



Analytical epidemiology of Spotty Liver Disease

Final Project Report | AUGUST 2022

A report for Australian Eggs Limited by
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Australian Eggs Limited Publication No 1BS004

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ISBN 978-1-920835-90-3

Project Title: Analytical epidemiology of Spotty Liver Disease

Australian Eggs Limited Project Number: 1BS004

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Published in March 2026

Foreword

This project was conducted to improve the understanding of the epidemiology of Spotty Liver Disease in cage-free layers in Australia, and to identify key determinants for its occurrence and control.

This project was funded from industry revenue, which is matched by funds provided by the Australian Government.

This report is an addition to Australian Eggs Limited's range of peer reviewed research publications and an output of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Acknowledgments

The assistance of farm owners, managers and consulting veterinarians in the collection of the data and in some cases, samples, for the surveys is gratefully acknowledged. The survey questionnaire was extensive and involved much time in completion and we are grateful for the generosity of time of the co-operators. We cannot name them under the necessity for confidentiality promised for the conduct of the survey.

Australian Eggs Limited provided the funds that supported this project.

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Abbreviations

ACTH	Adrenocorticotrophic hormone
AGP	Alpha-1 acid glycoprotein
APP	Acute phase protein
AVH	Avian Vibrionic Hepatitis
Bn	Barn
CC	Conventional cage
CGB	Corticosteroid- binding globulin
CH	<i>Campylobacter hepaticus</i>
cm	Centimetre
CPM	Counts per minute
CNS	Central nervous system
CRF	Corticotropin releasing factor
d	Day
FC	Furnished cage
FR	Free range
gm	Gram
h	Hour
ha	Hectare
HD	Hen day
HHP	Hen housed production
H/L	Heterophil:lymphocyte ratio
HPA	Hypothalamic-adrenal-axis
GR	Glucocorticoid receptor
km	Kilometre
m	Metre
MR	Mineralocorticoid receptor
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
POL	Point of lay
PVN	Paraventricular nucleus
RIA	Radioimmunoassay
SLD	Spotty Liver Disease
SEM	Standard error of the mean
TI	Tonic immobility
wk	Week

Executive Summary

While some epidemiological work has been conducted on Spotty Liver Disease (SLD) since the identification of the causative organism, this has been of the descriptive kind only. Descriptive epidemiology seeks to examine factors associated with the host, the causative organism and the environment – in effect to describe the disease’s natural history: i.e. the who, when and where of the disease’s occurrence. Analytical epidemiology, on the other hand, involves the application of surveys and observational studies in the actual population at risk. The major purpose of analytical epidemiology is to provide information on which “rational decisions for the prevention and/or control of disease in animal populations is based” (Martin et al. 1987).

Analytical epidemiology aims to identify risk factors for the occurrence of the disease in question (i.e. factors that are statistically associated with the occurrence or absence of the disease in each flock). Some factors may be beyond the ability of the farm to control (e.g. weather). Factors that can be modified by farm management are called ‘key determinants’. The objective of this project was therefore to identify as many ‘key determinants’ as possible from the data obtained from three surveys conducted in commercial free range and barn layer flocks across Australia.

Survey 1 observed rearing flocks and recorded as much information as possible on management, shed and environmental conditions. The rearing flocks were then followed through the production phase in Survey 2. A combined analysis was then conducted across these two surveys, searching for risk factors for SLD occurrence that may have been operative in rearing or in lay. Laying flocks were assigned to a category of ‘Case’, where clinical SLD was observed to occur (occurrence of clinical and postmortem signs typical of SLD, and an associated egg production drop prior to 40 weeks of age) or ‘Control’ where no clinical appearance of SLD was experienced. Presence of *Campylobacter hepaticus* in all flocks was assessed by PCR detection from cloacal swabs, pooled faeces and dust in sheds in some flocks.

Of interest, during the survey it was observed that some flocks that were classified as Controls had detectable *C. hepaticus* in their cloacal swabs, flocks could break with SLD prior to release to range or in barn sheds, and a brand-new site that had never had chickens before broke with SLD in its first flock. These observations add information to understanding SLD as not simply just due to the presence of *C. hepaticus* in the flock or environment, and that the range area is not necessarily the major source of exposure to this organism.

Thirty-two rearing flocks were enrolled, and this resulted in 24 laying flocks being followed through, as some layer flocks received birds from a combination of smaller rearing flocks. Twenty-nine factors in rearing (12 categorical and 17 continuous data variables) were considered along with 30 factors in lay (15 categorical and 15 continuous variables). An immediate finding was that all 13 flocks that had a scratch area in the shed (i.e. had only a partial slat covering of a section of the floor) experienced SLD, while only five out of 11 flocks with a fully slatted floor experienced SLD.

This total SLD occurrence in scratch area sheds confounded study of any other factor in such flocks. However, it did identify having a scratch area as a strong risk factor for SLD occurrence. This makes biological sense as SLD is transmitted via the faecal-oral route, and a scratch area provides much more direct access for birds to faecal material than does a fully slatted shed.

Further analysis was restricted to fully slatted sheds in Surveys 1 and 2 but this was limited by the now low sample size (11 flocks only). Some indications of the contribution of some flock factors were suggested but full confidence in these observations was not possible due to the restricted sample size

causing strong correlation between most of the factors present. Factors that were possibly involved in increased risk for SLD included a natural ventilation system in lay (as opposed to tunnel ventilation), smaller flock size (this also related with ventilation system), breed (also correlated with flock size and ventilation system) and nest brand.

Survey 3 was thus undertaken to search for more putative factor involvement for the risk of SLD in fully slatted sheds. Forty-nine fully slatted sheds were enrolled in Survey 3, and selected observations were recorded for each shed and analysed across the occurrence or absence of SLD (as defined for Survey 2). Thirty-three factors were evaluated (11 categorical and 22 continuous variables) across the occurrence or absence of clinical SLD. A strong risk of SLD was identified for sheds having natural ventilation compared to tunnel ventilation. This supported the preliminary indication seen for this factor from Surveys 1 and 2. It has also been recently reported that lowering shed temperature at the onset of an SLD outbreak could reduce the severity of the condition. This would obviously be more possible with a tunnel ventilation design. The finding from Survey 3 goes further in suggesting that the better environmental control possible with tunnel ventilation may be protective against an outbreak in fully slatted sheds. Further considerations were conducted after removing the tunnel ventilated sheds from the database. Forty-one flocks remained within this section of the study. Several factors were identified for closer study, and a multiple logistic regression analysis was applied to enable consideration of multiple effects of combined factor presence, confounding between these and interactions between the significant factors. Three factors contributed to the most parsimonious model developed, being the allocation of nest space, shed stocking density and the time between birds arriving at the layer farm and the flock reaching 60% Hen Day production (HD). Nest space allowance and shed stocking density were confounded as sheds with more nest space had less useable floorspace. Statistically, the stocking density estimation did not fit the data well and nest space allowance was a more significant effect. Therefore, we observed that higher numbers of birds per nest space was a significant risk factor for the occurrence of SLD in fully slatted, naturally ventilated layer houses. From the data available in the survey, a recommendation of a maximum nest space allowance of 112 birds/m² nesting space was determined.

There was also an indication that flocks experiencing SLD would have a longer time between arrival at the layer facility and onset and continuation of egg production (possibly 0.5–0.6 weeks later in SLD flocks). It is unclear whether this represents a cause or effect, but farmers can consider that a flock that is coming into lay slightly later (at 20 weeks rather than 19 weeks) is more at risk of developing SLD subsequently.

A further survey looking at sheds with a scratch area is necessary to search for factors that might decrease the severity of SLD outbreaks in that type of facility.

Overall Conclusions

Three key determinants and one predictive factor for the occurrence of Spotty Liver Disease in free range or barn flocks were identified from this project. These were:

- The presence of a scratch area in the shed is a strong risk factor for the occurrence of an SLD outbreak.
- In fully slatted layer sheds, the risk of an SLD outbreak under natural ventilation is higher than under tunnel ventilation.
- Within fully slatted, naturally ventilated layer sheds, a higher number of birds per square metre nest space increases the risk of an SLD outbreak (maximum suggested allowance is 112 birds/m²).
- Flocks in fully slatted, naturally ventilated sheds that come into lay and progress in lay somewhat later than usual may be at higher risk of developing an SLD outbreak subsequently.

1 Introduction

1.1 Background

Spotty Liver Disease (SLD) has been substantively reviewed recently (Courtice et al. 2018). SLD emerged as a serious problem in adult hens, both commercial egg layers and broiler breeder females, in Australia through the late 1990s (Jenner 2001). It is considered that this disease is not 'new' and that it represented the re-emergence of a problem that was well established in the 1950s, then described as Avian Vibrionic Hepatitis (AVH) or Avian Infectious Hepatitis (Sevoian et al. 1958; Hofstad et al. 1958). The definitive causal agent of AVH was never identified before the condition disappeared associated with the widespread introduction of cages for egg production. Early descriptions of a focal necrotic hepatitis in chickens in USA from 1953–55 with similar clinical, gross and histological signs as SLD were described where the condition yielded either no bacterial growth but was transmissible (Sevoian et al. 1958), or associated with an organism described as a vibrio (Peckham 1958), which was susceptible to streptomycin and tetracyclines and slightly susceptible to penicillin but resistant to bacitracin (Moore 1958). The disease came to be called Avian Vibrionic Hepatitis and was most often noted as occurring about one month after egg production began. The disease could be reproduced in young chicks and could be seen occasionally in young pullets (Winterfield et al. 1958). The organism grew in incubating eggs, killing the embryo and producing typical liver lesions (Sevoian et al. 1958; Winterfield et al. 1958). Partial immunity seemed to be produced from one exposure in chicks (Peckham 1958).

