 2014b). Many animals did not require physical restraint during this procedure. Individual swabs were placed in labelled screw cap tubes, chilled as soon as possible and frozen at -20^{o}C for medium-term storage.

Live-weights were usually collected following saliva swabbing and application of animal health treatments. Each animal was weighed individually to a precision of 0.5kg in a weigh box, or on a weigh platform. The weigh platforms utilised pairs of electronic load cells and weights and EID’s were captured using EID reading wands or panel readers recorded on a Gallagher TSi Smart Scale. There was never more than 1 week difference between live-weight and CARLA sampling.

4.5 CarLA IgA antibody measurement

Samples for a single year of progeny were all submitted in a single batch to the CARLA Saliva Test Unit at the AgResearch Hopkirk Research Institute (Palmerston North).

The sample preparation and analysis was carried out as described by Shaw (et. al., 2012) and Mackintosh (et. al., 2014).

4.6 Trait definitions

CARLA06 is a late autumn/pre-winter CARLA level collected in May or early June before progeny are transitioned to winter feeding.

W0506 is the live weight collected at the same time as CARLA06.

CARLA10 is the CARLA level collected 7-10 days before the cohort is slaughtered, in mid-late spring.

W101112 is the live weight at the time CARLA10 is collected. The W101112 trait represents the final weight of each year's full birth cohort (all sexes), prior to slaughter.

W08 is the weight around 20 August, which is when young deer (especially males) can rapidly increase their growth rate coming out of winter photoperiodic control.

W0910 is a spring weight.

Gain0510 is the live weight gain (g/day) between W0506 and W0910 (i.e. late-autumn and mid-spring).

Table 2. Live-weight trait descriptions and abbreviations

Trait Type	Trait abbreviation (units)	Trait description
Live weight	WWT (kg)	Live weight at weaning (mean age 101 days)
	W0304 (kg)	Live weight Mar/Apr, 6-weeks post weaning (mean age 141 days)
	W0506 (kg)	Start-winter weight (May/Jun mean age 187 days)
	W08 (kg)	End-winter weight (mean age 267 days)
	W101112 (kg)	12-month weight (mean age 352 days)
	WPreSltr (kg)	Pre-slaughter live weight (mean age 355 days)
	WWTtoW05 (gday)	Growth rate between WWT and pre-winter weights (86 days)
	WWTtoW10 (gday)	Growth rate between WWT and October weights (217 days)
	W05toW08 (gday)	Growth rate during winter (80 days)
	W05toW10 (gday)	Growth rate between pre-winter and October weights (131 days)

4.7 Statistical analysis

All progeny had DNA pedigrees determined, which were extracted from the DEERSelect database for use in the analysis.

CARLA data were transformed to natural logarithms. All zero responses for CARLA were assigned a value of 0.1 units/mL, which was the smallest reliable response measurable by the assay, and then log transformed.

Data was analysed using the $\log_e$CARLA and live-weight traits in the package ASREML (Gilmour et. al., 2009) which is also used in DEERSelect. Analysis then followed the procedure described by Ward (et al., 2015), with animal fitted as a random effect in all models.

gBreed, as previously described by Cullen et al. (2013), was tested in the model for preliminary analysis. This indicated that sire-type (maternal or terminal) was much less precise than gBreed in discriminating breed effects and therefore gBreed was included in the final models used. There are three gBreed types: Eastern European red deer, North American Elk (wapiti) and English red deer, with the two maternal (red) types (Eastern and English) being included in the ASREML model and the third (wapiti) predicted by difference.

Herd by year combination, sex, post-weaning mob and proportion Eastern and English was the standard (base) model used. For the weight gain trait (weaning to ten month) weaning weight was an additional covariate in the model.

Twins were excluded from the analysis, and data from animals with contradictory mob information or missing trait data were excluded from analysis where relevant. CARLA06 (pre-winter) data from Whiterock 2011 and Invermay 2013 cohorts was excluded as very few results were above the CARLA antibody zero detection/response level.

The analysis using ASREML was a two stage process. Univariate (single trait) models were run first to estimate direct and maternal (if applicable) heritability and variance, and gBreed proportion regression factors. Then trivariate (three trait) models were run to estimate average phenotypic and genotypic correlations and the heritability estimates (h^2).

Sire best linear unbiased predictions (BLUPs; effectively breeding values) were estimated for a selection of traits using the same methodology as Ward (et al., 2015): Running ASREML with gBreed included, then adjusting the BVs by breed regression factors (effects) pro-rata for proportion of Eastern and English, effectively added back in the breed effects.

The CARLA profiles of the sub-sampled progeny from 2014, have not been analysed, but are presented as an observational dataset only.

5. Results and Discussion

5.1 Single trait CARLA analyses

The regressions on gBreed are less stable for logeC06 than they are for logeC10. The regression of $\log_e$C10 on gBreed stayed much the same in the 3-trait analyses, whereas there was more movement in the regression of $\log_e$C6 on gBreed. While the regression slopes for the English and Eastern European red deer are both negative (Table 3), these were not statistically significant. This indicates no significant effect of breed on CARLA response pre-winter or between 10-12 months of age within the DPT.

Table 3. Single trait genetic parameters of CARLA traits: logeC06 (pre-winter) and logeC10 (pre-slaughter).

