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Report on the use of the CARLA® saliva test in different Australian climatic zones

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Abstract

Breeding sheep for enhanced immunity to gastrointestinal is one potential means of controlling parasites as the rate of anthelmintic failure increases. The CARLA® Saliva test measures a protective antibody response to nematode L3s and can be used to breed sheep that are more resistant to parasite infection.

CarLA specific IgA antibody levels in saliva were measured on a regular basis in 8 Information Nucleus Flocks located across Australia. The aim of the CARLA® testing was to determine for a range of different localities, when good CARLA® responses and thus adequate exposure to larval challenge occurred.

This study showed that good CARLA® responses can be measured in flocks at a variety of different locations. The CARLA® saliva test would be an option for selecting parasite resistant sheep under Australian conditions.

Executive summary

The primary objective of this project was to assess the applicability of the CARLA® Saliva test as an alternative to faecal worm egg counts for making young sire selections in Australian sheep breeds across different climatic zones.

Parasitism with gastro-intestinal nematodes is a significant limitation on animal productivity. Unfortunately, the regular and sustained widespread use of a limited number of drench families to control parasites has led to the development of anthelmintic resistance. In Australia especially, failure of anthelmintics is widespread. The challenge for researchers is to develop new technologies for parasite control that are effective, sustainable, have little if any impact on productivity and are farmer friendly.

The CARLA® Saliva test measures levels of IgA antibody in saliva to the nematode L3-specific surface molecule known as CarLA (carbohydrate larval antigen). CARLA® levels in saliva were measured on a regular basis in 8 Information Nucleus Flocks (INF) located across Australia. Using the information obtained, we were able to follow the development of this protective immune response to nematode parasite challenge in young lambs after weaning and could also assess seasonal changes in parasite larval challenge faced by the flocks at each of the different sites.

The CARLA® responses between and within flocks varied widely. Based on the responses in young lambs and Sentinels, some flocks appeared to have good larval challenge present all year round, e.g. Kirby, Cowra, Rutherglen and probably Katanning. Other sites had low CARLA® responses for most of the year e.g. Trangie, Straun and Turretfield. At these sites CARLA® responses and thus larval challenge appeared to be at their highest levels in June and September.

In young lambs post weaning it took 3-4 months of exposure to larval challenge before CARLA® responses matched that of co-grazing Sentinel animals. This is similar to the length of time it takes for the CARLA® response to develop in lambs in New Zealand sheep breeds.

Potentially the CARLA® saliva test may be of benefit in place of WEC (worm egg counts) testing in Australia as it would allow testing for a parasite resistance trait while also applying drench treatments for controlling parasites, breech soiling etc.

All CARLA® data has been provided to the Sheep CRC for detailed analysis. The results from this will be reported by the Sheep CRC. WEC data was provided to us from Kirby. The WEC – CARLA® correlation was very encouraging at this site.

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1 Background

The CARLA® Saliva test measures levels of IgA antibody in saliva to the nematode L3-specific surface molecule known as CarLA (carbohydrate larval antigen). Extensive research in New Zealand has shown that a CarLA specific antibody response in the intestinal mucosa of sheep prevents the establishment of *Trichostrongylus colubriformis* L3's (Harrison et al., 2003a & b, 2008). CarLA has been found on all the L3s of gastrointestinal parasites infecting livestock in New Zealand although the levels present may differ from one parasite species to another. Measurement of mucosal CarLA specific IgA (and IgG) can be done on saliva samples from sheep. The CarLA antibody response in saliva has been shown to be favourably associated with reduced faecal egg counts and to be moderately heritable (Shaw et al., 2012, 2013).

The CARLA® saliva test has been available as a commercial test for use by sheep stud breeders in NZ since 2010. Over 40,000 sheep have been tested for CARLA® for research purposes or commercially. Currently about 18-20 breeders are using the CARLA® test to test mainly ram lambs and generate estimated breeding values (eBV) for the trait. The CARLA® test offers an alternative to the use of the faecal egg counts (FEC or WEC in Australia) to select animals with improved host resistance to nematode parasite infection. The heritability of the CarLA IgA response is in the range of 0.25 to 0.30 and has a genetic correlation with FEC of approximately 0.5. Because the genetic correlation with FEC is not perfect selection for lowered FEC based on the CARLA® Saliva test would not be as fast as using FEC. However measuring CarLA-specific IgA levels would be a more flexible option than FEC for selection of parasite resistant sheep since animals exposed to larval challenge may be expressing a CarLA IgA response at any time, while elevated FEC is limited to a short period, typically 4-6 weeks post-anthelmintic treatments. Furthermore animals to be tested for a CarLA IgA response can be drenched before the effects of an adult burden begin to limit production as can occur with FEC testing. CARLA® testing may also enable better strategic use of drenches that can assist to control breech soiling and reduce the risk of fly strike. Other advantages include removing the user dislike of faecal sampling, the fact that there is no need to re-sample sheep and the ability to store saliva samples frozen. When larval challenge is high we have often see favourable correlation between CARLA® and productivity traits like weight gain and ability to get pregnant.

