Field Trials of a Spotty Liver Disease Vaccine

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Spotty Liver Disease (SLD) has occurred for decades in Australia and possibly over the world, with *Campylobacter hepaticus* being the bacteria identified only recently as the causal agent (Crawshaw et al., 2015; Van et al., 2016; Van et al., 2017; Kotiw et al., 2018). The disease appears to largely affect layer chickens of free-range or barn systems. SLD can affect any age flock during its first occurrence on a farm, although once established on a farm it occurs almost exclusively in flocks at peak of lay. Egg production can decrease in the flock by 10-35%, and mortality increase by 10-15% (Crawshaw and Young, 2003; Burch, 2005; Grimes and Reece, 2011), although these extremes are not always the case. Dead and sick/cull birds have characteristic small, white-grey and/or red spots on the liver, which can also be swollen with a fibrin covering. Ova are usually always inflamed to some extent (Figure 1).

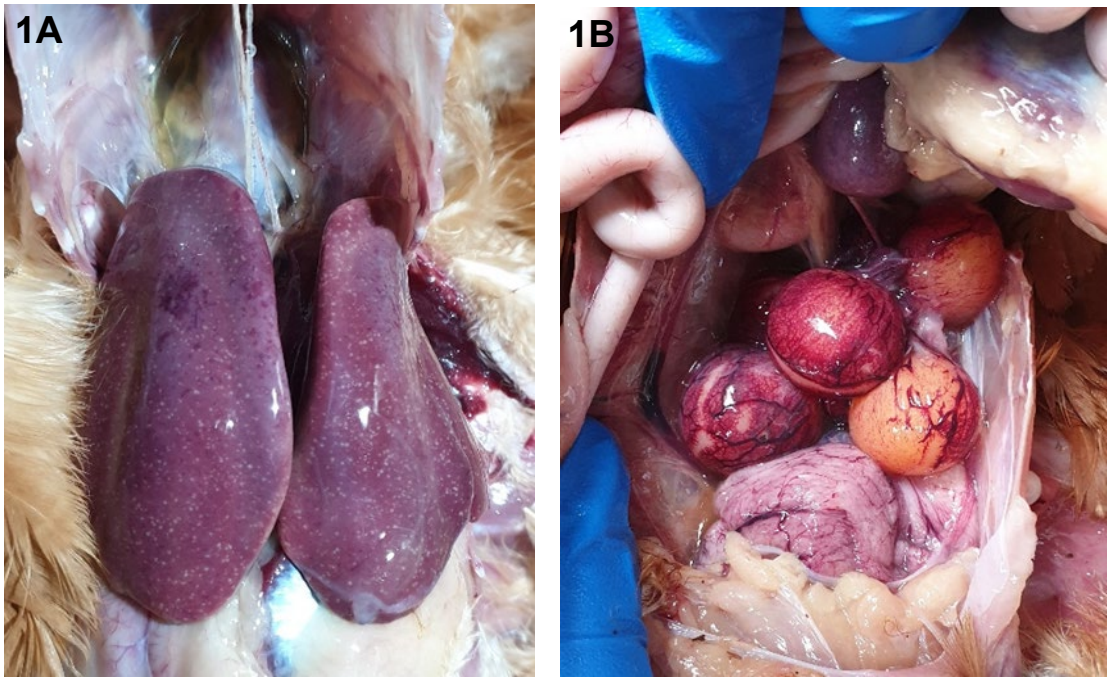

Figure 1A: Abnormal liver from a layer chicken infected with SLD. Note excess pericardial fluid, hepatomegaly, and fibrin at the base of the liver lobe. 1B: erythematous ova of a layer that died of SLD.

The financial cost of this disease is significant and can vary from flock-to-flock. Across 55 recent SLD outbreaks in peak-of-lay birds of flocks in Australia, the disease has been calculated to cost between $0.5/bird in the flock to $4.29/bird in the flock. This is $5,000-$42,900/10,000 birds. The largest factor identified to affect cost of the disease was mortality; as dead birds obviously don't lay eggs for the rest of the flock life. Other factors calculated in cost of the

disease were eggs lost during the egg production drop, costs of in-feed or in-water additives trialled to prevent SLD, and any treatments given to the flock. Extra labour costs were not calculated in these figures, but feed savings of dead birds were. Interestingly, across the vast number of in-feed and in-water additives trialled (probiotics, prebiotics, acids, clays, essential oils and others), none prevented, or significantly reduced the mortality associated with SLD in flocks. The effect of additives was somewhat hard to analyse, as mortality from SLD was always lower in the cooler months of winter.