The modern re-introduction of cage free production systems (free range and barn) has seen the major re-emergence of a similar clinical syndrome, now described as SLD. The syndrome was rarely reported once cage systems were adopted but reports were seen in broiler breeders in the late 1980s (Jenner 2001) and re-emerged with the advent of free range and cage free layer systems (Grimes & Reece 2011). SLD has been reported as capable of causing egg production declines of up to 35% hen day and mortality of up to 15% (Groves 2010, Grimes & Reece 2011; Courtice et al. 2018). SLD classically occurs around peak production, which manifests as 'sudden death' in "laying birds in good condition most likely at the peak of lay" (Grimes & Reece 2011; Scott 2016). Scott (2016) reported outbreaks ranging between 22 and 80 weeks of age but averaging 33.8 weeks.

The precise causative organism of SLD remained elusive until the identification of a slow growing *Campylobacter*-like organism by Crawshaw et al. (2015) in the UK. Excellent work in Australia by Professor Rob Moore's group at RMIT University identified an identical organism from SLD cases in Australia, characterised it, and established and named (Van et al. 2016) the existence of a new species: *Campylobacter hepaticus*. The group was able to confirm Koch's postulates with this organism (Van et al. 2017). It is accepted that the mechanism of infection with *C. hepaticus* is oral-faecal (Phung et al. 2020). Attempts are underway to develop vaccines against *C. hepaticus*.

Descriptive epidemiological studies so far have described the disease as occurring predominantly in warmer and humid weather, often following rain episodes, most commonly in early lay, affecting hens but not roosters (Courtice et al. 2018). There are also descriptions of strain variations (Courtice et al. 2018; Kotiw 2019; Van et al. 2019). Analytical epidemiologic studies have not been thoroughly performed to date but there have been anecdotal associations of SLD occurrence with wet litter, inadequate feed space, consumption of muddy water, overcrowding, nest box problems, cannibalism, parasites, and birds below breed weight standards (J. Courtice, pers. comm.). This report is of the first systematically designed analytical epidemiological study of SLD yet undertaken.

1.2 Rationale for an analytical epidemiological study

The discovery of the causative organism for Spotty Liver Disease (SLD), *Campylobacter hepaticus* (Crawshaw et al. 2015; Van et al. 2016; Van et al. 2017), and the development of molecular tools to detect it (Van et al. 2016) have provided a unique opportunity for a greater understanding of this disease. Analytical epidemiological studies have been a substantial gap in our knowledge. The preceding projects may have allowed some descriptive and molecular epidemiological information but there have been, to date, no analytical epidemiological studies conducted. SLD remains a problem for the cage-free and free range egg industries, still causing high mortality and egg production drops in many farms. This requires the use of antibiotics, and the condition sometimes recurs on the same property. Although *C. hepaticus* is recognised as a necessary cause of SLD, little is known about what would constitute 'sufficient causes' for it. There may be intervening factor(s) needed for the organism to cause the clinical disease. This at present is unknown. Few attempts have been made to identify risk factors for SLD (more recent results have shown that flocks that have recovered from clinical SLD harbour the organism permanently without showing disease; Courtice et al. 2018). Another recent field finding has shown that on a farm where SLD has never occurred, *C. hepaticus* could be detected in its rearing flocks. Both findings suggest a complex epidemiological basis to SLD which is not at all understood.

The objective of this project was to identify one or more of these possible 'intervening factors' or 'stress factors' with potential outcomes of discerning the most cost effective and least interventionist control measures for SLD. Potential factors to be examined range from bird health, shed infrastructure, resource availability, nutrition, husbandry, biosecurity, and so on. In economic terms, success of this project will decrease the amount of antibiotic use in cage free layer flocks, decrease mortality losses, and avoid egg production drops due to the outbreak of serious SLD. In social terms, success would remove one of the major disadvantages of the free range system and improve the welfare of free range flocks in reality. In environmental terms, the reduction of antibiotic use will remove a contributor to the development of antibiotic resistant organisms in a major food chain industry, and decrease the need to dispose of large numbers of mortalities in landfill or via other disposal mechanisms.

1.3 Epidemiology

Epidemiology is defined as "the study of the relationships of various factors determining the frequency and distribution of diseases in a community (Blood & Studdert 1999). Epidemiology is a human disease term but the animal equivalent, epizootiology, is rarely used. It is an holistic approach to understanding disease in populations (Martin et al. 1987). Its main purpose is to develop information upon which logical and informed decisions in animal disease control can be based (Martin et al. 1987).

Epidemiological investigations are always part of an ongoing cycle, with initial studies searching for information on disease frequency and occurrence, leading to hypotheses that can be tested using formal studies designed to examine relationships between disease and factors that determine its manifestation. Each step in the cycle should build understanding of the disease and how it operates in a population of animals.

Epidemiology has two basic strands. Descriptive epidemiology, which gathers information about the dynamic between the host, the causative agent and the environment. It asks who is affected, when are they affected, where are the affected animals and attempts to quantify the rates of disease occurrence and spread. Analytical epidemiology, however, is a "statistical analysis in an attempt to establish relationships between causative factors and disease incidence" (Blood & Studdert 1999). Most diseases have multiple factors involved, which lead to the expression of the clinical condition.

Analytical epidemiology seeks to expose the really important factors that are associated with the occurrence of the disease in a particular animal population. Having identified several factors, it then aims at identifying those that are 'key determinants'. Many factors may not be able to be controlled (e.g. weather, genetics in the short term, the physiological state of the host). A key determinant is a factor that can be manipulated by management to modify the manifestation of a disease. Finding one or more key determinants can allow for improved control of the disease.

A further definition is necessary here. This is to discriminate between 'necessary' and 'sufficient' causes of SLD. A 'necessary cause' is one that has to be present for the disease to occur. Hence in this case, the necessary cause is the presence of *Campylobacter hepaticus* in the bird. However, as we have seen from some of the descriptive epidemiology, *C. hepaticus* can exist in the bird without causing disease (i.e. present in recovered flocks which are asymptomatic). A 'sufficient cause' however is one that ALWAYS causes the disease, and these are seldom single factors. A necessary cause will always be part of any sufficient cause, but the latter will usually be a combination of this and one or more other factors (key determinants), which when present together will produce disease. There may be more than one sufficient cause. A further objective of this study will be to gain information leading to the identification of at least one sufficient cause of SLD.

1.4 Project design and concept

While some epidemiological work has been conducted on Spotty Liver Disease (SLD) since the identification of the causative organism, this has been of the descriptive kind only. This has only partially been achieved to date.

Three survey periods were originally proposed. As mentioned above, epidemiological studies are usually a developing cycle of studies, each subsequent study being informed by the preceding one(s) and developed as knowledge increases. This turned out to be the case with our study, and activities were much affected by the COVID-19 pandemic restrictions established during the conduct of the field work.

Participation of several free range egg producers was acquired: in New South Wales, Queensland, Victoria, South Australia and Western Australia.

Phase 1: Co-operating farms were identified and approached to enrol in the study. Particularly, operations that do not regularly (or at least infrequently) experience SLD outbreaks were targeted, as well as those that regularly experienced SLD. The importance of the 'Control' group (i.e. not experiencing SLD) must be recognised here – this is usually ignored but the reason(s) that it remains healthy is of extreme interest to this study. The co-operators were asked to notify the investigators if there was an occurrence of SLD in a flock between November 2019 and May 2020 (covering the summer period).

Survey 1: The importance of the experience of the rearing flocks cannot be underestimated. A cross-sectional survey of rearing flocks likely to contribute birds to the co-operating layer flocks from Step 1, were visited from September 2019 to December 2019. This timeframe covered rearing flocks likely to provide birds in the actual Case-Control study (Survey 2). Cloacal swabs were collected from a statistically valid number of birds for *C. hepaticus* PCR and an extensive flock questionnaire completed by participating rearing farms (Appendix 1). Thirty-two rearing flocks were recruited in this survey.

Survey 2 (major study): A prospective Case-Control study was initially intended. The summer of 2019–2020 turned out to have extensive outbreaks of SLD; more than in the preceding years. The initial potential case and non-case farms from the co-operating flocks were targeted from their

histories of SLD (late rearing to just on arrival = Survey 1). From arrival of birds at the layer farm, co-operators were asked to notify the research team if SLD occurred or was suspected. Visits were to be made to flocks experiencing SLD over the course of the outbreaks and their resolution where cloacal swabs were collected for SLD PCR. Twenty Case flocks were sought along with 10 non-case flocks (Controls) from the targeted flocks at 35–40 weeks of age (i.e. Case = experienced an outbreak of SLD between POL and current age or was treated for such; Control = no experience of SLD or only low level occurrence not requiring treatment to current age).