The CARLA® Saliva test can be used as a relative measure of larval challenge. Once sheep develop the CarLA IgA response, the level of antibody measured varies depending on the level of larval challenge they are exposed to. This has the advantage of allowing researchers to virtually continuously measure changes in the larval challenge a flock is exposed to. To monitor the larval challenge adult "Sentinel" sheep with a fully developed immune response can be co-grazed with young lambs and their CARLA® response recorded.

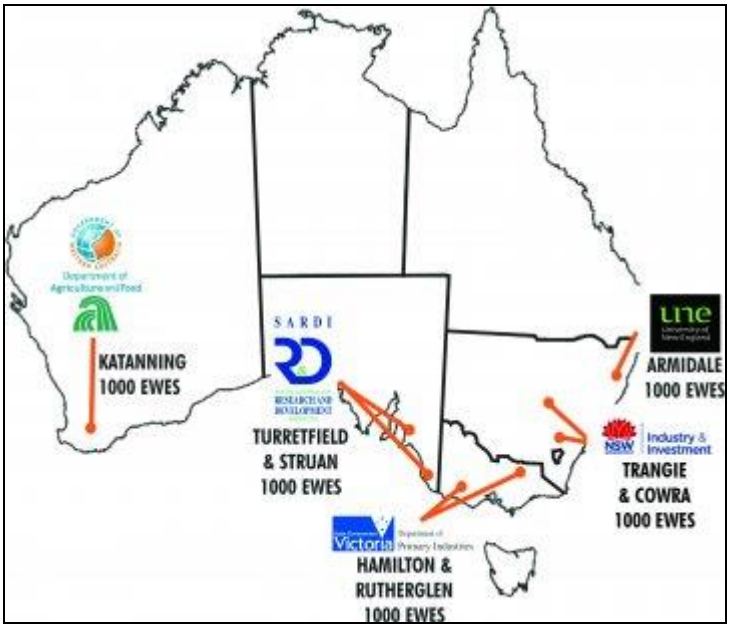
2 Project objectives

This MLA/Sheep CRC/AgResearch study was designed to see if the CARLA® Saliva test could be used under a variety of Australian climatic conditions. The intention was to undertake saliva sampling in the Information Nucleus Flocks at approximately the same time as flocks were sampled for WEC measurement. Monitor samples were also to be collected regularly from 2011 born animals and adult Sentinels co-grazing with the young stock to assess when may be the best times to sample flocks under differing climatic conditions.

3 Methodology

Each of the INF sites was supplied with saliva sampling kits and a set of instructions. Prior to the trial starting, staff at 3 of the sites were also briefed in person (Richard Shaw & Ken Geenty) about the CARLA® saliva test, trial design and sample collection and handling. The aim was to collect samples from 2010 born animals at 15-17 months of age, from 2011 born lambs at or soon after weaning, and then sample these same animals at 15-17 months of age. Ideally saliva samples would be collected at the same time as normal WEC sampling of the sheep (or within the next 4 weeks). Adult Sentinel sheep, typically wethers, would graze with the 2011 born lambs and at 4-6 weekly intervals these would be sampled along with 20 randomly selected lambs. As the costs of sending samples to NZ for CARLA® testing was prohibitive, samples were sent typically in 2 batches over the period of the trial.

Location of the 8 Information Nucleus flocks in Australia



After import into New Zealand, all saliva samples were treated as restricted biological products of animal origin and handled and disposed of appropriately.

Saliva samples were assayed for CarLA specific IgA using the standard assay (Shaw et al., 2012) except that in anticipation of relatively low CARLA® responses

compared to those normally observed in NZ, samples were diluted 1/10 rather than 1/20. The samples were then assayed using 1/10 and 1/25 dilutions and the mean of CARLA® value for each dilution reported. Samples which were above the standard curve range of the assay were diluted further and re-assayed. Individual animal results were reported as units/ml. The assay measured CARLA® levels upwards from ~0.15 units/ml. For this report CARLA® Flock frequencies have been reported in bands of 0.0 to 0.5, 0.5 to 1.0, 1.0 to 5.0 and >5.0 units/ml. These bands correspond to zero or trace, low, medium and high responses respectively.

Regular monitor samples were not collected at all the sites and at some sites WECs were not measured due to flocks not achieving acceptable WEC trigger levels.