The above-mentioned financial cost of the disease, along with the welfare implications, antibiotic use and effect on staff general well-being, easily justifies the effort required to undertake vaccine trials against SLD in Australia.

VACCINE TRIAL 1: Small scale Trial

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45 Hy-Line brown commercial pullets of 16 weeks of age were used for the vaccine dosage and challenge trials. All chickens were pre-screened for *C. hepaticus* via PCR of rectal swabs and leg banded and wing tagged for identification.

Chickens were housed in four climate-controlled rooms, with groups of birds being:

- 9 x negative controls (no vaccination or challenge)
- 9 x killed vaccine A + *C. hepaticus* oral challenge
- 9 x killed vaccine B + *C. hepaticus* oral challenge
- 9 x killed vaccine C + *C. hepaticus* oral challenge
- 9 x positive controls (vaccine adjuvant only + *C. hepaticus* oral challenge)

Chickens were vaccinated at 16 and 18 wks of age, and challenged at 21 wks of age. No dead or morbid birds occurred during the trial. All chickens were culled and sampled 13 days post challenge. Livers were scored between 0-5 for gross pathological lesions, with 5 being comparable to that of an SLD dead bird in a field outbreak. Bile and liver samples were cultured on sheep blood agar and in Bolton broth. Bile, liver, duodenal, jejunal, caecal and rectal samples were tested for *C. hepaticus* on PCR. Liver samples were collected in formaldehyde for histopathology.

Of the positive control birds, 6/9 (67%) had gross pathological changes on the livers, consistent with various stages of SLD. These livers were scored between 1 and 4, with examples shown in Figure 2. Of the 27 vaccinated birds, only 2 birds from one of the vaccine groups had gross pathological changes consistent with SLD. These livers were both given score 1, and are shown in Figure 3.

On culture, positive control and vaccinated bird groups had some amount of growth from the bile and liver, with bile being most fruitful. Growth from the bile of the positive control birds was significantly higher than that seen with two out of the three groups of vaccinated birds. Negative control birds had no growth.

On PCR, birds from each of the positive control and vaccinated groups had *C. hepaticus* present in all organ samples: bile, liver, duodenum, jejunum, caecum and rectum. Statistically, the positive control group had significantly more of the organism in the caecum than two of the vaccine groups. The negative control birds had no *C. hepaticus* identified in organs at all.

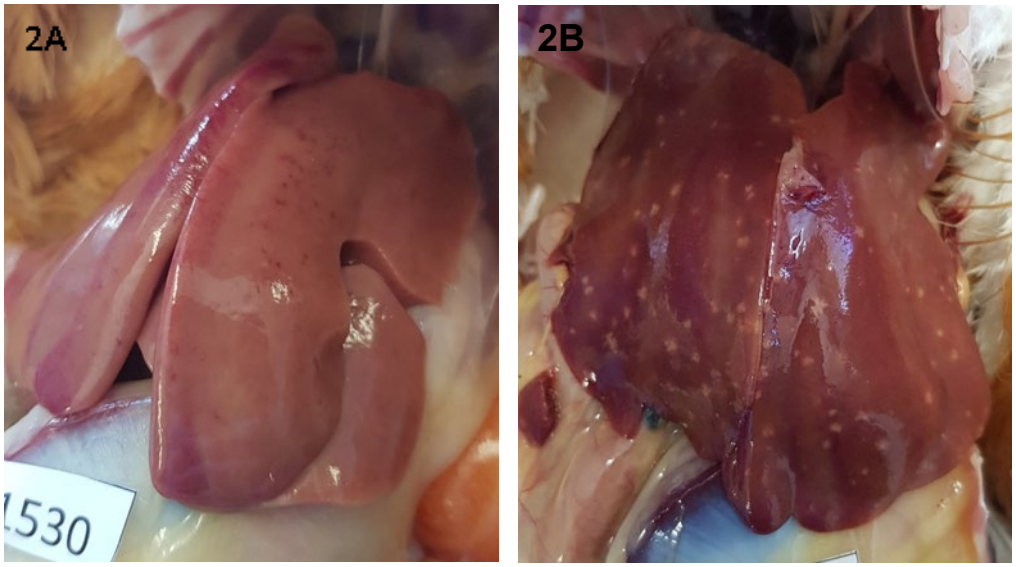

Figure 2: Examples of gross pathological findings of positive control chickens. The liver on the left is score 2, and the liver on the right is score 4.