	$\log_e$C06	$\log_e$C10
Heritability	0.099 ± 0.066	0.353 ± 0.090
Total variance	0.852	1.16
Regr: Eastern	-0.332 ± 0.257	-0.591 ± 0.329
Regr: English	-0.272 ± 0.251	-0.038 ± 0.315

5.2 Three trait CARLA analyses

All three trivariate analyses produced very similar values for logeC06 and logeC10, as the univariate analysis; logeC06 had a low non-significant ($p<0.05$) heritability ~0.10, whereas logeC10 heritability was significant ($p<0.05$) and moderate ~0.35. The heritability estimates for the growth traits were all higher than the CARLA traits.

The genetic correlations for the two CARLA traits with each other were high ~0.80 and significant at the 5% level (Tables 4-6). The only other significant genetic correlation was logeC06 with growth rate from weaning to October (Table 4). This was a positive (favourable) genetic correlation and was moderately high.

There were no other significant genetic correlations as the genetic correlations were low or moderate and the error terms were relatively large (Tables 4-6). The direction of these non-significant genetic correlations were positive or around zero, suggesting favourable or at worst neutral genetic correlations between CARLA and the live weight and growth traits analysed, so there should not be negative impacts on growth traits caused by selecting for CARLA traits.

There were small significant phenotypic correlations between the two CARLA traits and between live-weight 10-12 month-of-age and growth rate, from weaning to October (Tables 4 & 6). This indicates a very small but significant ($p<0.05$) growth advantage over the post-weaning to October period, even under anthelmintic control.

As the CARLA10 trait was moderately heritable, not unfavourably genetically correlated, and lowly favourably phenotypically correlated with live weight or growth traits, on a population basis would be the more useful of the two CARLA traits to select for. Given the low non-significant heritability estimate of logeC06 there would be no advantage selection for CARLA pre-winter.

Table 4. Three trait genetic parameters of CARLA traits heritability estimates in bold on the diagonal, phenotypic correlations above the diagonal, genetic correlations below, traits logeC06 (pre-winter), logeC10 (pre-slaughter) and WWTtoW10 (growth rate from weaning to 10-months old). Significant ($p<0.05$) heritability estimates or correlations marked with an asterisks (*).

	log_eC6	log_eC10	WWTtoW10
log_eC6	**0.107 ± 0.060**	0.284 ± 0.031*	0.128 ± 0.033*
log_eC10	0.773 ± 0.208*	**0.347 ± 0.090***	0.083 ± 0.032*
WWTtoW10	0.574 ± 0.241*	0.059 ± 0.196	**0.430 ± 0.103***

Table 5. Three trait genetic parameters of CARLA traits heritability estimates in bold on the diagonal, phenotypic correlations above the diagonal, genetic correlations below, traits logeC06 (pre-winter), logeC10 (pre-slaughter) and W08 (live weight at end-winter). Significant (p<0.05) heritability estimates marked with an asterisks (*) – no significant correlations.

	log_eC6	log_eC10	W08
log_eC6	**0.086 ± 0.056**	0.284 ± 0.031*	0.056 ± 0.036
log_eC10	0.863 ± 0.230*	**0.352 ± 0.090***	0.052 ± 0.034
W08	0.215 ± 0.301	0.053 ± 0.171	**0.753 ± 0.110***

Table 6. Three trait genetic parameters of CARLA traits heritability estimates in bold on the diagonal, phenotypic correlations above the diagonal, genetic correlations below, traits logeC06 (pre-winter), logeC10 (pre-slaughter) and W101112 (live weight pre-slaughter). Significant (p<0.05) heritability estimates or correlations marked with an asterisks (*).

	log_eC6	log_eC10	W101112
log_eC6	**0.091 ± 0.058**	0.285 ± 0.031*	0.084 ± 0.035*
log_eC10	0.836 ± 0.224*	**0.350 ± 0.090***	0.094 ± 0.033*
W101112	0.256 ± 0.288	0.082 ± 0.174	**0.675 ± 0.109***

5.3 Estimated breeding values (EBV) for DPT sires

The EBV calculated for the DPT sires were estimated using a data on a logarithmic scale, due to the nature of the CARLA response data. This means that the EBV (Table 7) need to be back-transformed for the sire EBV to reflect the actual CARLA response measurement scale. This back-transformation may make it difficult to reconcile the CARLA response BV values in relation to actual CARLA responses, but with all EBV the most important consideration is the ranking.

There was a very similar range of sire CARLA EBV for the two different sire types, which is contrary to earlier findings of Mackintosh et al. (2011) which showed poor CARLA responses in wapiti-crossbred progeny. This difference may reflect varying management practices of the animals in the different trials carried out so far. This new data indicates as much within sire-type variation as across sire-type variation.

There was a clear gap in the EBV between the highest ranking sires for each sire-type and the next highest ranking (Table 7), the gap was not as large for the lowest ranking individuals. The range of sire EBV for CARLA10 was much greater than for CARLA06, indicating that the increased levels of CARLA response of the R1 progeny increased with age. This range of sire variation demonstrates an opportunity to use EBV for CARLA response of R1 animals to select for CARLA response.

Given that these CARLA10 response trait is moderately heritable and did not have negative genetic correlations with growth traits, and did have small significant positive phenotypic correlations with growth to October. At the very least selecting for CARLA10 should not compromise selection for growth and may provide a very small growth advantage in R1 farmed deer between weaning and October (main growth periods for venison production systems).