4 Results

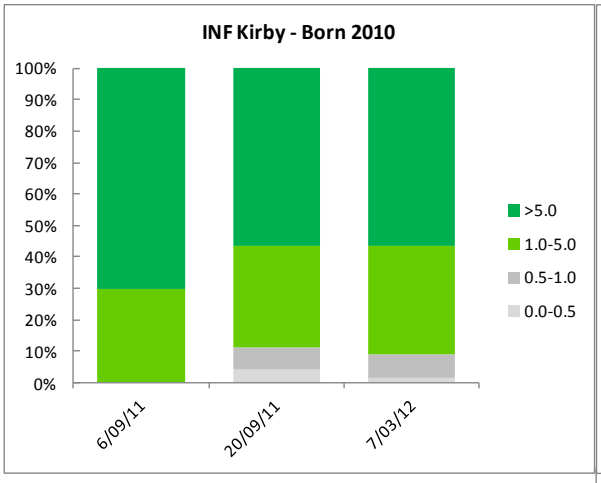
4.1 INF Flock 01/A –Kirby near Armidale in northwest NSW

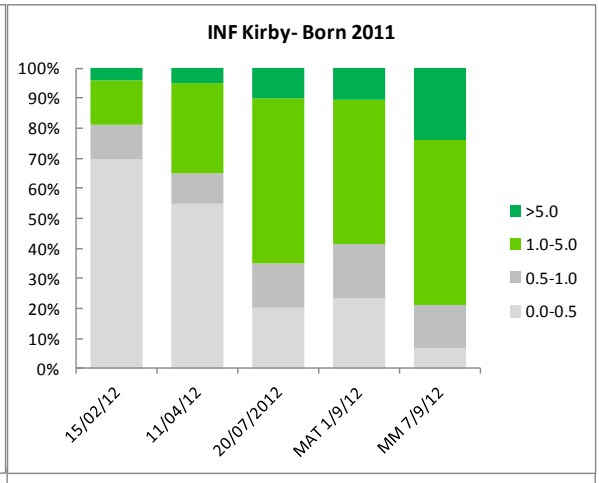
Sampling dates

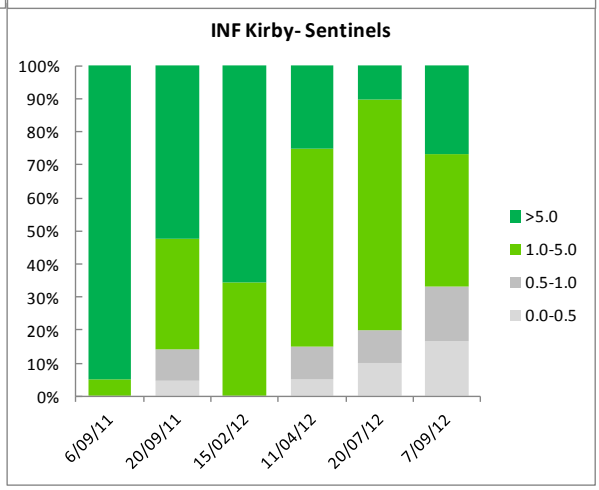
2010 born animals sampled 6/9/11 (Monitor), 20/9/11 (>15 month sampling) & 7/3/12
2011 born animals sampled 15/2/12 (post weaning sampling), 11/4/12 (Monitor), 20/7/12 (Monitor), 1 & 7/9/12 (>15 month sampling)
Sentinels sampled 6/9/11, 20/9/11, 15/2/11, 11/4/12, 20/7/12 & 7/9/12

Results

Graphs of frequency of CARLA® response for different animal classes







The CARLA® responses in the Sentinels suggest that there was a good larval challenge present in this environment at all saliva sampling times. Over the 12 months of monitor sampling the response in the Sentinels did wane marginally suggesting a gradual drop-off in larval challenge over time. This is further supported by the strong CARLA® responses in the 2010 born animals which were at least 12 months of age. The 2011 born animals showed a gradual increase in CARLA® response as their immunity to larval challenge developed and by September 2012 their CARLA® response was similar to the Sentinels.

WEC data was provided for animals at Kirby.

For the 2010 born animals, WEC and saliva sampled in March 2012, animals with greater than 2.0 units/ml of CARLA® (80% of the flock) had on average 57% lower WEC after transformation by loge(WEC+40). Larval cultures carried out with this sampling consisted mainly of 34% *Haemonchus* and 63% *Trichostrongylus*.

For the 2011 born animals, which were sampled for WEC sampling in January 2012 and CARLA® in February 2012, animals with >2.0 units of CARLA® (11% of the flock) had on average 22% lower WEC. Larval cultures carried out in January consisted of 45% *Haemonchus*, 25% *Trichostrongylus* and 30% *Ostertagia*.
At the September 2012 sampling, MAT animals with >2.0 units of CARLA® (38% of the flock) had on average 31% lower WEC. In the MM animals 58% of the flock had >2.0 CARLA® units and had 17% lower WEC. Larval cultures taken at about this time consisted of 60-61% *Haemonchus* and 33-35% *Trichostrongylus*.
In Sentinel sheep WEC was collected at 2 samplings. Sentinels with >2.0 units of CARLA® predicted 58% and 62% reduction in WEC in July and September 2012 respectively.

