THE CARLA® SALIVA TEST – A NEW TOOL FOR PARASITE CONTROL ON NEW
ZEALAND FARMS

Ian Sutherland, Mike Tate and Richard Shaw
The Hopkirk Research Institute, AgResearch
Private Bag 11008,
Palmerston North 4442, New Zealand

Email: ian.sutherland@agresearch.co.nz
Introduction

In September 2010, the CARLA® Saliva Test was made commercially available to New Zealand sheep farmers. The basis of the test is the quantification of salivary antibodies specific to a carbohydrate larval antigen (CAR_L_A) which is present on all infective-stage larvae of gastrointestinal nematodes infecting livestock (Harrison et al., 2003a). The test has application in identifying animals with enhanced protective response to internal parasite larvae, experimental and field trials show animals with high levels of the antibodies can reduce or prevent larval establishment (Harrison et al., 2003b, 2008) and tend to have lower fecal egg count and better growth under parasite challenge (Shaw et al., in press).

The test may also provide a surrogate measure of larval challenge in ewes, as once protective immunity has developed, antigen levels reflect the larval challenge animals are receiving from pasture.

What is CARLA®?

CARLA® is a molecule found on the surface of all internal parasite larvae (L3s) infecting livestock. It is very tough and able to withstand passage through the rumen. CARLA® is only present for a few days after worms are ingested. Later stages of the worm life-cycle (L4 and adult) do not have the molecule.

What are CARLA® antibodies?

CARLA® antibodies are produced by the sheep's immune system in response to larval challenge. In immune sheep, high levels of CARLA® antibodies are present in saliva and gut mucus; these antibodies bind to CARLA® on the surface of ingested L3 and prevent establishment. This results in fewer adult worms in the gut, which in turn results in less damage to the intestine and the passing of fewer eggs onto pasture. Furthermore, the high responding animals usually perform more productively.

The ongoing challenge for researchers and of course, veterinarians, is to utilise the CARLA® technology to obtain maximum benefits for farmers. This paper will introduce
the major applications for the technology suggested thus far, and discusses how they can be applied on New Zealand sheep farms.

In order to do so, it would be valuable to consider some of the characteristics of the test – each of which has an influence on how it may be utilised.

1. The response is to an antigen found only on parasite larvae – animals which have not been under previous challenge will not have the antibodies in their saliva. This means that young animals i.e. at weaning, are unsuitable for sampling. By contrast, most older animals (e.g. greater than 6 months old) have developed a detectable response.

2. The response is labile and will drop down to undetectable levels within a few days if animals have been put onto e.g. clean pastures such as crop, and are therefore not under challenge.

3. There is no requirement for farmers to extend the period between drenches, as may be the case when faecal egg counts (FEC) is used for selection purposes. Regular or suppressive drenching will have an overall effect on larval numbers, which may slightly delay the development of the response.

4. The CARLA® response can be measured in ram and ewe lambs.

5. The CARLA® response has a more favourable correlation with dags and productivity than FEC.

6. ELISA technology is more automatable and repeatable format than FEC for testing large numbers of samples.

1. CARLA as a selection tool for stud breeders

Studies have determined that while most animals eventually express a detectable salivary CARLA® response, the timing and intensity is highly variable (Shaw et al., in press). Some lambs may develop a detectable response within a month post-weaning, while others, under a similar larval challenge, have continued to respond poorly several
months post-weaning. The observation that this trait was heritable led to the assessment of the CARLA® Saliva Test as a means of selecting animals for breeding purposes.

Individual measurements of salivary CARLA® antibodies, taken 2-6 months post weaning, correlate favourably with subsequent faecal egg output and growth under challenge. Lambs that develop a strong early CARLA® response have 20-30% lower faecal egg count and better growth rates during the late summer and autumn. The trait is heritable (h²=0.3) and has a favourable genetic correlation with FEC (r²=-0.5).

Selection of breeding stock with higher levels of CARLA® antibodies will result in progeny with better protection from larval challenge.

These progeny will tend to have lower faecal egg counts and similar or better performance under high larval challenge than the progeny of unselected animals. Because of the good heritability (30%), substantial year-on-year gain is expected. CARLA®results can be used to identify individual rams and ewes with a better immune response to parasites.

However, the best way to apply CARLA® is within a planned programme that calculates CARLA® breeding values (through Sheep Improvement Limited (SIL)) and which tests a cross-section of the breeding flock (e.g. at least 10 animals per sire used).

Useful results for the CARLA® saliva test can be obtained from lambs between February and June (i.e. 5-9 months of age). The best results are obtained at the peak of larval challenge (typically in late May or June). This is the best time to sample if breeding values are not being used.