Presently there is limited knowledge on the impact of increased CARLA response on deer production systems. The work of Mackintosh et al. (2014b) indicated that CARLA response levels >2 correlated with reduced adult abomasal GI nematodes, and may, therefore, be protective against parasitic abomasal damage.

The DPT was not able to greatly add to that knowledge because the experimental design of the DPT was to allow expression of meat and growth traits under animal health regimes, not under levels of parasite challenge that may have significantly compromised the growth of some or many individuals. Although for both CARLA traits there were small positive phenotypic correlations with growth, post-weaning to October and live weight at 10-12 months-of-age indicating a small, but significant ($p<0.05$) growth advantage with increased CARLA response. The mechanism for this cannot be explained by the DPT trial, and the question still remains as to what does an elevated CARLA response mean in relation to production outcomes in an environment of parasite challenge, without the protection of anthelmintic? The Tomorrow's Deer study plans to investigate this question, using high and low CARLA response EBV sires from the DPT.

Table 7. Estimated breeding values (EBV) of DPT sires for two CARLA response traits at pre-winter (CARLA06) and pre-slaughter (CARLA10), as estimated (as a natural log) and back-transformed sorted by sire-type and CARLA10 EBV descending.

	Sire-	Log		Back-transformed	
DPT Sire ID	type	CARLA06	CARLA10	CARLA06	CARLA10
8078.08174.2008	Maternal	0.03	0.79	1.03	2.20
8007.00Y278.2000	Maternal	-0.13	0.52	0.88	1.68
8019.06259.2006	Maternal	0.13	0.40	1.14	1.50
8019.09182.2009	Maternal	-0.01	0.37	0.99	1.45
8019.06318.2006	Maternal	-0.07	0.30	0.93	1.36
8109.75/10.2010	Maternal	-0.08	0.29	0.92	1.33
8019.06306.2006	Maternal	-0.03	0.26	0.97	1.30
8005.31/04.2004	Maternal	-0.05	0.23	0.95	1.26
8085.05068.2005	Maternal	-0.19	0.17	0.83	1.19
8109.144/05.2005	Maternal	-0.15	0.13	0.86	1.14
8019.09116.2009	Maternal	-0.09	0.03	0.91	1.03
8019.10570.2010	Maternal	-0.25	-0.12	0.78	0.89
8086.449/10.2010	Maternal	-0.23	-0.17	0.79	0.84
8019.MFCF-08-122.2008	Maternal	-0.28	-0.39	0.75	0.68
8065.04374.2004	Maternal	-0.38	-0.45	0.68	0.64
8065.02077.2002	Maternal	-0.24	-0.47	0.79	0.62
8086.259/06.2006	Maternal	-0.23	-0.49	0.79	0.61
8007.02P162.2002	Maternal	-0.40	-0.57	0.67	0.56
8065.03556.2003	Maternal	-0.46	-0.60	0.63	0.55
8019.MFCF-08-378.2008	Maternal	-0.47	-0.67	0.63	0.51
8125.205/07.2007	Maternal	-0.49	-0.72	0.61	0.49
8019.11134.2011	Maternal	-0.43	-0.76	0.65	0.47
8007.06015.2006	Maternal	-0.44	-0.78	0.64	0.46
8065.99540.1999	Maternal	-0.71	-1.53	0.49	0.22
8334.42/08.2008	Terminal	0.30	0.95	1.36	2.58
8304.WH05043.2005	Terminal	0.16	0.33	1.18	1.39
8304.PU06086.2006	Terminal	0.24	0.30	1.27	1.36
8304.PU97201.1997	Terminal	0.13	0.28	1.14	1.33
8334.209/08.2008	Terminal	0.05	0.25	1.06	1.28
8304.PU06105.2006	Terminal	-0.02	-0.17	0.99	0.85
8302.G33.2004	Terminal	-0.11	-0.23	0.90	0.79
8115.03B923.2003	Terminal	-0.17	-0.28	0.84	0.76
8334.49/07.2007	Terminal	-0.18	-0.36	0.83	0.70
8314.14/00.2000	Terminal	-0.25	-0.78	0.78	0.46
8314.749/07.2007	Terminal	-0.38	-1.04	0.69	0.35

5.4 Plots of change in CARLA over time

These following figures show how the CARLA response levels have changed at different sampling times over the year. The interpretation of the individual CARLA IgA responses uses the same scale as is used for sheep, with 0.0 -0.5 being none or trace, 0.5-1.0 being low, 1.0-5.0 medium and >5.0 high. The scale/interpretation of responses for deer cannot yet be determined without further research.