Many of the expected 'Control' flocks experienced SLD. Of the 24 enrolled flocks, 18 experienced SLD ('Cases') and only 6 remained clinically unaffected ('Controls'). Each flock was visited and observations made regarding facility specifications (floor space, feed space, drinker provision, nest space, scratch area and slatted area, range area and shade, presence of perches and platforms and their dimensions; bird plumage cover was scored, nutrition information was collected). Samples were collected from 12 random birds within the flock (cloacal swabs) for *C. hepaticus* PCR and attempted culture, a feed sample for analysis and 60 random eggs for albumen corticosterone analysis.

A comprehensive questionnaire (Appendix 2) was designed to gather information on the history of the outbreak (Case flocks), rearing information (location, vaccination history), shed design and range area size, nest space, feeder space and style, perches used, lighting program, , information on other flocks supplied from same group and current flock details (egg production data, presence of any other diseases or parasites, worming program, any treatments in feed (probiotics, prebiotics, mannan-rich fragments of carbohydrate products, essential oils), feed source, feed type, feed intake (gm/bird/day), time of feed changes, , age allowed to range, and production history.

Stratified contingency table analyses were performed on all factors with Case or Control as the dependent variable (statistically compared using χ^2 or Fisher's exact test). This indicated several factors that either individually or interactively modify the occurrence of SLD in the field (i.e. either increase or decrease the risk of the disease occurring). Due to the introduction of travel restrictions and lockdowns for COVID-19 across Australia in early 2020, many of the planned farm visits could not take place. Interstate surveys were carried out online where possible, and local veterinarians were asked to collect the appropriate samples and consign them to the research team. Most participating flocks in NSW were able to be visited by the research team. The difficulties and risks proposed by COVID-19 and their unwillingness to have visitors caused one flock to be withdrawn from the survey

Survey 3: Selected risk or protective factors identified in Survey 2 were proposed for further studies: flocks with the absence of a factor contributing to a sufficient cause discovered in Survey 2 were identified and determined if they had (Case) or had not (Control) experienced SLD until 40 weeks of age during 2021–22. A retrospective Case-Control study was performed to seek identification of further possibly contributory factors that could not be completely assessed during Survey 2. Flocks in NSW were enrolled and were visited, and samples to detect *C. hepaticus* (cloacal swabs, environmental samples as above) were collected. The survey questionnaire is detailed in Appendix 3.

Contingency table analysis and stratified Mantel-Haenszel analysis provided output on the association of each factor with the occurrence of SLD. Multiple logistic regression and Mantel-Haenszel stratified analyses were used to examine confounding and interactions.

2 Phase 1 – Surveys 1 and 2

2.1 Introduction

Examining farms and flocks that are unaffected is important for an epidemiological approach. It is essential to search for factors that may differentiate diseased and healthy units so that factors that may contribute to their status can be compared. The intention was to enrol farms into the survey representing both affected and unaffected flocks. Expectations of their SLD status was based on the previous history of the farms in relation to the occurrence of the disease. Field veterinarians were approached to identify likely farms that could fit into both categories. The original intention was to incorporate 20 unaffected (Control) flocks and 10 affected (Case) flocks and conduct a Case-Control study across these. However, the period chosen (summer 2019 to 2020) eventuated as one of high SLD incidence, and many of the expected Control flocks experienced disease outbreaks. As a result, the study design could be regarded as cross-sectional.

2.2 Methodology

Surveys 1 and 2 were conducted under the supervision of the Animal Ethics Committee of the University of Sydney (protocol number 2019/1589) and the Human Ethics Committee of the same institute (protocol number 2019/662).

2.2.1 Survey 1 – rearing flocks

Thirty-two flocks rearing for birds destined for a free range laying flock were enrolled for the study. The flocks were based in NSW, Queensland, Victoria, South Australia and Western Australia. Each flock was visited between the age of 12 weeks till the end of rearing (usually 16 weeks of age). An extensive questionnaire designed using REDCap (Appendix 1) was completed on each visit. A publication summarising the variation in style, furnishings and management is planned from these data. There were more rearing flocks surveyed than laying flocks, as more than one of the smaller rearing flocks contributed to a single layer flock and sometimes birds reared in one flock were distributed to more than one layer facility. All rearing flocks hatched between July and November 2019. The rearing survey was completed before the COVID-19 pandemic took effect in Australia.

2.2.2 Survey 2 – flocks in lay

Layer flocks that were supplied with point of lay birds from the surveyed rearing flocks were enrolled in the layer survey. Twenty-four flocks were eventually surveyed and included. Due to COVID-19 pandemic travel restrictions, which stopped interstate travel in 2020 and restricted local movements within NSW, flocks were only visited by the research team where possible. Interstate flocks were interviewed remotely, where questionnaires were sent to participants to complete and responding veterinarians were asked to assist with sample submission. As a result of the travel restrictions and COVID-19 concern by some farm owners, some flocks were withdrawn from the survey. Consequently, some data were incomplete. All flocks in this part of the survey had birds transferred to the laying quarters between October 2019 and March 2020. Survey visits occurred between January and September 2020. The target visit age was 35–40 weeks of age, which would allow for any likely incursion of SLD to have occurred on these farms. A detailed questionnaire (Appendix 2) was completed and 12 cloacal swabs from random birds were collected for detection of *C. hepaticus* by PCR.

Participating flocks were categorised as 'Cases' or 'Controls'. The Case definition used was that the flock experienced a rise in mortality and a decline in egg production associated with the occurrence of typical gross pathology of SLD: viz. multiple focal necrotic lesions (spots) in the liver, a fibrinous perihepatitis possibly with icterus.

2.2.3 *C. hepaticus* PCR

A PCR protocol developed and published by RMIT University researchers (Van et al. 2017) was used to detect *C. hepaticus* from faecal material on cloacal swabs. Twelve cloacal swabs were collected randomly from each flock at the time of visit for detection of *C. hepaticus*, once in rearing and again in lay. Matching environmental samples such as pooled fresh faeces and dust were also collected from some flocks.

In 2021, RMIT University researchers announced the discovery of another *Campylobacter* species capable of causing SLD, named *Campylobacter bilis* due to its ability to be isolated from bile (Moore 2021). It is believed that the PCR method used in this study also detects the presence of *C. bilis* (R. Moore, pers. comm.). This means that the recent discovery of *C. bilis* would not compromise this study, as we were able to detect an organism known to cause SLD from samples collected, except we were unable to differentiate between *C. hepaticus* and *C. bilis*.

2.2.4 Statistical analyses

Data were transferred to MS Excel and uploaded in STATSTICA v6 (Statsoft Inc., 2003) for analysis.

All survey variables in both rearing and laying compartments were assessed in a univariate analysis using a contingency table analysis for categorical variables or Student's t-test for continuous variables with the Case or Control definition as the dependent variable. Any variable displaying a probability of an association being due to chance value of < 0.25 (as suggested by Hosmer et al. 2013) was selected for further inclusion in any multivariate model building approach. This high probability value is used as a screening level for selection of possibly important factors, the significance of which may be hidden within the complexities of the data. Pearson χ^2 tests were used to assess probability due to chance, however, given the restricted sample size, Fisher's exact test (2-tailed) was used when an expected value was < 5 . Continuous variables expressing interest (as there were numerous factors with $P < 0.25$, a slightly lower selection level of $P < 0.20$ was used) were broken into ordinal categories, using the median value as the break point and these were further analysed as categorical variables.

With a survey that has a small sample size and a vast number of independent variables, confounding is to be expected. Where confounding was to be expected, the usual approach to understanding this is to use the Mantel-Haenszel stratified analysis technique (Martin et al. 1987; Thrusfield 2013). The intention thereafter was to include all selected variables and their interaction terms into a stepwise multiple logistic regression model to attempt to develop the most parsimonious model for factors associated with the occurrence of clinical SLD from the available database. The multiple logistic regression model would be analysed using JMP statistical software (SAS 2022).

2.3 Results

The association between observed factors in the rearing and laying section of the survey were cross tabulated against the occurrence ('Case') or non-occurrence ('Control') of clinical SLD outbreaks in the surveyed flocks. There were 32 rearing flocks surveyed, which supplied birds to 24 flocks in lay. In some situations, more than one rearing flock supplied a single layer flock and in others, a single rearing flock contributed birds to more than one layer flock. No cloacal swabs from rearing flocks returned a

positive result although *C. hepaticus* was detected in pooled faeces from one rearing flock. Table 1 shows the proportion of cloacal swabs that gave a positive detection of *C. hepaticus* by PCR from the laying flocks (one flock's samples were not submitted due to difficulties with COVID-19 restrictions).