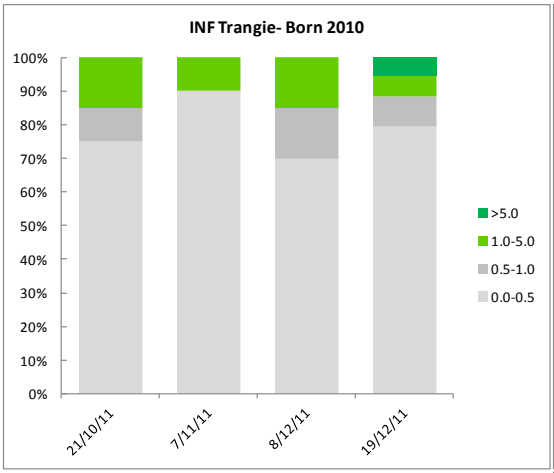
4.2 INF Flock 02/T Trangie – 360 km northwest of Sydney NSW

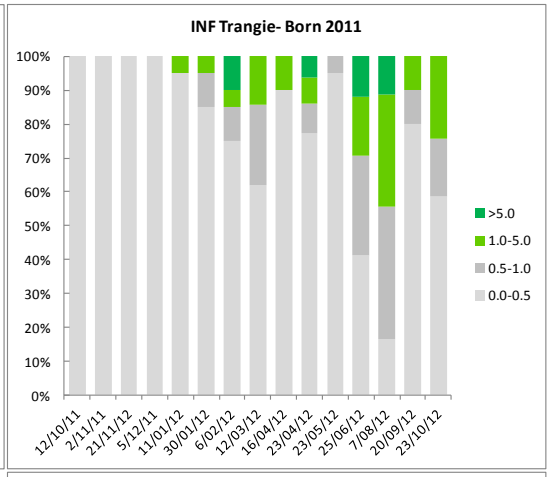
Sampling dates

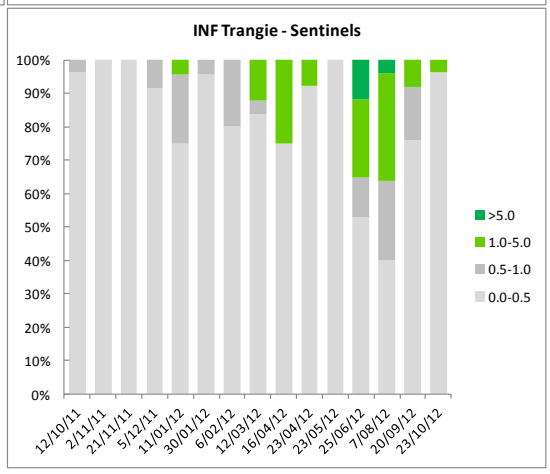
2010 born animals sampled 21/10/11 (Monitor), 7/11/11 (Mon), 8/12/11 (Monitor) & 19/12/11(>15 month sampling)
2011 born animals sampled and Sentinels were monitor sampling from 15 times between 12/10/11 and 23/10/12. All the 2011 born animals were sampled on the 23/4/12.

Results

Graphs of frequency of CARLA® response for different animal classes







In contrast to Kirby, the CARLA® responses at Trangie were relatively weak. The CARLA® responses in the Sentinels suggested that over the sampling period the larval challenge did increase a little. This is further supported by a weak although slightly better response in the 2010 born animals observed in late 2011. The 2011 born animals showed a gradual increase in CARLA® response from February 2012 as their immunity to the larval challenge developed and this matched that of the Sentinels for the most of the monitor samplings.