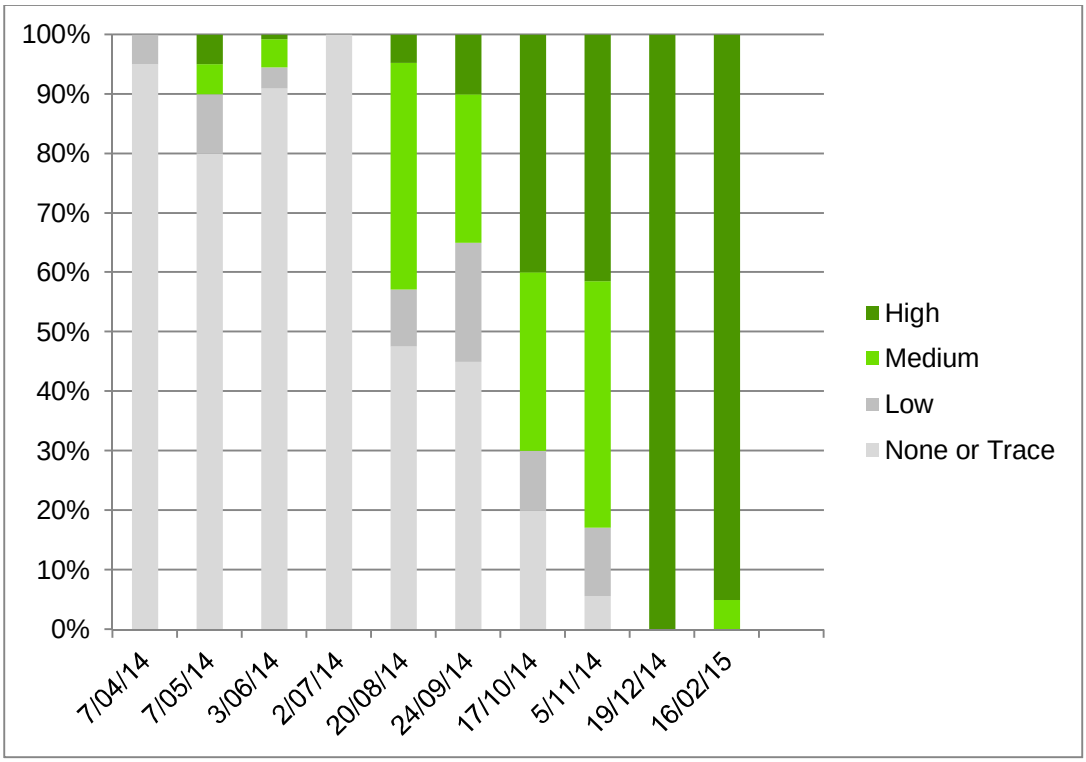

Figure 1. Percentage of sampled Invermay 2014 born rising yearling DPT progeny, expressing levels of high, medium, low, trace or no CARLA IgA antibody. Note only 3/06/2014 and 17/10/2014 represent the entire cohort, the rest are subsamples of approximately 20 random individuals.

The last two measurements for Invermay (Figure 1) and the last for Haldon Station (Figure 2), are from maternal female progeny only. In both herds most females expressed relatively high levels of CARLA response. This aligns with conventional understanding that breeding hinds develop a natural resistance to parasites as they reach puberty.

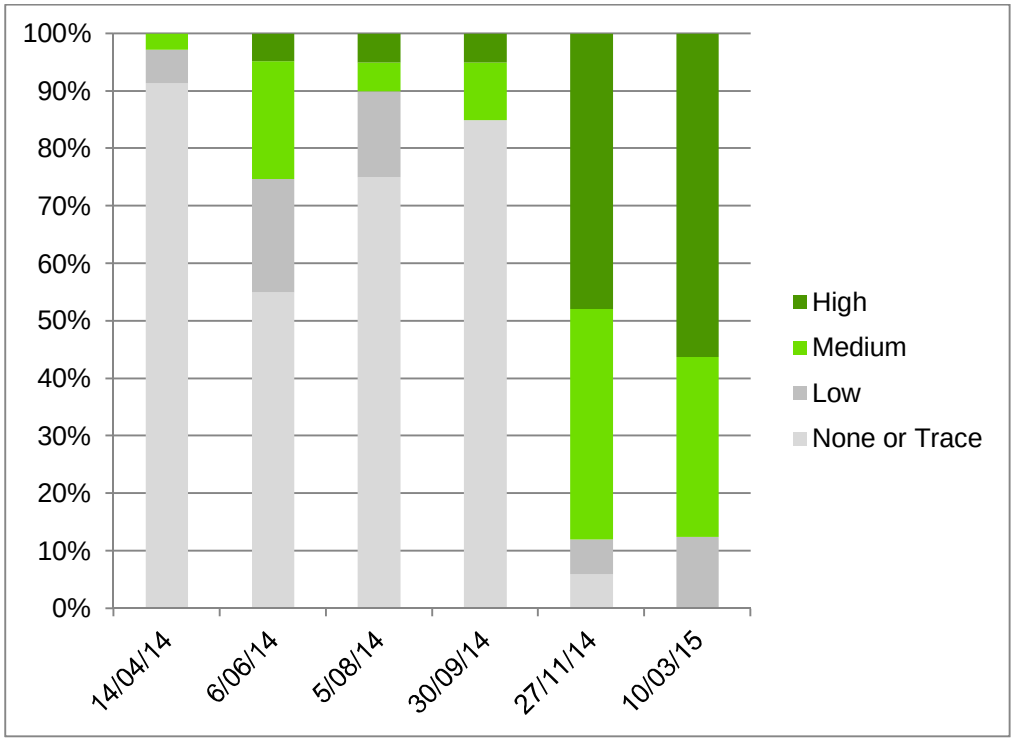

Figure 2. Percentage of sampled Haldon 2014 born rising yearling DPT progeny, expressing levels of high, medium, low, trace or no CARLA IgA antibody. Note only 06/06/2014 and 27/11/2014 represent the entire cohort, the rest are subsamples of approximately 20 random individuals.