Table 1 Number cloacal swabs (N=12) positive for *C. hepaticus* by PCR in laying flocks

Flock category	No. flocks positive	Number of cloacal samples positive (N=12)			
		Mean	Minimum	Median	Maximum
Case	17	8.88	4	9	12
Control	6	0.67	0	0	2
Mann-Whitney U test p=		0.0004			

Case flocks had significantly higher numbers of cloacal swabs giving positive *C. hepaticus* detection than did Control flocks (variances in this dataset were not homogeneous, hence the nonparametric Mann-Whitney test was used to compare groups). All of the Case flocks had positive cloacal swabs (at least 4/12). As we had only 12 cloacal samples per group, the confidence in detecting at least one positive result in this sample size was limited. A zero detection out of 12 samples would only provide 95% confidence that the actual level was below 25% of the population, hence declaring a flock to be 'negative' on this basis is not valid. Noting this condition, out of the six Control flocks, four gave zero positive swabs while two Control flocks had two positive swabs. The Control flocks had significantly fewer positive cloacal swabs but there were still positive birds present in at least two of these flocks.

Some interesting observations of the occurrence of SLD emerged during the survey:

- Many of the predicted Controls developed as Cases in the survey. Prediction of the outcome was based on the previous history over several years. This particular year saw an increase in the incidence of SLD, not only in the survey, but also anecdotally in the industry.
- Certain sheds on a farm would be Cases while others stayed unaffected.
- SLD occurred in a barn flock and also often broke out in free range flocks before the birds were let out to range (commonly this is after peak production). These instances suggest that SLD may be more associated with something inside the shed rather than outside in the range area, as has commonly been thought (Phung et al. 2020).
- One flock in the survey was a brand new aviary system built in a very isolated location, by a company that had never experienced SLD in its other FR sheds in its history.

Cross tabulated results for the association of rearing variables with later Case or Control classifications are shown in Table 2, and those for variables described in laying flocks are shown in Table 3. Tables 4 and 5 summarise Student's t-test analysis for continuous variables for rearing and laying flocks respectively.

This was a large survey in terms of the number of exposure factors measured and recorded. There were substantial missing data in many records. The survey was conducted throughout 2020 during the COVID-19 pandemic in Australia which was responded to by heavy restriction of people movement both interstate and regionally within states. This prevented the research team from completing field visitations, and relying on local farm staff and veterinarians to complete the survey questionnaires. The effects of COVID-19 restrictions also heavily impacted farm activity and staff availability, making completion of the in-depth survey burdensome and difficult for some co-operators. In many cases this has led to missing data points within the dataset. This somewhat limited analyses to those factors that had close to complete data entries (only these are shown in Tables 3–7).

Table 2 Categorical variables association with Spotty Liver Disease occurrence for all flocks surveyed – rearing flocks

Variable – rearing shed features	Variable Level	No. of flocks		Odds ratio	Pearson Chi-Sq	Fisher's exact 2-tail [†]
		Case	Control		P=	P=
Hatchery	A	9	2	3.375	0.34	
	B	2	0	Undefined		
	C	4	3	Reference		
Other animals on property	Yes	9	6	0.00	0.07	0.12
	No	7	0			
Rear and laying location	Same farm	2	0	Undefined	0.41	1.00
	Remote farm	14	5			
Rearing shed type	Aviary	7	0	Undefined	0.07	0.12
	Barn	9	5			
Rearing ventilation style	Natural	7	3	0.52	0.53	0.64
	Tunnel	9	2			
Platforms in rearing shed	Yes	9	3	0.86	0.88	1.00
	No	7	2			
Perches in rearing shed	Yes	12	4	0.75	0.82	1.00
	No	4	1			
Feather pecking in rearing	Yes	1	0	Undefined	0.57	1.00
	No	15	5			
Smothering during rearing	Yes	7	2	1.17	0.88	1.00
	No	9	3			
Biosecurity plan in rearing	Yes	14	4	1.75	0.68	1.00
	No	2	1			
Vehicle disinfection on rearing farm	Yes	14	2	10.50	0.03	0.06
	No	2	3			
Rearing shed resting time	up to 14 days	5	2	0.45	0.88	1.00
	28 days or more	11	2			

[†] Where an expected value was <5, Fisher's exact test value is shown.

Table 3 Categorical variables association with Spotty Liver Disease occurrence for all flocks surveyed – flocks in lay

Variable – layer shed features	Level	No. of flocks		Odds ratio	Pearson Chi-Sq	Fisher's exact 2-tail [†]
		Case	Control		P=	P=
State	WA	1	0		0.22	
	SA	1	0			
	NSW	7	6			
	Vic	4	0			
	Qld	3	0			
Brown egg layer strain	Strain A	13	2	5.20	0.09	0.15
	Other	5	4			
Layer shed style	Barn	1	0		0.45	
	Aviary	3	0			
	Free Range	14	6			
Shed ventilation system	Natural	13	3	3.25	0.23	0.32
	Tunnel	4	3			
Slats	Partial	13	0	Undefined	0.002	0.003
	Full	5	6			
Slat brand	A	1	0	Undefined	0.68	
	B	6	2	2.25		
	C	1	0	Undefined		
	D	6	1	4.50		
	E	4	3	Reference		
Pop hole position	Both sides	14	4		0.42	0.58
	One side	3	2			
Feeder type	Pan	7	4	0.32	0.24	0.36
	Chain	11	2			
Drinker type ¹	A	5	2		0.87	
	B	1	0			
	C	1	0			
	D	1	0			
	E	2	1			
Water source	Bore	6	2		0.89	
	Town	9	2			
	River	2	1			
	Dam	1	0			
Range vegetation type	Grass Yes	4	0	Undefined	0.30	0.55
	Grass No	14	4			
	Shrubs Yes	8	2	0.80	0.84	1.00
	Shrubs No	10	2			
	Tree Yes	6	2	0.50	0.53	0.6
	Trees No	12	2			
Access to water on range	Puddles	12	1	6.00	0.15	0.22
	No water	4	2			

IB vaccination in lay	Yes	11	4	0.92	0.93	1.00
	No	6	2			
Feed additives for SLD ²	Additives	14	1	17.50	0.007	0.15
	No additives	4	5			
Light type	Warm					
	White	9	1	4.50	0.10	
	Cool White	6	3			

[†] Where an expected value was <5, Fisher's exact test value is shown.

¹ Drinkers included Ziggity, SKA, Lubing, Big Dutchman and Impex (not in order shown).

² Feed additives used as a preventative for Spotty Liver Disease (SLD) including probiotics, organic acids, prebiotics, yeast extracts.

From Tables 2 and 3 it can be readily discerned that one particular variable showed a highly significant association with the occurrence of clinical SLD in the layer flock (the presence of partial slatted floor compared to fully slatted flooring in the layer house; $P = 0.003$, Table 3). This is also echoed in Table 4 where total slat percentage coverage of the shed floor has also a highly significant statistical association with Case flocks. The cross tabulation for this variable reveals a zero cell value (for Control flocks where partial slats were present) and hence the Odds Ratio (Ψ) for this association is undefined (infinite). Hence, within the framework of this survey, **all flocks with only a partially slatted floor in the layer house (hence those that have a scratch area within the house) showed the occurrence of clinical SLD**. Thus, the presence of a scratch area in the layer house can be considered to be a key determinant (Martin et al. 1987) of SLD.

Table 4 Student's t-test for continuous variables in rearing flocks

Rearing flock variable	Case mean	Control mean	t-value	df	p	Valid N Cases	Valid N Controls
Property size (ha)	157.47	245.10	-0.776888	15	0.449	12	5
Maximum age difference across farm (wk)	11.88	11.20	0.693605	19	0.496	16	5
Shed floor area (m ²)	1421.07	1303.12	0.355505	19	0.726	16	5
Platform area in house (m ²)	267.72	1.06	1.314244	19	0.204	16	5
Total space available for birds (m ²)	1688.84	1797.72	-0.292082	19	0.773	16	5
Total perch length (m)	1869.59	363.69	1.363775	18	0.189	15	5
No. birds	24245.13	23095.80	0.192101	18	0.850	15	5
Age birds delivered to laying (wk)	15.81	16.00	-0.326042	19	0.748	16	5
Pan feeder space (cm/bird)	0.58	0.81	-0.525956	20	0.605	17	5
Chain feeder space (cm/bird 2 sides)	3.76	1.91	0.857225	21	0.401	18	5
Calculated feed space (cm/bird)	5.18	2.72	1.300920	18	0.210	15	5
Drinker space (birds/nipple)	8.36	9.63	-0.826983	17	0.420	14	5
Perch space (cm/bird)	9.17	1.42	1.459888	17	0.163	14	5
Stocking density (birds/m ²)	17.06	18.25	-0.334187	17	0.742	14	5
Total number of light units/shed	303.42	32.00	0.615292	12	0.550	12	2
Maximum temperature recorded in shed (°C)	51.61	47.20	0.266184	18	0.793	15	5
Maximum temperature recorded outside shed (°C)	62.53	68.20	-0.361604	18	0.722	15	5