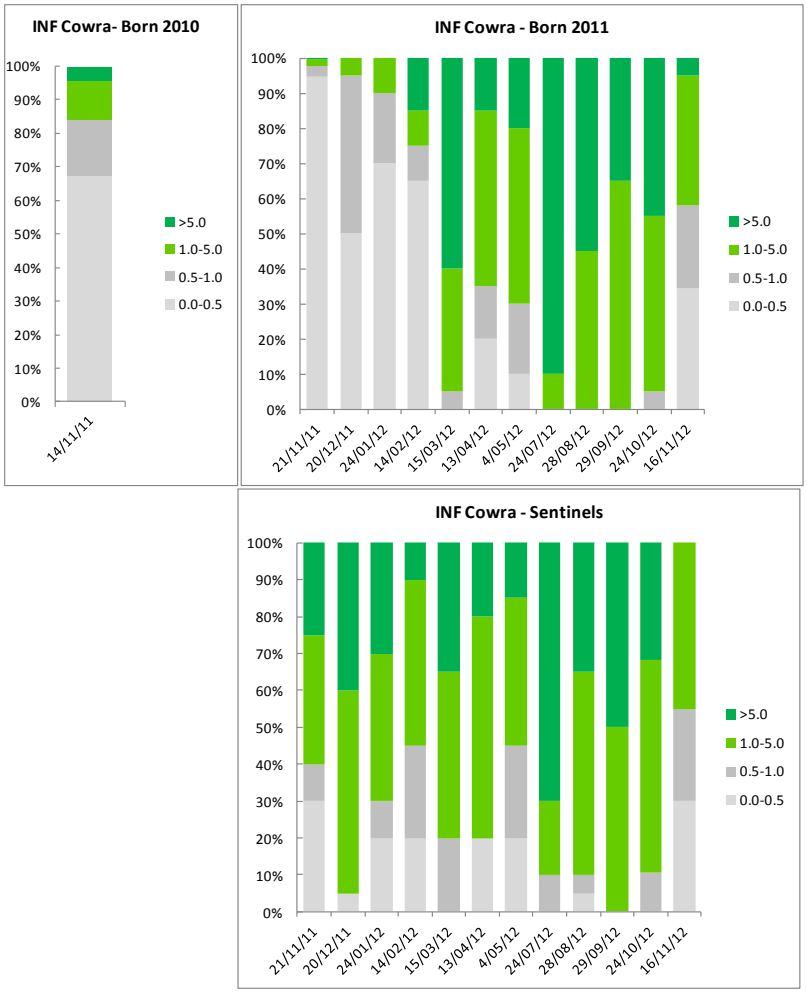
4.3 INF Flock 03/C Cowra – 300 km west of Sydney, NSW

Sampling dates

2010 born flock sampled 14/11/11 (>15 month sampling)
2011 born flock sampled 21/11/11 (post weaning sampling) and 16/11/12 (>15 month sampling), including Sentinels.
In between these dates monitor samplings were taken 10 times from 2011 born animals and Sentinels.

Results

Graphs of frequency of CARLA® response for different animal classes



The CARLA® responses from the Sentinels suggest that there was a good larval challenge present in this environment on most saliva sampling dates. The larval challenge appeared to be strongest from July to October 2012. The 2011 born animals showed a gradual increase in CARLA® response as their immunity to the larval challenge developed and from March 2012 onwards their CARLA® response was quite similar to the Sentinels.

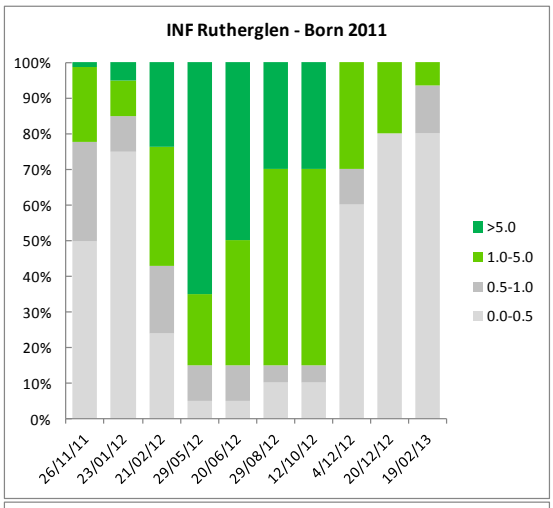
4.4 INF Flock 04/R Rutherglen – 250 km northeast of Melbourne, Victoria

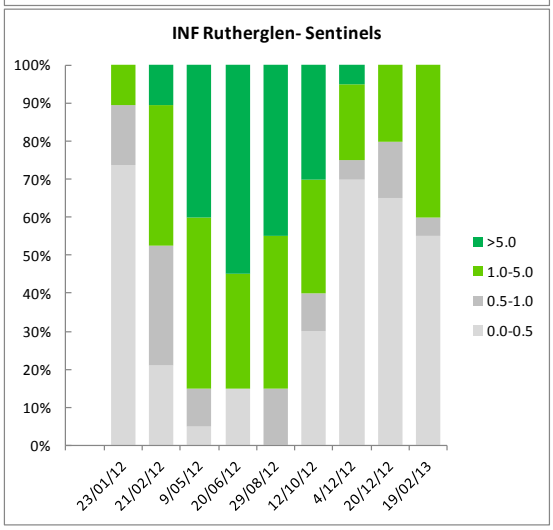
Sampling dates

2011 born flock sampled 26/11/11 (post weaning sampling) and 19/02/13 (>15 month sampling).
In between these dates monitor samplings were taken 8 times from 2011 born animals and Sentinels.