Levels of CARLA increased as the R1 progeny increased in age. Some of this may relate to the level of natural parasite challenge, as response levels appear to drop over the winter months. The recording of none or trace levels of CARLA from the Invermay subsample 02/07/2014 (Figure 1) would be consistent with a lack of challenge as the animals had been grazing a swede crop for the previous 6 weeks. The response at Haldon Station also dropped in the two late-winter/ early spring sub-samples 05/08/2014 & 30/09/2014 (Figure 2). These animals remained on very short pastures over winter and were fed lucerne silage. A combination of seasonal/climatic and forage type probably resulted in reduced parasite challenge (reflected in the reduced CARLA responses) in both of these cases.

5.5 Plots of CARLA between farms in the same year

The following graphs show the differences in CARLA response between farms, using the same (DPT) sires, but a different breeding hind base in different farm systems and environments. Whiterock Station in particular had two very different parasite challenges in the two different years (Figures 3, 4), and also the lowest CARLA responses both years. The between year variation could be due to the climatic conditions, or differences in the farm system.

The low response is most likely due to a combination of the farm system, in particular the animals health programmes, and feeding environments (extensive high country pre-weaning, intensive post weaning, brassica crop winter to spring), and climate extremes. Invermay by comparison has a less extreme climate and does not lose soil moisture as readily as Whiterock

Station, whereas Haldon Station experiences the more extreme climate, but has irrigated pastures available post-weaning and crops are not used in winter.

Whiterock Station also was the first to slaughter progeny each year, either mid-October (2012) or early-November (2013), whereas Haldon Station retained progeny for 6 or more weeks, slaughtering them at the start of December. This meant that Haldon Station progeny had a longer opportunity to be exposed to parasite challenge(s) and develop immune responses to those challenges.

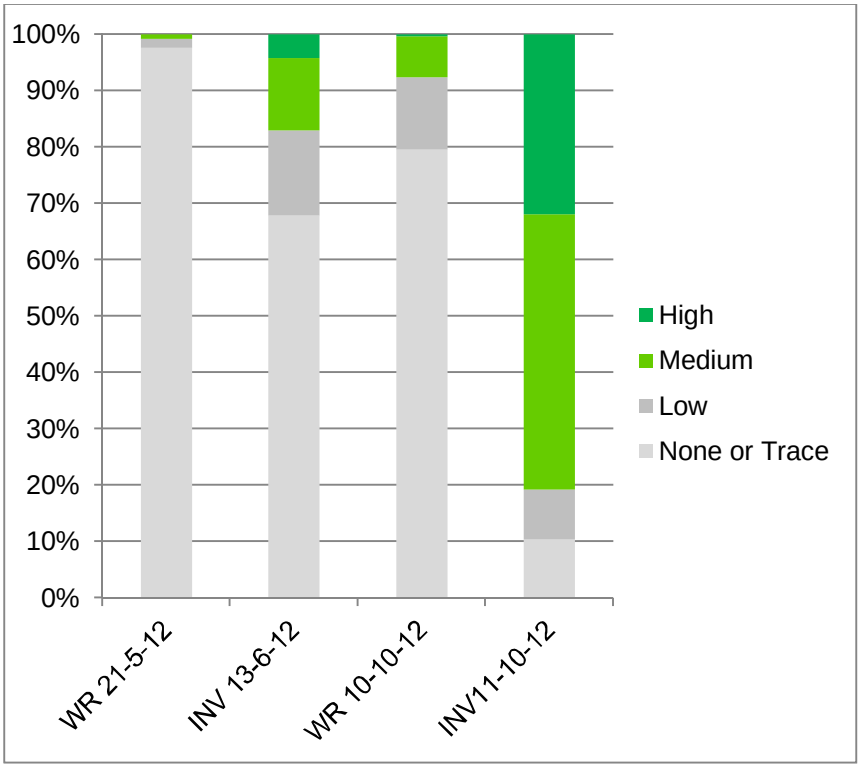

Figure 3. Percentage of Whiterock Station (WR) or Invermay (INV) 2011 born rising yearling DPT progeny, expressing levels of high, medium, low, trace or no CARLA IgA antibody at two times of the year.

The low levels of response at Whiterock Station meant that the 2011 cohort data (Figure 3) were excluded from some analysis, as noted earlier. The low CARLA06 (4/06/13) responses and high CARLA10 (25/11/13) at Haldon Station in 2013 (Figure 4) suggest that a low response prior to the first winter of R1 deer should not necessarily prevent a high CARLA response at 12-months of age (presumably in the presence of parasite challenge.). The low CARLA06 (21/05/12) and CARLA10 (10/10/12) responses at Whiterock Station in 2012 (Figure 3) may indicate low parasite challenge that year.