Timing is less critical if CARLA® breeding values are being calculated because breeding values from February – June samplings are all highly correlated with each other. The recommended sampling strategy is to check the larval challenge level by sampling 20-30 animals from the flock, approximately a week before the intended flock sampling.
date. These samples are then sent to AgResearch to confirm there has been sufficient larval challenge to deliver good results.

2. **CARLA® as a tool for selecting ewe replacements**

Using the CARLA® Saliva test to select replacement ewes, as well as sires, will maximise genetic progress towards a flock with enhanced protective immunity to parasite challenge. The CARLA® Saliva test allows for easy selection of replacement ewe lambs without affecting productivity (as in FEC selection). There are also preliminary indications that ewes with elevated CARLA® responses have an improved reproductive performance.

3. **CARLA® as a tool for estimating pasture contamination**

The CARLA® response is induced by the presence of ingested worm larvae, so a pasture challenge is required for the antibody to be detectable in saliva. This led to the hypothesis that antibody levels may actually reflect the level of larval challenge from pasture, implying the test could be a useful predictor of pasture contamination.

To test this hypothesis, groups of two-tooth ewes were grazed on replicated paddocks which were assumed to have one of three levels of contamination i.e. very low, medium or high. The sheep rotated around these treatments for several weeks, and were saliva sampled before and after each move. The CARLA® results obtained were then compared with those from traditional pasture-pluck sampling.

In summary, the antibody levels showed a clear differentiation between the treatments, reflecting the relative intensity of larval challenge. Interestingly, if it is assumed the relative levels of contamination were as expected, and the CARLA® results appear to confirm this was the case, then the results obtained from pasture-plucks were highly variable, and were a poor reflection of larval numbers.

The results also highlighted that CARLA® is related to larval intake rather than overall contamination present on pasture: towards the end of the experiment, the antibody levels measured were highest in the animals grazing the ‘very low’ paddocks. It was
assumed this was due to over-grazing, as the pasture cover in these paddocks was significantly less than in the other treatments. This would be expected, as larval numbers are significantly higher close to the ground (Callinan and Westcott, 1986), and is a good example of the benefit of maintained an acceptable sward height where possible.

The logistics of the test would be to use older animals known to express detectable antibody levels: regardless of their recent exposure, sampling after approximately 7 days of grazing the ‘test paddock’ would provide an indication of the level of contamination i.e. if the animals started off high and remained high, or started low and became high, then there is significant contamination. Conversely, low CARLA® levels after 7 days must indicate low contamination.

Potential applications of the CARLA® test as a measure of pasture contamination include the following:

1. As a measure of contamination prior to rotating stock. Heavily contaminated pastures may be more suitable for older animals than young, growing stock.
2. Similar to 1, paddocks with minimal levels of contamination may be suitable for finishing animals.
3. Co-grazing 2-tooth or mixed aged ewes with lamb flocks could determine when the young animals have reached their immune potential i.e. when the hoggetCARLA® response matches the older animal response.

Using the CARLA® Saliva Test in other host species

As the test was developed using research funding from Ovita Ltd., its use is primarily intended to benefit New Zealand sheep farmers. However, given that there is likely to be a high degree of conservation in the immune response to GIN across all grazing animals, preliminary work is underway to assess the utility of the test in cattle, deer and goats. While each of these projects is at an early stage, the early indications are that each species does produce a detectable antibody response, which is variable between
individuals. Deer appear to produce a response which is similar to that observed in sheep. The response in goats is weaker, while cattle tend to be somewhere in between. Where we have been able to look at parasite data, the CARLA® response has been correlated with lower FEC (cattle).

An area which we have not yet investigated is whether the various host species produce an antibody response following challenge with those GIN species which are unable to establish. For example, would cattle express a similar response under challenge with either *Teladorsagia circumcincta* or *Ostertagia ostertagi*? Conversely, would sheep express the response in *Cooperia* spp. which had been cycling in cattle? While interesting scientifically, answering these questions has direct relevance to the use of the test to monitor pasture contamination.

In summary, the CARLA® Saliva Test is proving to be an effective method of selecting ram lambs for breeding purposes. The ‘CARLA trait’ confers enhanced immunity to GIN, resulting in increased productivity and reduced pasture contamination with parasites. Furthermore, the research has not identified any negative productivity factors involved in sheep production.

While sire selection is central to the CARLA® Saliva Test business, the technology platform can be utilised in a number of alternative approaches. Ewe lambs with enhanced immunity can also be selected, enabling farmers to make much more rapid genetic gain if allied to the use of high-CARLA® sires. Furthermore, the characteristics of the response means the test can be utilised to estimate the extent of pasture contamination – a tool which could be of significant value in pasture and parasite management. Finally, while most of the research thus far has been conducted on sheep, the technology is showing significant promise in all of the other major grazing livestock species in New Zealand. What the research team are keen to achieve, however, is a partnership approach with veterinarians and their clients, to ensure the CARLA® Saliva Test is used to its full potential.


