SPOTTY LIVER DISEASE

Progress in pathobiology and genetic epidemiology of *Campylobacter hepaticus* the aetiological agent of Spotty Liver Disease

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A. Refresher on our past research findings on spotty liver disease

1. Disease progression and recurrence in a single flock

It has been our experience that following an initial outbreak of SLD, which may affect sexually mature layers of any age, the disease usually, becomes recurrent in replacement flocks with outbreaks consistently occurring in the characteristic peak of lay period. The disease generally persists on a property, even in the face of total flock replacements, application of biosecurity measures and a variety of management interventions. We have rarely seen recurrence of SLD within a single flock and these episodes tend to be within a few weeks of an outbreak that has generally been treated with antibiotics. We suggest that SLD recurrence in such early episodes may be related to the pace of the disease spreading through relatively large flocks, or perhaps due to incomplete effective treatment of the flock (which could be related to the mode of antibiotic delivery). However, SLD in our experience does not appear to recur over longer periods within a single flock, which suggests that a protective immunological response may be induced by the initial infection, and consequently suggests that vaccination may be an effective means for preventing or reducing the impact of the primary infections at point of lay poultry.

2. Potential mechanisms for disease persistence

We have demonstrated that the microscopic morphology of *C. hepaticus* bacteria alters from the characteristic vegetative vibrioid form to a more inert coccoid form. It appears that the main factors that induce the change in morphology are the age of the bacterial culture and exposure to less hospitable conditions such as atmospheric levels of oxygen. We have found in laboratory conditions that older bacteria or repetitive sub-culturing of the organism induces it to change to the coccoid shape and eventually we fail to be able to grow the bacteria. Coccoid forms in campylobacter (including *C. jejuni)* have been previously described, *with* some suggestion that it represents a degenerative form of the bacteria and so may account for the loss of culturability in laboratories. However, it seems more probable that the coccoid form acts in a manner similar to bacterial spores and allows *C. hepaticus* to survive and persist in a hostile environment, whilst the vibrioid form (which is very susceptible to exposure to atmospheric oxygen) is the vegetative and disease-causing phase of the bacteria.

3. Methods for bacterial isolation and species confirmation

C. hepaticus is a difficult bacterium to isolate, keep alive and store (which may be the reason that US cultures of 'vibrioid' organism were lost following frozen storage in the 1950s and 1960's. In a laboratory, the microbe is best grown at 41^{o}C to 42^{o}C in a micro-aerobic atmosphere (1, 2). In fact, prolonged exposure to normal air appears to inhibit growth of the bacteria. Laboratory cultures are generally slow growing and may need to be incubated for up to 7 days (whereas the more common *Campylobacter jejuni* usually grows within 2-3 days). This unusually long incubation period may account for the reason why earlier attempts to isolate a bacterial cause of SLD failed (1, 2, 3). There are laboratory techniques that can assist isolation of *C.hepaticus* and includes initial growth in enrichment media. This procedure is often used to select for *C.hepaticus* and suppresses other competing bacteria that may be present. There are a few rapid laboratory tests that if positive suggest that the suspect bacterial colony is likely to

be a campylobacter. These tests include microscopically seeing the typical vibrioid shape, as well as a couple of biochemical tests (catalase and oxidase). However, confirmation is best achieved by using DNA identification, which can usually be completed in one day. The test we most commonly use is the Polymerase Chain Reaction (PCR), which is very sensitive, as primers used are very specific for *C.hepaticus*. We often include DNA from *C.jejuni* and *C.coli* as controls in the PCR assay to ensure the accuracy of our test.

4. **Preliminary findings on the stability and other genetic information about *C. hepaticus***

There is emerging evidence that there is genetic variability between *C.hepaticus* strains (3,5), as well as anecdotal evidence that there is variability in the virulence of disease outbreaks. The application of such comparative bacterial genetic analytical techniques on *C.hepaticus* isolates has enabled us to report some very preliminary data relating to: the stability and or variability of *C.hepaticus* isolates in a temporal manner; in relation to strain commonality between different properties; and potential variability in virulence. We have very preliminary data that suggests both genomic commonality and variability for isolates from distant spaced properties genetic variability over the years within single properties, as well as between separate properties. We have also detected a genetic difference in an isolate from a putative avirulent outbreak. Further genomic analysis with particular emphasis on the epidemiology of SLD outbreaks is continuing.

B. Recent research findings

a. Infectivity and persistence of C. hepaticus

We have shown data, which suggests that following an outbreak of SLD in a flock that subsequently the entire flock becomes infected with *C. hepaticus,* although the layers remain without any evidence of overt disease, suggesting that the birds have become asymptomatic carriers of the bacteria and that a possible immunological response has prevented recurrence of invasive disease.

b. The coccoid form of *C. hepaticus* appears to be the means by which the bacteria persists in the inert environment and the main mechanisms of SLD transmission.

***In vitro* data**: We have performed *in vitro* experiments that can induce vibrioid (vegetative) forms of *C. hepaticus* to transform into coccoid forms and reverse the process by resurrecting the coccoid forms of the bacteria back to the vibrioid form.

***In vivo* data**. We have recently demonstrated that following an oral challenge of sexually mature layers coccoid *C. hepticus* induced onset of SLD and that *in vivo* the bacteria reverted to the vibrioid form. This data strongly suggests that the inert coccoid form is a survival mode of the bacterium in the inhospitable inert environment when ingested by the layer is induced to revert to the vegetative (potentially invasive) form of the bacteria.

c. Epidemiological tracking of C. hepaticus as sources SLD outbreaks and environmental reservoirs of the bacterium

We have demonstrated by fingerprinting of genomic DNA, the existence of at least 2 genetically different clades of *C. hepaticus* that may be associated with recurrent outbreaks of SLD on a single property or in different properties within SE Queensland. One particularly prominent clade was detected on multiple farms in SE Queensland and on a property in NSW

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