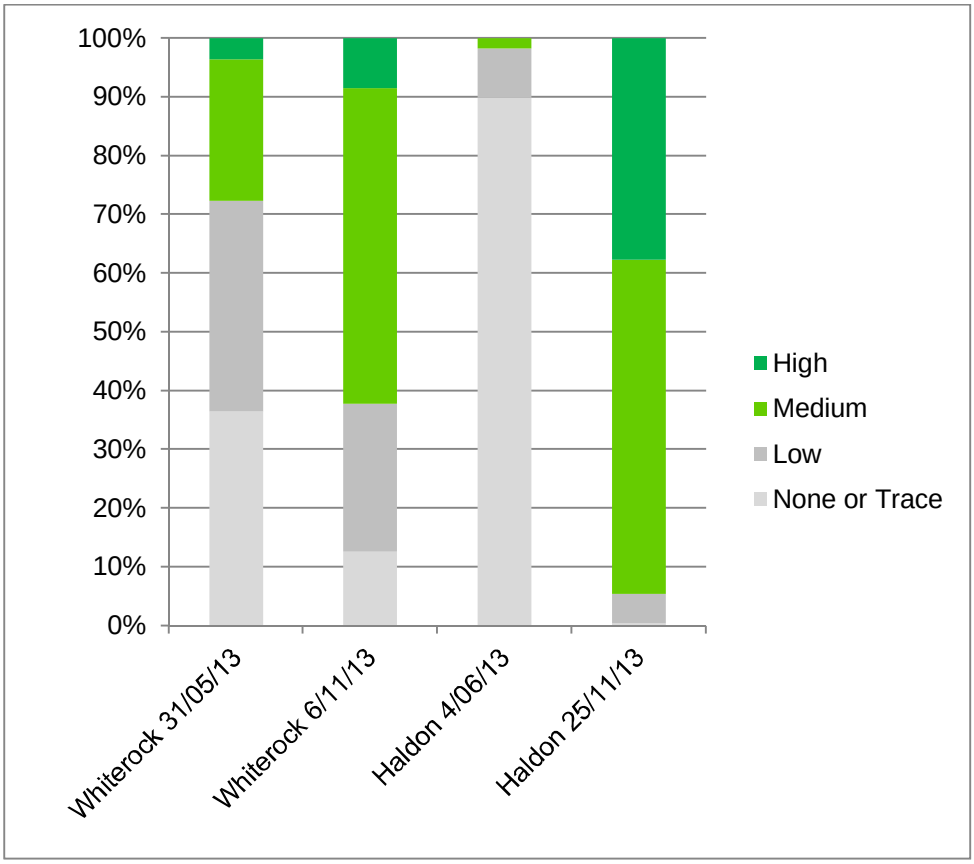

Figure 4. Percentage of Whiterock or Haldon Station 2012 born rising yearling DPT progeny, expressing levels of high, medium, low, trace or no CARLA IgA antibody at two times of the year.

5.6 Plots of CARLA between sexed mobs on the farm in the same year

Each DPT farm operated different mob management policies for the DPT. In 2014 Invermay and Haldon Station managed the progeny in sexed mobs. There was no effect of sex on CARLA response reported from the first two year's birth cohorts of the DPT (Ward et al.,2014a). Assuming this, then any observed differences between sexed mobs (Figures 5, 6) were likely due to different levels of parasite challenge resulting from differences in mob management (e.g. paddock effects). Although the data in Figures 5 and 6 has not been tested statistically there appears to be little difference between the CARLA responses between mobs, most notably at Haldon Station (Figure 5).

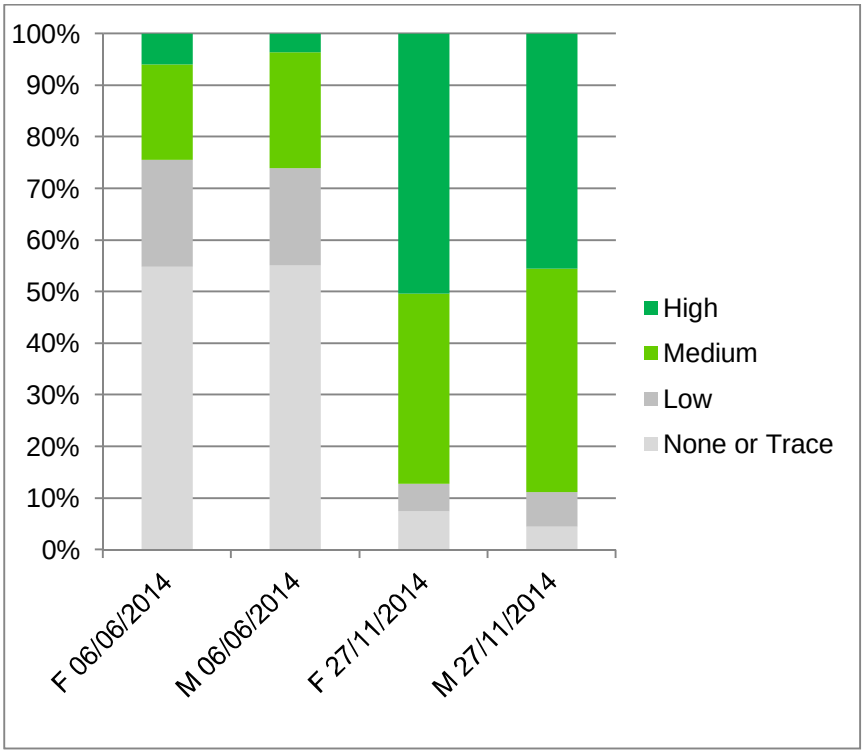

Figure 5. Percentage of Haldon Station 2013 born rising yearling DPT progeny in two sexed mobs (F=female, M=male), expressing levels of high, medium, low, trace or no CARLA IgA antibody at two times of the year.