Table 5 Student's t-test for continuous variables in laying flocks

Layer flock variable	Case mean	Control mean	t-value	df	p	Valid N Cases	Valid N Controls
Distance from rearing farm (km)	211.30	71.25	0.84039	19	0.411	17	4
Shed slat coverage (%)	67.23	100.00	-2.24326	17	0.038	13	6
First age of ranging (wk)	20.81	20.00	0.50914	16	0.618	16	2
Duration of ranging (h)	11.09	11.25	-0.24327	16	0.811	16	2
No. birds transferred to layer shed	19390	19900	-0.08906	22	0.930	18	6
Feed space (cm/bird)	5.86	3.60	1.24986	19	0.227	16	5
Drinker space (birds/nipple)	10.30	11.29	-0.46244	19	0.649	16	5
Perch space (cm/bird)	9.41	4.86	1.48203	21	0.153	18	5
Nest space allowance (birds/m ²)	121.68	72.68	3.32579	15	0.005	14	3
Stocking density inside (birds/m ²)	11.70	10.71	0.57981	19	0.569	15	6
Range area stocking rate (birds/ha)	4605	1808	1.67731	17	0.112	16	3
Feed intake at 5% HD (g/bird/d)	78.86	74.40	0.66454	10	0.521	7	5
Feed intake at 60% HD (g/bird/d)	94.86	91.20	0.92527	10	0.377	7	5
Feed intake at peak HD (g/bird/d)	110.64	116.00	-0.69160	14	0.500	11	5
Maximum age difference across farm (wk)	11.88	11.20	0.69360	19	0.496	16	5

The presence of a zero-cell value causes major difficulties for any multiple factor analysis as it seriously inflates standard error values. Hence this factor would strongly confound the putative effect of any other associated factor in houses with a scratch area. Hosmer et al. (2013) suggest methods for dealing with this problem statistically, including collapsing the categorisation within the levels of the exposure factor or removing the members of the data with this zero value. The separation in Cases and Controls between full and partially slatted houses (i.e. those with or without a scratch area) was complete and there is no obvious way to collapse the categories any further. Some Cases and Controls were observed in fully slatted sheds. Hence our only solution was to remove all houses with a scratch area for further analysis, further looking only for other factors that may be associated with SLD occurrence in fully slatted sheds. This reduced the sample size to only 11 flocks; five (45.5%) of which were Cases. Further analysis was hoped to identify other putative risk factors that might contribute to the occurrence of SLD in only houses with fully slatted flooring.

Table 6 shows the univariate analyses for rearing flock exposure factors, and Table 7 shows these for layer flock exposure factors (including some continuous variables transformed to categorical or ordinal variables as described above) and Fisher's exact (2-tailed P values for each cross tabulation as the small sample size produces expected values < 5). Notably, the small sample size remaining leads to the occurrence of many zero cells, producing Odds Ratios that are either zero or infinite. There were a number of exposure factors producing probability due to chance values < 0.20 and several of these < 0.05. With the small sample size, the presence of these exposure factors within the same flock is highly concurrent and their effects are considered highly confounded by each other. Applying the stratified Mantel-Haenszel technique was not satisfactory as the coincidence of potential associations with SLD occurrence was complete (i.e. all the Case flocks share the same exposure factor distribution).

Table 6 Categorical variables association with Spotty Liver Disease occurrence for flocks with full slat cover (N=11) – rearing flocks

Variable – rearing farm features	Level of variable	No. of flocks		Odds ratio	Fisher's exact P (2-tailed)
		Cases	Controls		
Hatchery	Hatchery A	5	3	Undefined	0.18
	Hatchery B	0	3		
Rearing house style	Aviary	1	0	Undefined	1.00
	Barn	4	5		
Rearing house ventilation system	Natural	5	0	Undefined	0.44
	Tunnel	0	2		
Rearing lights able to be dimmed	Yes	1	4	0.06	0.21
	No	4	1		
Perches in rearing house	Perches present	1	4	0.06	0.21
	No perches	4	1		
Rearing feeder type	Pan	4	3	2.67	1.00
	Chain	1	2		
Beak trimming frequency	Once (hatchery)	2	3	0.44	1.00
	Twice (hatchery and 10-12 weeks)	3	2		
Rearing feed type	Mash only	3	2	Undefined	0.14
	Crumbles only	2	0	Undefined	
	Crumble starter, then mash	0	2		
Rearing feeding program	<i>Ad libitum</i>	3	0	Undefined	0.17
	Restricted	2	4		
Probiotics used in rearing feed	Yes	1	2	0.38	1.00
	No	4	3		
Rearing water disinfection	Chlorine dioxide	2	2	1.00	1.00
	No treatment	3	3		
Rearing transport hygiene	Crate disinfection	4	5	0.00	1.00
	No disinfection	1	0		
Vehicle disinfection	Yes	3	2	2.25	1.00
	None	2	3		
Resting time for rearing house between batches	Up to 14 days	1	2	0.13	1.00
	28 days or more	4	1		
Vehicle disinfection on site	Yes	3	2	2.25	1.00
	No	2	3		
Distance of rearing farm from laying house	<70 km	1	3	0.22	1.00
	>70km	3	2		

Table 7 Variables association with Spotty Liver Disease occurrence for flocks with full slat cover (N=11) – flocks in lay

Variable – layer shed features	Level of variable	No. of flocks		Odds ratio	Fisher's exact P (2-tailed)
		Cases	Controls		
State	NSW	4	6	0.00	0.45
	WA	1	0		
Flock size (bird number)	Flock <16080	5	0	Undefined	0.002
	Flock ≥16080	0	6		
Brown egg layer strain	Strain A	5	2	Undefined	0.06
	Strain Other	0	4		
Layer shed type	Barn	1	0	Undefined	0.45
	Free range	4	6		
House ventilation system	Tunnel ventilation	0	3	0.00	0.18
	Natural ventilation	5	3		
Available floor space	Space <1500 m ²	4	1	4.00	1.00
	Space ≥1500 m ²	1	1		
Slat type in fully slatted house	Slat type E ¹	0	3	0.00	0.18
	Other slat types	5	3		
Platforms in house	Platforms in shed	0	2	0.00	0.45
	No platforms	5	4		
Perches in house	Perches in shed	3	5	0.30	0.54
	No perches	2	1		
Automatic nest type	Brand X	4	1	20.00	0.08
	Other brands	1	5		
Drinker type	Nipple drinkers	4	6	0.00	0.45
	Bell drinkers	1	0		
Drinker space	<10.7 birds/nipple	8	2	2.00	0.63
	≥10.7 birds/nipple	6	3		
Water source	Town water	2	2	1.00	0.71
	Other ²	3	3		
Water chlorination	Water treated	3	5	0.00	0.44
	Water not treated	2	0		
Feeder type	Pan feeders	3	7	0.43	1.00
	Chain feeders	2	2		
Pop hole position	Pop hole one side	2	2	2.00	0.45
	Pop hole both sides	2	4		
	No pop holes	1	0		
Light types in house	Cool white lights	0	3	0.00	0.14
	Other lights ³	4	1		
Vegetation on range area	Grass in range	4	4	0.00	1.00
	No grass in range	1	0		
	Shrubs in range	1	2	0.25	0.52
	No shrubs	4	2		
	Trees in range	2	3	0.44	1.00
	No trees	3	2		

Infectious bronchitis vaccination during lay	Vaccination in lay	1	4	0.17	0.53
	No vaccine in lay	3	2		
Feeding regime	Varied feed run times	1	3	0.17	0.52
	Feed times not varied	4	2		
Feed additives for SLD	Probiotics	1	0	Undefined	1.00
	No probiotics	4	2		
	Prebiotics	1	1	0.25	1.00
	No prebiotics	4	1		
	Organic acids	3	0	Undefined	0.43
	No organic acids	2	2		

¹ Slat types present in the study include Salmat, SKA, Big Dutchman, Vencomatic, Facco and Roxell.

² Other water sources include bore, rainwater and dam water.

³ Includes warm white, or a mixture of warm and cool white lights in the house.

Only factors from the univariate analyses with full data were considered further as the sample size is now small. From Tables 6 and 7, the factors with a Fisher's exact test $P < 0.20$ with full data include hatchery and layer strain (these two factors are identical hence only strain was considered), flock size, ventilation system, and slat type and nest box type (the latter two factors are always supplied by the same manufacturer and hence are the same).

From this analysis SLD in fully slatted sheds occurred **more frequently** in:

- flocks of less than 16,080 birds
- layer strain A
- in sheds with natural ventilation systems
- using nests/slats of a particular type.

However, these factors are highly confounded in their occurrence. All the smaller flocks ($n = 5$) were naturally ventilated and used layer strain A and four used nest/slat type X. This high level of confounding made discernment of the priority importance of any of the factors impossible, and attempts at multiple factor analysis gave unstable estimates and failed to provide statistical validity.

Using a forward stepwise multiple logistic regression analysis with slat type, layer strain, flock size and ventilation system as model variables, indicated that ventilation system may be the factor of most interest in this limited dataset for the occurrence of SLD in fully slatted sheds, however, estimates were unstable (Table 8 shows the JMP analysis outcome). Table 8 shows extreme standard error and odds ratio values indicative of the strong confounding of the variables. The effect summary in Table 8 does suggest that of the factors, natural ventilation may be more at risk of SLD than tunnel ($P = 0.025$), but this needs to be confirmed by further studies.