Results

Graphs of frequency of CARLA® response for different animal classes





The CARLA® responses from the Sentinels suggest that there was a good larval challenge present in this environment from May to October 2012. The 2011 born animals showed a gradual increase in CARLA® response as their immunity to larval challenge developed and from May 2012 onwards their CARLA® response was quite similar to the Sentinels. From December 2012 to the last sampling in February the CARLA® response in both the 2011 born and Sentinel animals was noticeable lower then through the winter months.

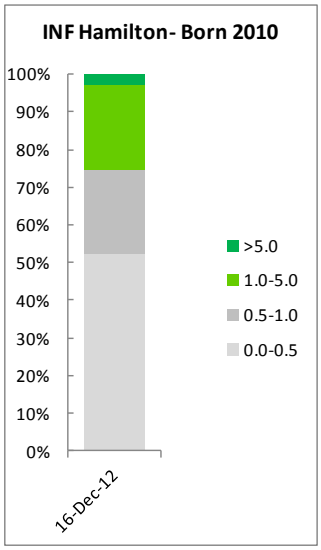
4.5 INF Flock 05/H Hamilton – 260 km west of Melbourne, Victoria

Sampling date

2010 born animals sampled 16/12/12 (>15 month of age sampling)

Results

Graphs of frequency of CARLA® response for 2010 born animals



There was a moderate CARLA® response in the 2010 born animals at 15-17 months of age.

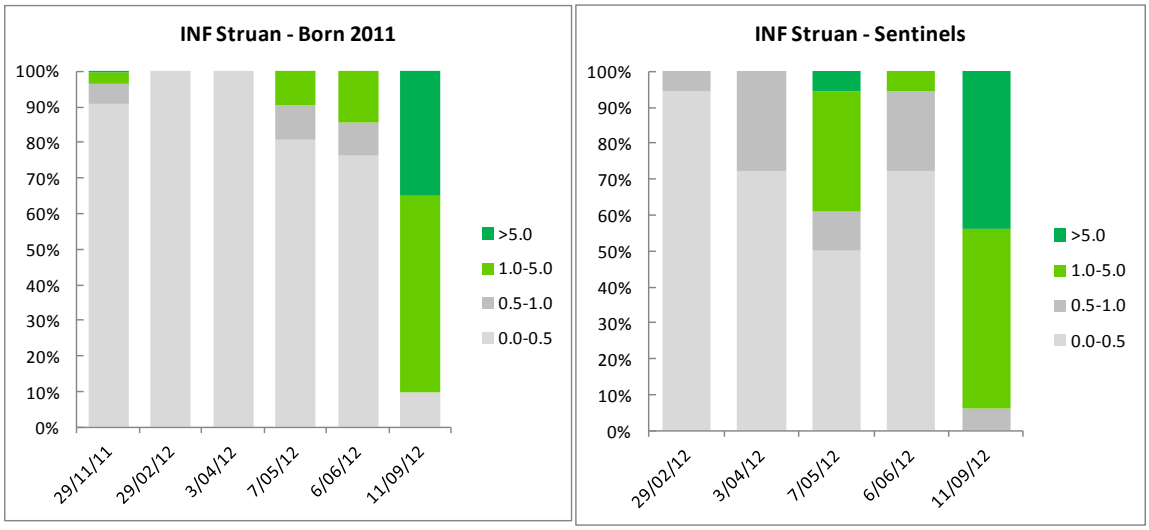
4.6 INF Flock 06/S Struan – 300 km southeast of Adelaide, South Australia

Sampling dates

2011 born animals sampled 29/11/11 (post weaning sampling).
Monitor samplings were taken 5 times from 2011 born animals and Sentinels.

Results

Graphs of frequency of CARLA® response for different animal classes



From February to June 2012 the CARLA® responses at Struan were low. The strong CARLA® responses seen in both the 2011 born and Sentinels in September suggested that between June and September climatic conditions changed so as to be favourable for the development of a good larval challenge. By September 2012 the CARLA® response of the 2011 born animals was similar to the Sentinels.

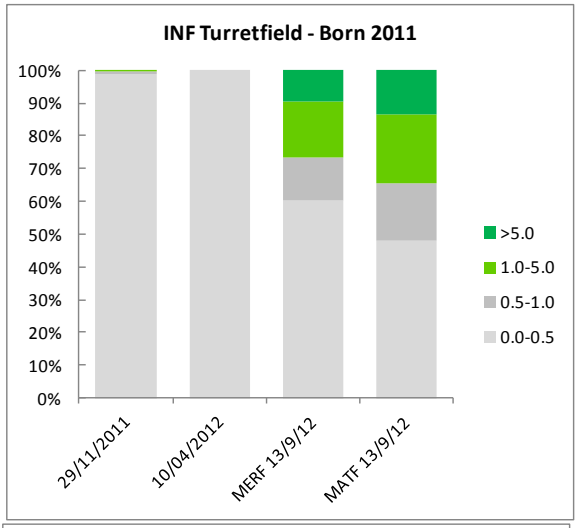
4.7 INF Flock 07/F Turretfield – 50 km north of Adelaide, South Australia