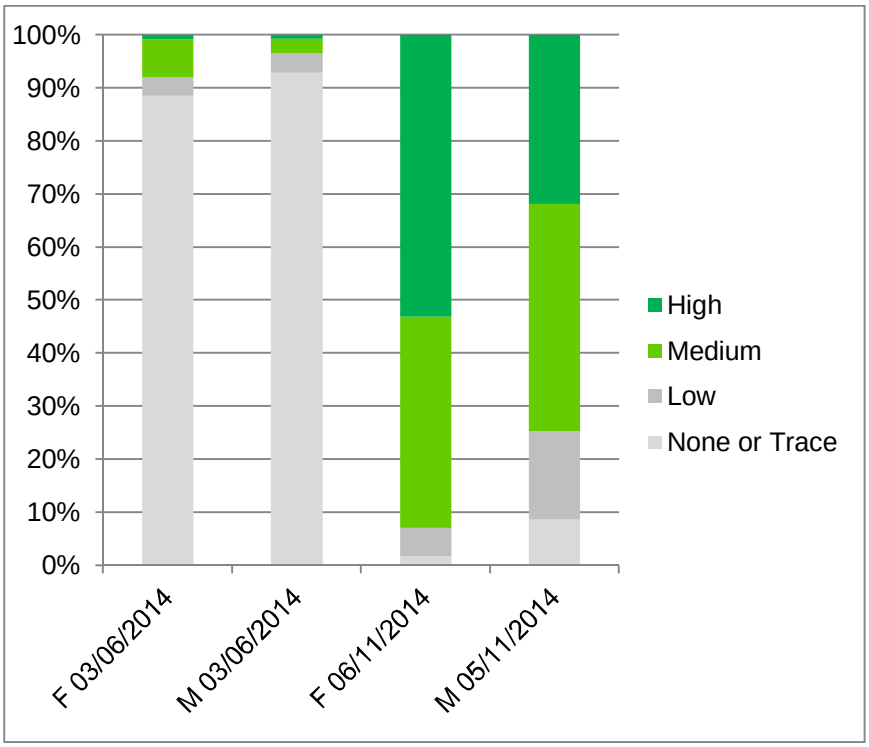

Figure 6. Percentage of Invermay 2013 born rising yearling DPT progeny in two sexed mobs (F=female, M=male), expressing levels of high, medium, low, trace or no CARLA IgA antibody at two times of the year.

5.7 Protective CARLA responses

The percentage of each DPT birth cohort on each farm that had attained a CARLA10 response ≥2.0 is presented in Table 7 below. A CARLA response of ≥2.0 was considered protective by Mackintosh et al., (2014b) as above that level mean numbers of adult abomasal GI nematodes were significantly reduced. Whiterock Station had very low levels of attainment of this protective CARLA response at 10-months.

Table 8. Percentage of DPT progeny on each DPT farm for each birth cohort that attained a 10-month CARLA response ≥2.0.

Farm	Cohort	CARLA10 > 2
Whiterock Station	2011	2%
Invermay	2011	62%
Whiterock Station	2012	31%
Haldon Station	2012	76%
Invermay	2013	70%
Haldon Station	2013	73%

The low responses on Whiterock Station (Table 8) were possibly related to a low level of parasite challenge due to a combination of climatic and farm management conditions. Invermay and Haldon Station had much higher levels of CARLA response, with >60% of progeny attaining the abomasal protective response (Table 8). Farm system interventions may provide an opportunity to reduce deer parasite challenge in the future, but this could only be conclusively demonstrated if a tool became available to accurate measure levels of on-farm parasite challenge (i.e. populations).

The DPT sires were submitted by stud breeders as representative of current sires they were using within their stud breeding herds. That would suggest that these sires are above industry average for growth traits and it cannot be assumed that they were an industry average representation even for previously unmeasured traits such as CARLA response. It is therefore unlikely that the range of sire CARLA responses of the 35 sires in the DPT represent the full range of possible CARLA responses within the entire NZ deer industry, but as evidenced by the work of Mackintosh et al., (2014b) there must (at least) be some sires within the industry with much poorer progeny CARLA responses than those measured in the DPT.

6. Conclusions and Recommendations

The CARLA immune response saliva test offers an interesting opportunity to the NZ deer industry. Presently there are few tools available for parasite diagnosis in R1 deer past their first winter (e.g. faecal egg or larval counts). The DPT has demonstrated that CARLA immune response at 10-12 months of age is moderately heritable and not unfavourably correlated with selected growth traits.

There is sufficient sire variation of CARLA responses to estimate breeding values and those responses are as great within-breed as across-breed. The CARLA10 trait is moderately heritable and not negatively genetically correlated with growth traits (W08, W101112 and GR WWT to W10). So including a CARLA10 trait in a selection objective would not detract from selection for these growth traits.

Both CARLA response traits are highly correlated with each other, so selecting for one will positively influence the other, although the CARLA06 trait has a lower heritability and was non-significant ($p<0.05$). However CARLA06 was significantly positively correlated with the growth traits, being moderate-highly genetically correlated from weaning to 10-months. Genetic and phenotypic correlations with other DPT measured traits will be reported in the second round of DPT genetic parameter estimations in June 2016.

We do not know what the CARLA response may confer, or how it may or may not advantage production in the absence of anthelmintic parasite/nematode control, other than that responses >2.0 have previously been shown to reduce/minimize abomasal damage/adult GI nematode numbers. It should be noted that in the presence of anthelmintic control there was a significant ($p<0.05$) very small positive phenotypic correlation with weaning to 10 month growth rate and live weight at 10 to 12 months. There would need to be further research undertaken to understand the factors if the NZ deer industry wants proof of the benefit that CARLA may confer to parasite challenge. However, if breeders or breeder groups have a desire to undertake selection for improved CARLA response then producing EBV would be possible at the present time.