Table 8 Forward stepwise logistic regression on factors of interest in fully slatted sheds with the occurrence of SLD (JMP analysis outcome)

Nominal logistic fit for 'Case' – forward stepwise analysis
Effect summary likelihood ratio tests – converged in gradient after 17 iterations

Source	Nparm ¹	Degrees of freedom	Log worth ²	p value
Ventilation system	1	3	1.597	0.025
Slat type	3	1	0.841	0.144
Layer strain	2	2	0	1.000
Flock <10608 birds	1	0		

¹ Nparm = no. non-zero estimates associated with the effect

² LogWorth = $(-\log_{10}(\text{p-value}))$ and measures strength of the contribution of the factor to the model.

Parameter estimates

Term	Level	Reference group	Status	Estimate	Std Err	Odds Ratio	Chi-sq	P=
Intercept			Biased	-9.101	5363		0.00	0.998
Slat brand	A	D	Biased	9.101	11969	8,964.25	0.00	0.999
	B	D	Biased	9.101	9107	8,964.25	0.00	0.999
	E	D	Biased	-27.304	6537	0.0000	0.00	0.997
Layer strain	A	C		-2.659E-08	8455	1.00	0.00	1.000
	B	C		0.00896	5593	1.01	0.00	1.000
Ventilation system	Natural	Tunnel	Biased	18.203	5013	80,438,220	0.00	0.997
Flock <16080 birds			Zeroed	0	0			

2.4 Discussion

Finding the presence of *C. hepaticus* in Control flocks, although at lower prevalence than the Cases, is suggestive that the pathogen is widespread, possibly ubiquitous. Phung et al. (2020) have shown that birds can be infected for quite some time before disease occurs. This is suggestive that factor(s) other than the presence of the organism in the environment or affecting the birds are necessary for the disease to manifest. When clinical SLD occurs, however, *C. hepaticus* can be detected in more cloacal swabs from average birds, perhaps indicating a more active spread of infection.

The discovery of the importance of having a scratch area on SLD occurrence is a major finding. All flocks with scratch areas in the survey became Cases. Free range and barn houses that have a scratch area are at much higher risk of clinical SLD occurrence than those that have a floor area fully covered by slats. As a scratch area allows much closer contact of the birds with their faeces and a slat coverage can restrict this access, this observation does make biological sense and is an important effect. Presence of a scratch area can therefore be considered a 'key determinant of SLD' (i.e. a factor strongly associated with a disease that is amenable to management; Martin et al. 1987). A 'sufficient cause' of a disease is defined as a set of factors, including any 'necessary cause(s)', that when they occur together will always cause the disease (Martin et al. 1987). These data indicate that the presence of a scratch area in a free range or barn layer house when *Campylobacter hepaticus* is present (the necessary cause), comprises a sufficient cause of SLD. There may be more than one sufficient cause in the ecology of any disease, and others obviously exist as we observed SLD in 45% of houses with full slat cover over the floor.

Unreliable data, due to strong confounding of factors within the small sample size of fully slatted sheds, might point to ventilation system as being important. In this study, naturally ventilated sheds which were smaller on average, may be more at risk of SLD in fully slatted facilities. This may make some sense as SLD was often noted in warmer weather (Business Queensland 2018). Other anecdotal reports also suggest that keeping the shed cooler during an outbreak of SLD can decrease the severity, which is not an option with natural ventilation. But this needs a larger study to confirm this concept.

A further study with a much larger sample size, focused on houses with no scratch area, is needed to identify further risk factors and key determinants under these conditions.

We observed that SLD can become clinical in barn sheds and also before birds are allowed access to the range area in FR systems. As *C. hepaticus* has a faecal-oral mechanism of spread (Business Queensland 2018), this finding regarding the scratch area makes biological sense, as there would be much higher chance of bird access to fresh faeces in the scratch area than there would be on a fully slatted floor where faeces pass through the slats efficiently. We can propose then that the existence of a scratch area in a barn or FR flock where *C. hepaticus* (or perhaps *C. bilis*) is present constitutes a 'sufficient cause' for SLD. As 45% of sheds without a scratch area (full slats) also developed SLD, there are obviously other factors besides a scratch area that contribute to Cases as part of other sufficient cause scenarios, and a search for these needs to continue.

2.5 Conclusions from Surveys 1 and 2

Having a scratch area (i.e. a section of the floor exposed for the birds to dust bathe) is identified as a strong risk factor for the occurrence of SLD in a cage-free flock. This factor is so strong that the presence of a scratch area, if *Campylobacter hepaticus* is present, constitutes a sufficient cause for SLD.

SLD will also occur in sheds with full slat coverage and further factors that contribute to this need to be identified. Other factors were considered as potential risk factors under fully slatted flooring but could not be satisfactorily assessed due to strong correlation between them with this sample size.

A further survey examining possible factors under fully slatted conditions was necessary.

3 Survey 3 – fully slatted floor survey

3.1 Introduction

Following on from the initial cross-sectional survey, a second survey examining flocks in lay in free range sheds with fully slatted floors was undertaken to identify other factors that might modify the expression of SLD in this type of housing. As per Surveys 1 and 2, it was identified that sheds with scratch areas contribute to a sufficient cause of SLD. However, even in sheds with fully slatted floors, almost half of the flocks developed clinical SLD. It was hypothesised that there are potential further risk factors that could lead to clinical diseases of SLD.

3.2 Materials and methods

The survey was conducted under the supervision of the Animal Ethics Committee of the University of Sydney (protocol number 2021/1898).

3.2.1 Survey design

A more focused questionnaire was designed based on the preliminary findings of Survey 1 and 2, with a heavy focus on shed infrastructure, resource availability and bird production data. Due to the on-and-off COVID-19 travel restrictions, interviews of NSW flocks were carried out in person, where all interstate flocks were interviewed remotely. The questionnaire was drawn up in a Microsoft Excel spreadsheet to aid the ease of data collection, as REDCap used in the preceding survey was found to be difficult to use for the participants. Data were collected retrospectively, where one flock was enrolled for each of the fully slatted sheds identified and included in the survey. If the existing flock was less than 40 weeks of age at the end of sampling and data collection period, then the most recent finished flock was enrolled in the study. An example of the questionnaire is included in Appendix 3.

Participating flocks were categorised as 'Cases' or 'Controls'. The Case definition used was that the flock experienced a rise in mortality and a decline in egg production associated with the occurrence of typical gross pathology of SLD: viz. multiple focal necrotic lesions (spots) in the liver, a fibrinous perihepatitis possibly with icterus.

3.2.2 Statistical analyses

Data were transferred to MS Excel and uploaded into JMP (SAS 2022) for analysis.

All survey variables were assessed in a univariate analysis using a contingency table analysis for categorical variables or Student's t-test for continuous variables with the Case or Control definition as the dependent variable. Any variable displaying a probability of an association being due to chance value of < 0.20 (as suggested by Hosmer et al. 2013) was selected for further inclusion in any multivariate model building approach. This high probability value is used as a screening level for selection of possibly important factors, the significance of which may be hidden within the complexities of the problem. Pearson χ^2 or Fisher's exact 2-tailed tests were used to assess probability of associations being due to chance. Continuous variables of interest (as there were numerous factors with $P < 0.20$ in Student's t-tests) were selected for further analysis.

Multiple logistic regression is an iterative statistical test that allows assessment of the effect of factors after controlling for the presence of other factors, thus assisting in eliminating extraneous effects. It

also allows assessment of confounding and interaction between factors (Dohoo 1992; Hosmer et al. 2013). All selected variables were considered in a univariate logistic regression, which is able to consider several levels of a variable (e.g. slat brands). Variables showing a statistical association with occurrence of clinical SLD were then added to a multivariate logistic regression analysis along lines suggested by Dohoo (1992). Variables that showed a probability due to chance of < 0.05 were then examined for confounding and then subjected to a forward stepwise analysis including interaction terms to identify the key factors. A final multivariate analysis was then performed to define the major factors involved in risk of SLD in fully slatted houses.

3.3 Results

Shed ventilation system was flagged as of interest from the small sample number of fully slatted sheds examined in Survey 1. Out of 49 sheds enrolled in Survey 2, eight were tunnel ventilated and the remaining 41 were of conventional naturally ventilated design. Table 7 shows that this factor involved no Cases occurring in tunnel ventilated sheds. Hence being in a naturally ventilated fully slatted shed is identified as a putative high-risk factor for SLD. As was found in Survey 1, the zero cell value confounds all other factors within tunnel ventilated sheds. Only naturally ventilated sheds could be considered further for the analysis. This reduced the sample size to 41, which we consider to be a useful number for further analyses.

Breeds were not evenly distributed across the shed ventilation types and only one layer breed was present in 39 of the naturally ventilated sheds. This distribution negates any breed comparison in this study.

Table 9 shows categorical variables in contingency tables, showing numbers of naturally ventilated sheds experiencing SLD ('Case') or not ('Control'). Thus, categorical variables selected for further assessment included nest/slat brand, closure of nests at night, and nutritionist used.