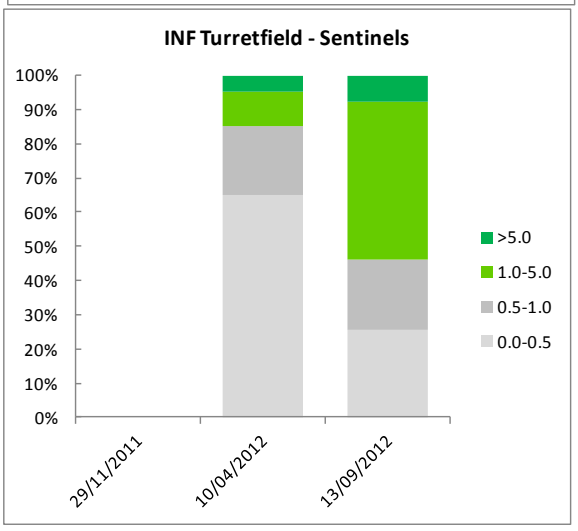
Sampling dates

2011 born animals sampled 29/11/11 (post weaning sampling) and 13/9/12 (>15 month of age sampling). Monitor samples were taken in April 2012 from 2011 born animals only. Monitor samples were taken from Sentinels in April and September 2012.

Results

Graphs of frequency of CARLA® response for different animal classes





With the limited number of monitor samples taken it is difficult to draw any strong conclusions from these results. In November 2011 either there was insufficient larval challenge or the 2011 born animals were too young to have developed any CARLA® antibody response. By April 2012 the 2011 born animals still had not developed any CARLA® response however the sampling of the Sentinels did suggest that a reasonably larval challenge was present. By September 2012 the 2011 born animals had developed a good CARLA antibody response.

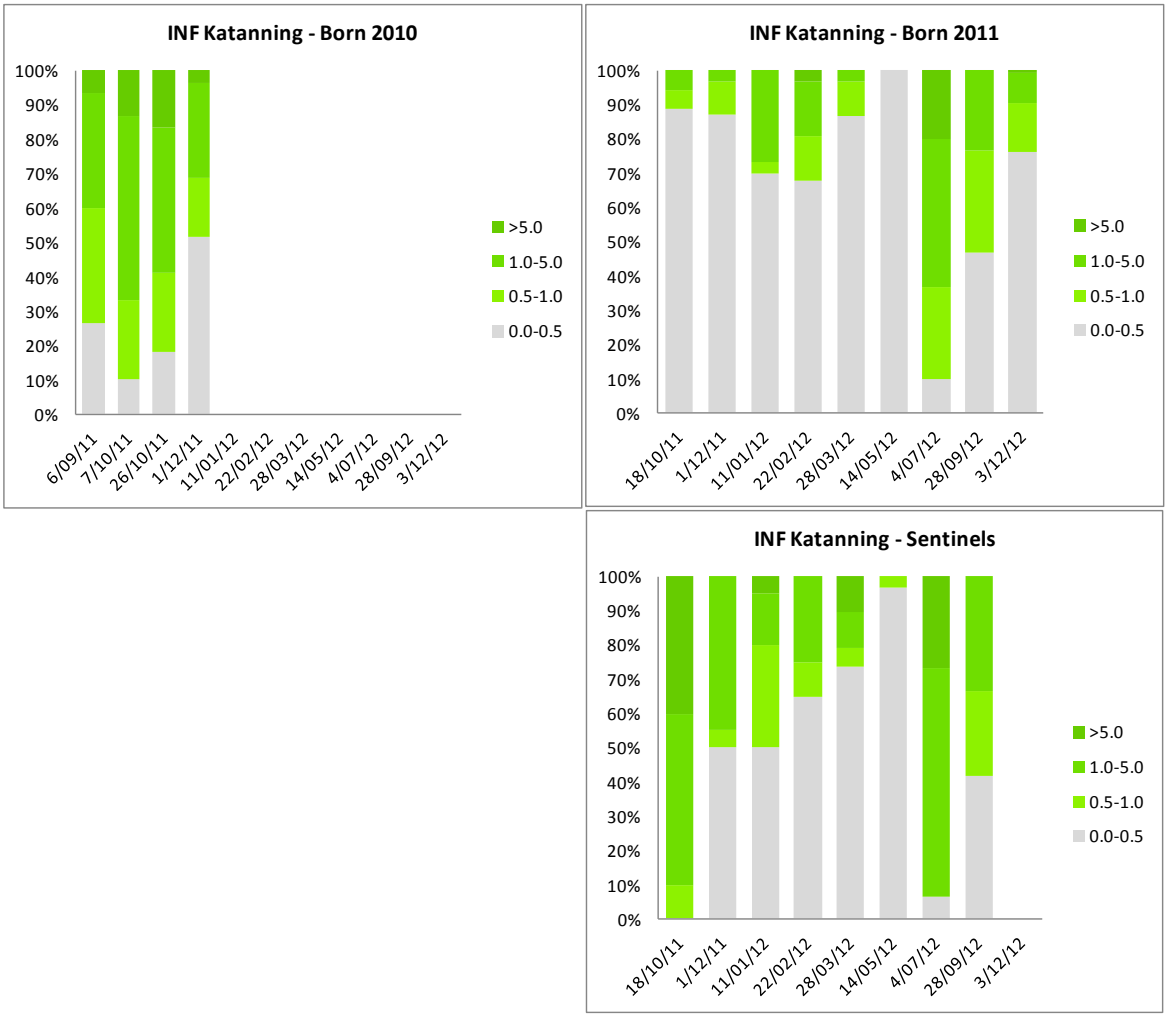
4.8 INF Flock 08/K Katanning – 300 km southeast of Perth, Western Australia