At present CARLA response is not a tool to reduce reliance on anthelmintic by breeding for resistant hosts, but it may be in the future. The DEEResearch Tomorrow's Deer research programme is going to further investigate the CARLA response and progeny of four DPT sires: two high (8078.08174.2008 & 8334.42/08.2008) and two low (8125.205/07.2007 & 8314.749/07.2007) are being bred in the 2016 season.

We recommend that the industry gives consideration to making CARLA available as a trait for the deer stud breeding industry to measure and select for in rising yearlings. The CARLA10 trait is moderately heritable should not have a negative genetic impact on growth selection and could improve in a very small way growth performance to 10-months-of-age. Breeders and commercial producers need to be informed of the knowledge gaps that exist around CARLA response and production outcomes for deer at the present stage, so they can make informed choices. If breeder groups express a desire to adopt CARLA phenotyping then a facility should be made available in DEERSelect to estimate breeding value(s) for the trait(s).

The inclusion of the CARLA response phenotype in the Tomorrow's Deer programme is a very good first step in understanding the production outcomes of deer with different CARLA responses.

7. Acknowledgements

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DPT Farms, owners, farmers and staff at AgResearch Invermay, Haldon Station and Whiterock Station.

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9. Appendices

9.1 Recommendations for deer breeders considering CARLA sampling (Sep 2014)

CARLA® Saliva test for Deer

Anthelmintic resistance looms as a major issue for the New Zealand deer farming industry. The ability to easily identify deer for breeding that have developed immunity to gastrointestinal nematode parasites would be very useful alternative to option for controlling parasite infection. Unfortunately the traditional means of identifying such animals, faecal egg counting has been shown not to be very useful in deer due to poor correlations with worm burden. The CARLA® saliva test has been developed for the NZ sheep industry as a means of identifying animals with enhanced protective immunity to gastrointestinal nematode parasites. In sheep this test measures an antibody response that is directly associated with protection to nematode larval challenge and we predict this is the case in other livestock species. Research by Colin Mackintosh has shown that animals with good CARLA® responses tend to have significantly less abomasal nematodes. Over the last 2 years the CARLA® test has been evaluated within the Deer Progeny Test (DPT). The CARLA® response in young deer sampled at about 10 months is comparable to that observed in sheep and has a heritability of 0.31.

The CARLA® saliva test unit at AgResearch is offering Deer stud farmers the opportunity to use the test before it is available as a fully validated commercial test. In return for making the test available at this pre-commercialisation stage, AgResearch requests the ability to use individual farmers test results and herd information in on-going test validation. AgResearch can supply kits containing all the material and instructions required to carry out Saliva sampling.

Deer farmers interested in using the CARLA® saliva test would need to consider the following.

- Animals to be tested need to be around 10 months of age and have been grazing pasture for at least 1-2 months.
- The CARLA® test measures the response to larval challenge (worms larvae picked up off pasture).
- Animals can be tested at any time irrespective of drench treatments as it is the larval challenge and not the accumulated worm burden that determines the response.
- Animals grazing crops will be exposed to low larval challenge and will thus have low CARLA® responses.
- To ensure a good larval challenge and thus maximise the CARLA® response try to have animals selected for testing graze pasture to low cover over 5-7 days.
- A monitor saliva sample of 20 animals should be taken and sent for testing before sampling larger numbers of animals to ensure a good CARLA® response is occurring. We can provides results and interpretation of these within 48 hours of receiving the monitor samples
- The monitor sampling is free. Herd testing currently costs $9.00 per sample for research based testing
- The CARLA® Saliva test is still in the process of being fully validated for use in deer, so AgResearch cannot offer any assurances with this testing.

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CARLA SALIVA TEST is a result of NEW ZEALAND FARMER Investment in Beef+Lamb New Zealand and Ovita

9.2 CARLA saliva sampling procedure

CARLA™ SALIVA TEST

Saliva Sampling Instructions

Sampling process

This procedure provides instructions on how to collect oral fluid (saliva) samples from farm livestock (especially sheep and goats) for the purpose of measuring antibody or other molecules present in saliva.

Oral fluid contains secretions from various salivary glands including secreted IgA antibody and Crevicular fluid. Crevicular fluid is an interstitial transudate passively transported from the capillary bed beneath the mucosa of the mouth into the oral cavity. It is composed of humoral and cellular components from blood, but at different concentrations due to the transudation process.

Animal selection and preparation

Saliva can be collected from most healthy animals. Animals with significant infection or adverse condition of the mouth should not be sampled i.e. severe scabby mouth infection or facial eczema that affects the mouth. Animals should be moved into a small pen or raceway for sampling.

Procedure

Materials required:

- Forceps, straight ~200mm for holding cotton roll (e.g. Forceps Rochester Pean: Shoof #203 056)
- Dental cotton rolls (e.g. Alan dental rolls No. 2:Global Science)
- Vial to place cotton rolls in (typically 5 ml vial or Sarstedt Salivette)
- Raddle to mark sampled animals

Restrain animal (much like as if about to drench animal)

- Clamp approximately 10 mm of one end of cotton roll in forceps
- While holding sheep under its jaw, insert forceps and cotton roll between jaw and cheek of the animal
- Gently manipulate forceps backwards and forwards by about 20 mm or alternatively gently rotate forceps
- Collect saliva for approximately 10 seconds
- Gently remove forceps from the mouth of the sheep
- Mark animal
- Place cotton roll in labelled vial or salivette
- Keep samples cool during sampling then freeze before the end of the day.