Table 10 shows outcomes of Student's t-tests conducted using Case or Control as the dependent variable and all continuous variables measured in the study in naturally ventilated sheds. Tables 9 and 10 were used to select factors for further analysis if the probability of the association with being a Case was less than 0.20 as a screening level (adapted from Hosmer et al. 2013). There were some missing data points in many variables due to differences in what farmers generally record and what could be provided. Obtaining full records was hampered by travel restrictions due to COVID-19 during 2021, especially with regard to flocks outside of NSW. Continuous variables considered further included stocking density (birds/m² useable floor space in shed), perch space in lay, nest space (birds/m² of nest space), the age at which birds were first allowed to range (weeks), range density (birds/ha), age (weeks) at which the flock reached 5% hen day production (% HD), age they reached 60% HD, the body weight of the birds at 60% HD, age at peak lay, and number of weeks between transfer to laying shed and reaching peak HD production.

Several of these variables are highly associated with each other. Notably factors related to bird age are correlated with body weights at that age, as birds continue to gain weight throughout lay: older birds are heavier. Five of the factors selected for further study fall under this association and data for all of them are incomplete, as weights are not always recorded for every week. Dohoo (1992) suggests a valid technique to deal with this type of autocorrelation by intuitively selecting one factor as representative of all of these related factors, based on the investigator's knowledge of the husbandry system and the relative P values of each variable with the dependent variable. In this case, we selected the time since transfer until the flock reached 60% HD production, based on this factor having the highest complete data number and the lowest P value of being due to chance in its association with the flock being a 'Case'. This gave us eight remaining variables to further assess within naturally ventilated sheds (Table 11).

Table 9 Categorical variables distributed across Case or Control sheds in naturally ventilated houses

Variable	Level	No. Case sheds	No. Control sheds	χ^2 or Fisher's exact 2-tailed test* P=
Season chicks hatched	Autumn	6	10	0.25
	Winter	6	3	
	Spring	5	5	
	Summer	1	5	
Perches in rearing shed	Yes	7	9	0.80
	No	11	12	
Nest and slat brand	Vencomatic	7	13	0.04
	Roxell	4	5	
	Big Dutchman	6	1	
	Facco	0	4	
	Salmet	1	0	
Slat shape	Rectangular	5	3	0.34
	Square	6	5	
	Oval	7	13	
Lights type	Cool white	10	8	0.61
	Warm white	7	8	
Nests closed at night	Yes	16	17	0.12*
	No	0	4	
Nutritionist	A	8	8	0.02
	B	1	5	
	C	3	8	
	D	5	0	
Feed mill	W	8	8	0.37
	X	6	5	
	Y	3	8	
	Z	1	0	
Water additives used until 40 weeks	Yes	4	5	0.91
	No	14	16	
Number of rations used in lay	1	12	13	0.76
	>1	6	8	
Perches in laying shed	Yes	14	14	0.48*
	No	4	7	

* Pearson χ^2 test but if an expected value was <5, Fisher's exact 2-tailed test was used.

Highlighted factors considered worthy of further statistical evaluation.

Table 10 Continuous variables distributed across Case or Control sheds in naturally ventilated houses (N=41)

Student's t-tests, independent variables*	Control mean	Case mean	t-value	df	p	Control valid N	Case valid N	Total N
Age transferred (wk)	16.09	16.10	-0.03	39	0.974	23	18	41
Stocking density (birds/m²)	10.17	9.61	1.44	39	0.158	23	18	41
Perch space in lay (cm/bird)	45.63	86.51	-1.77	37	0.084	21	18	39
Total nest space on record (m ²)	153.74	144.93	0.70	36	0.490	21	17	38
Nest space on record (birds/m²)	101.41	115.86	-2.44	36	0.020	21	17	38
Age of first access to nest boxes (wk)	16.63	16.57	0.21	35	0.836	21	16	37
First let out age (wk)	22.12	22.71	-0.69	32	0.497	17	17	34
Range size (ha)	7.06	5.65	1.07	36	0.291	21	17	38
Range density (birds/ha)	3232	4666	-1.59	36	0.122	21	17	38
Number of rations from arrival to 40 weeks of age	1.87	1.61	0.82	39	0.416	23	18	41
Body weight at arrival (kg)	1.38	1.39	-0.34	38	0.735	23	17	40
Age at first egg (wk)	19.02	18.84	0.49	32	0.629	18	16	34
Age at 5–10% HD (wk)	19.65	20.16	-1.34	35	0.190	21	16	37
Age at 60% HD	21.71	22.38	-1.59	34	0.122	20	16	36
Body weight at 60% HD (kg)	1.75	1.80	-2.06	28	0.049	17	13	30
Age at peak lay (wk)	28.86	27.55	0.86	34	0.394	19	17	36
Body weight at peak lay (kg)	1.85	1.90	-1.30	20	0.207	11	11	22
Time since transfer to 60% (wk)	5.40	6.16	-1.71	34	0.097	20	16	36
Body weight gain from transfer to 5–10%HD (g)	251	275	-0.72	29	0.477	18	13	31
Body weight gain from transfer to 60% HD (g)	341	418	-106.6	28	0.042	17	13	30
Body weight gain from transfer to peak lay (g)	487	494	-0.19	20	0.854	11	11	22
Range density (birds/ha)	3232	4666	-1585	36	0.122	21	17	38

* Highlighted factors deemed worthy of further statistical evaluation (P<0.20).

In a database with a relatively small sample size, confounding, interaction and multicollinearity between factors is a significant issue. These relational problems often produce aberrant analyses. These interrelationships need to be assessed to determine the most meaningful associations between factor presence and the disease outcome. Entering each variable into a logistic regression model can reveal erroneous issues where extreme values of standard errors lead to biased or unstable associations. Some variables had minimal valid numbers for either Case or Control (e.g. nest closure at night) and could not be validly analysed.

Table 11 shows a univariate logistic regression outcome for each of the variables selected for further analysis. This analysis showed that nest/slat brand and nutritionist produced large standard errors giving unstable estimates of effect (β) and extreme odds ratios (Ω) between types. Closure of nests overnight was only done in sheds, and was not valuable for estimation of odds ratio.

Table 11 Univariate logistic regression for selected factors for risk of SLD

Variable	Level	B ¹	Standard error	Ω ²	Wald's test	P=
Nest/slat brand	Big Dutchman	1.60	737.7	11.14	4.19	0.38
	Facco	-16.39	1475.5	1.7 x 10 ⁻⁷		
	Roxell	-0.41	737.7	1.49		
	Salmat	16.01	2660.0	2.02 x 10 ⁷		
	Vencomatic	reference	unstable			
Nests closed at night	Yes	Undeterminable				
Nutritionist	A	-5.26	608.2	5.00	2.53	0.47
	B	-3.65	608.2	1.47 x 10 ⁸		
	C	reference	unstable			
	D	13.55	1824	1.875		
Perch space in lay	cm/bird	0.008	0.0046	1.01	2.94	0.087
Range density	1000 birds/m ²	0.0002	0.0001	1.00	2.37	0.12
Stocking density in shed	Birds/m ²	-0.381	0.270	0.68	2.00	0.157
Nest space	Birds/m ²	0.086	0.057	1.09	2.297	0.130
Time from transfer to 60% HD	Weeks	0.476	0.305	1.61	2.437	0.119

¹ Logistic regression coefficient.

² Odds ratio for risk of SLD.

The associations of nest/slat brand, closure of nests at night and nutritionist delivered non-significant results and were removed for further analysis. Perch space in lay, stocking density in the shed, range density, nest space and time from transfer to 60% HD produced statistically valid estimates and odds ratios. These remaining terms with a were entered into a multiple logistic regression model (Table 12).

Table 12 Multivariate logistic regression for valid factors for risk of SLD

Variable	Level	B ¹	Standard error	Ω ²	Wald's test	P=
Intercept		-28.499	15.74			
Perch space in lay	cm/bird	0.007	0.013	1.01	0.30	0.581
Range density	1000 birds/m ²	0.340	0.331	1.40	1.06	0.304
Stocking density in shed	Birds/m ²	-1.532	0.608	0.216	6.34	0.012
Nest space	Birds/m ²	6.628	3.026	756.3	4.80	0.029
Time from transfer to 60% HD	Weeks	0.664	0.428	1.94	2.40	0.121

¹ Logistic regression coefficient.

² Odds ratio for risk of SLD.

This logistic regression model assesses each variable controlling for each other variable in the model. Controlling for the presence of other variables, perch space in lay and range density became non-significant and were dropped from the model. Table 13 shows a forward stepwise multiple logistic regression of the remaining three variables and interaction terms were added to the model. All three remaining individual variables contributed to the most parsimonious model, the interaction terms, although significant, resulted in a higher BIC (Bayesian Information Criterion) score for the overall model and hence are not considered further.