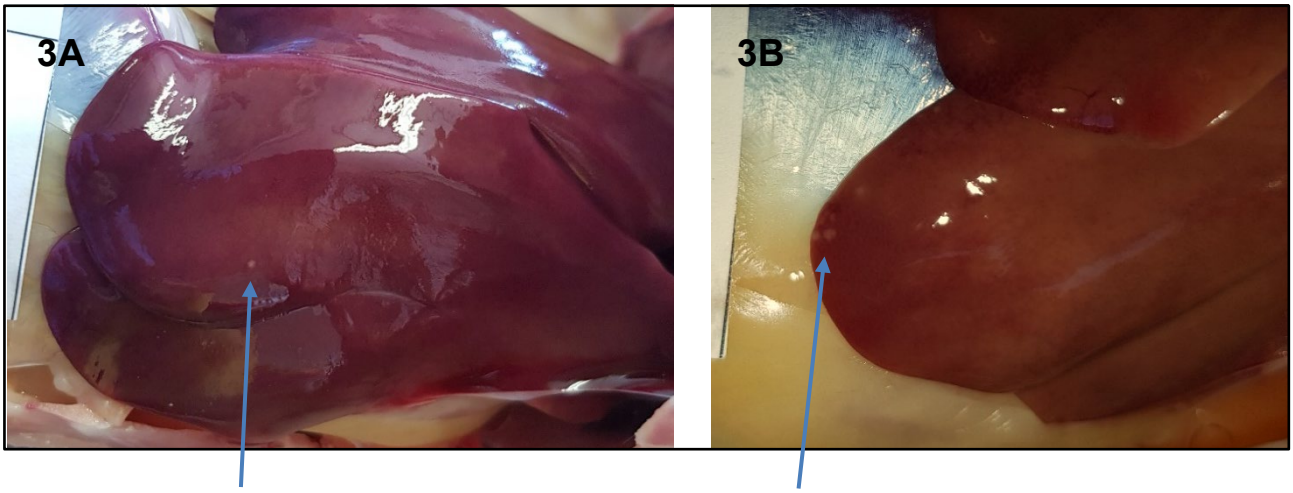

Figure 3: Gross pathological findings of the two vaccinated birds. Arrows denote minor changes

Microscopically, eight out of nine liver samples from the positive control group had inflammatory lesions, ranging from mild to classical SLD lesions. All liver samples from the negative controls and vaccinated birds had normal histopathology.

In conclusion, results of this small-scale vaccine trial have warranted further trials in the field/commercial setting. The vaccine adjuvant used needs to be approved by APVMA to progress.

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VACCINE TRIAL 2: Commercial Field Trial

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In an aviary rearing shed of 40,000 chickens, 20,000 birds were vaccinated twice at 12 and 16 weeks of age with an autogenous *C. hepaticus* killed vaccine. The isolates used to prepare this vaccine were derived from virulent outbreaks of SLD at the free-range farm where the trial took place, and where SLD occurs in every flock around peak of lay. The remaining 20,000 birds in the rearing shed were left as unvaccinated controls.

All rearing birds from this shed were shifted to the layer house at 16 weeks of age. Half the birds were on one side of the shed, and half on the other, with mesh separating the two. Egg production and mortality were recorded separately for the two groups. Birds were given access to the range at 20 weeks of age.

The first signs of SLD occurred in the shed at 27 weeks of age. Given the size of the shed, the outbreak lasted for 6 weeks, when production recovered to standard and mortalities from SLD stopped.

Final figures from the outbreak were that both vaccinated and unvaccinated decreased in production by 11% and then recovered, and mortality in the two groups from SLD was 3.09% and 3.67%, in favour of the control birds. Hence, in this case, the vaccine was not deemed to protect the birds from SLD. Interestingly though, serology showed that in the weeks after being vaccinated, all the birds, both vaccinated and unvaccinated, seroconverted.

This trial is currently being repeated in another flock, where, at the time of publication of this document, it is too early to report results.

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