Sampling dates

2010 born animals monitor sampled on 6/9/11, 7/10/11 and 1/12/11 and flock sampled 26/10/11 (>15 month sampling).
2011 born animals were flock sampled on 18/10/11 (post weaning sampling) and 3/12/12 (>15 month of age sampling). In between these samplings there were 7 monitor collected. In between these dates monitor samplings were taken 8 times from 2011 born animals and Sentinels.

Results

Graphs of frequency of CARLA® response for different animal classes



The CARLA® responses towards the end of 2011 in the 2010 born animals and the Sentinels suggest there was a good larval challenge peaking around October 2011. Based on the response in the Sentinels the CARLA® response and thus presumably the larval challenge waned through to May 2012. However by July 2012 the CARLA® response increased markedly from May in both the born 2011 animals and Sentinels.

5 Discussion

The CARLA® responses between flocks varied widely. Based on the CARLA® responses in young lambs and Sentinels, some flocks appeared to have good larval challenge present all year round, e.g. Kirby, Cowra, Rutherglen and probably Katanning. Other sites had low CARLA® responses for most of the year e.g. Trangie, Straun and Turretfield. At these sites CARLA® responses and thus larval challenge appeared to be at their highest levels in June and September. Only one sampling occasion occurred at Hamilton.

In young lambs after weaning it took around 3-4 months of exposure to larval challenge before CARLA® responses matched those of co-grazing Sentinel animals. Over the months post weaning the CARLA® response gradually increased. This is similar to the length of time it takes for the CARLA® response to develop in lambs in New Zealand sheep breeds and conditions. In dry seasons when larval challenge is limited, the CARLA® response may take longer to develop. The CARLA® responses at Trangie and Straun perhaps offer examples of delayed development of the CARLA ®response presumably due to dry summer/autumn conditions.

Analysis of trial data is being carried out by the sheep CRC, however some WEC data was provided (Kirby) and the association between the CARLA® response and WEC at this site was very good and similar to that which AgResearch has observed in New Zealand flocks, i.e. young animals with a CARLA® response greater than 2.0 units/ml had approximately 20-31% lower WEC. In older animals e.g. 2010 born and Sentinels, animals with greater than 2.0 units had approximately 60% lower WEC. The main parasite species present in larval cultures and presumably stimulating the CARLA® antibody response were *Haemonchus* and *Trichostrongylus*.

If the CARLA® saliva test is considered to be of practical use in the Australian environment then it is likely a commercial veterinary testing laboratory would need to be contracted to provide a CARLA® testing service there. Due to cost and practicability issues, sending saliva samples to New Zealand for processing is not an option. One potential limitation will be the practicability of carrying out monitor saliva testing before full flock testing given the long distances and potentially time it would take for monitor samples to get to testing labs.

6 Conclusions

The monitor program as reported here showed that testing for CARLA® can be carried in most of the different climatic environments tested as part of this trial. In presumably drier environments CARLA® testing may be limited to winter months when conditions are more suitable for larval challenge to develop to reasonable levels. Some of the sites used in this trial were not able to do full flock WEC testing for the same reason. One potential advantage the CARLA® may offer under Australian conditions is the ability to test for a parasite resistance trait while using drench treatments to restrict parasite induced breech soiling and thus reduce fly strike issues.

The detailed statistical analyses of this trial's data (being carried out by the Sheep CRC) will however be more important in determining the usefulness of the CARLA® under Australian conditions.

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