Table 13 Forward stepwise multiple logistic regression output for risk of SLD

Variable	Level	B ¹	Standard error	Ω ²	Wald's test	P=
Intercept		-18.697	30.293			
Stocking density in shed	Birds/m ²	-4.506	2.328	0.011	3.746	0.053
Nest space	Birds/m ²	6.628	3.026	1165.9	2.662	0.103
Time from transfer to 60% HD	Weeks	3.829	2.601	46.02	2.166	0.141
Stocking density x nest space		7.272	4.416	1439.9	2.712	0.010
Nest space x transfer to 60% HD		-7.895	5.248	0.0004	2.263	0.135
Stocking density x transfer to 60% HD		1.055	0.711	2.871	2.202	0.138
-Log Likelihood = 24.73	P= 0.0004	Lack of fit (D) =	0.678			

¹ Logistic regression coefficient.

² Odds ratio for risk of SLD.

High standard error values and extreme odds ratios (Table 12) were suggestive of confounding between these variables. The three remaining variables ($P < 0.20$) were examined for confounding in the model by a method as suggested by Dohoo (1992). The extent of change in the regression coefficients when one variable was added to the analysis was measured and confounding was considered significant if this change exceeded 30%.

Stocking density was strongly confounded by the addition of nest space to the model (41% change in coefficient estimate) and also by time from transfer to 60% HD (66% change). Nest space and transfer time to 60% HD were not confounded. Further, looking at the univariate logistic regression models for each variable, the deviance for the stocking density alone model was significant, indicating that this model did not fit the data well. Nest space and transfer time to 60% HD showed acceptable model fits. Hence the final model for assessment of risk factors for SLD in naturally ventilated fully slatted floor sheds contained only nest space (birds per m²) and time (weeks) from transfer to the laying quarters until the flock reached 60% HD. A term for the interaction between these remaining two variables was added to the model. The final model is shown in Table 14. The means and 95% confidence limits for nest space allowance for Control and Case flocks is shown in Figure 1.

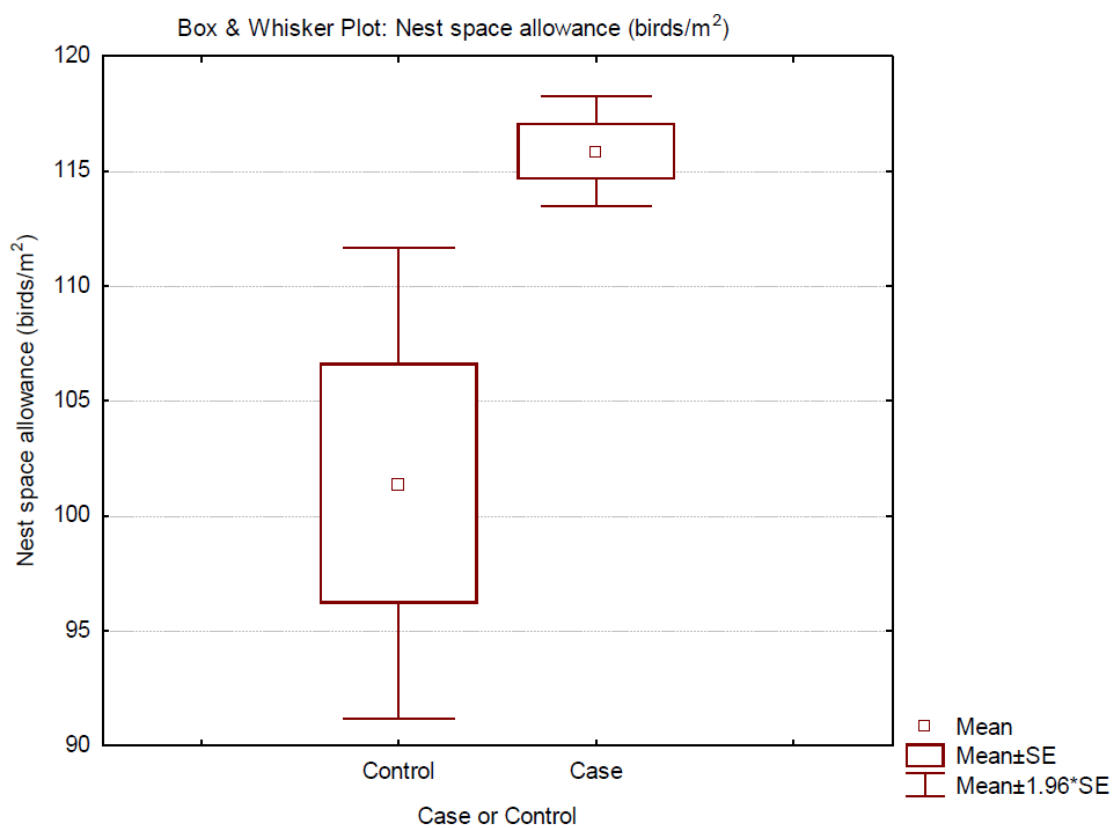


Figure 1 Comparison of means and 95% confidence intervals for nest space allowance for Control and Case flocks in naturally ventilated houses

Whisker lines represent $\pm$ 95% confidence limits.

Table 14 Final multivariate logistic regression analysis of identified variables associated with SLD

Term	B ¹	Std Error	Likelihood Ratio tests			Ω^3	Ω confidence limits	
			Chi-Square	P=	LogWorth ²		Lower 95%	Upper 95%
Intercept	-28.396	15.974						
Transfer to 60% (wk)	1.747	1.042	3.841	0.050	1.301	5.736	1.000	63.645
Nest space on record (birds/m ²)	0.158	0.100	5.910	0.015	1.822	1.172	1.017	1.471
Nest space on record (birds/m ²) x Transfer to 60% HD (wk)	-0.136	0.102	2.079	0.149	0.826	0.873	0.694	1.043
Whole model test								
	Model	-Log-Likelihood	DF	Chi-Square	P=	R-Square (U)	0.2401	
	Difference	5.93666	3	11.87332	0.0078	AICc ⁴	46.8782	
	Full	18.793956				BIC ⁵	51.922	
	Reduced	24.730617				Observations	36	

¹ Logistic regression coefficient.

² A statistic based on Chi-square to evaluate a contribution due to chance in the full model ($=(-\log_{10}(p\text{-value}))$) – higher values signify more contribution to the model.

³ Odds ratio for risk of SLD.

⁴ Akaike Information Criterion corrected for small sample sizes

⁵ Bayesian Information Criterion

3.4 Discussion

The data indicated that sheds with tunnel ventilation have some protection against the occurrence of SLD. Courtice (2022) reported that the severity of SLD can be much reduced if the shed temperature can be reduced by 8°C. This intervention will obviously be more possible in mechanically ventilated sheds than in those relying on open sided ventilation (termed 'natural ventilation' in the present report). The finding in the present study would suggest that protection against an outbreak of SLD may be possible if temperature control is optimal beforehand. The absence of clinical SLD in the present study in tunnel ventilated sheds meant that further analyses could only be considered across naturally ventilated sheds within the database. This observation was consistent with putative findings from Survey 2.

In continuing analysis for naturally ventilated houses, univariate analyses identified many factors within our probability capture range (those showing an association with SLD occurrence with a statistical probability of $P < 0.20$; Hosmer et al. 2013). These factors were then subject to multiple logistic regression techniques to draw out the most likely contributors and develop a parsimonious model for risk of SLD. A high level of confounding between factors was detected. Confounding occurs when a variable is associated both with the dependent (outcome) variable (in this case, occurrence of SLD) but this factor is also associated with another variable (e.g. stocking rate inside the shed and nest space available). Confounding gives rise to overestimation of coefficients and very high standard errors, tending to overestimate the importance of the factors. Once the confounder was removed, acceptable odds ratios were developed for the important factor. Interaction, where the outcome from one associated factor may be different in the presence of another factor, was not found to be complicating the final model.

Nest space (number of birds per m² of nest) emerged as the most important remaining factor ($P = 0.015$) in the model for naturally ventilated sheds within this survey. The time between transfer to layer quarters and when the flock reached 60% HD production was close to statistically significant ($P = 0.050$).

3.4.1 Nest space

An odds ratio of 1.172 for higher number of birds per square metre of nest space was estimated. That is, for every increase of 1 bird per m², the risk of SLD rises by 1.17 times. This was consistent with the preliminary findings from Survey 2. The mean nest space for Control flocks was 101.4 birds per m² of nest space compared with 115.9 for flocks that experienced SLD (Cases). Hence, using the means, the risk of increasing the number of birds from 101 to 115 is $(1.172)^{14} = 9.23$. In other words, increasing by 14 birds/m² nest space would increase the risk of SLD occurring by 9.23 times (i.e. a 923% increase in risk). The sample means here are an estimate of the actual population mean and the 95% confidence limits for the Control mean were between 90.5 and 112 birds/m² of nest space, while the Case 95% confidence limits were from 113.3 to 118.5 birds/m². The suggested maximum nest space allocation suggested by a free range brown egg layer breed management manual (HLB 2021) is 120 birds/m² in colony nests. To make a recommendation from the survey data, it would be advisable to not exceed 112 birds/m² of nest space in naturally ventilated sheds to avoid a higher risk of SLD occurring.

3.4.2 Time lag to production levels

There were several variables involving time for the birds to begin and continue into egg production and an association with the occurrence of SLD in the flock. These included age at 5% HD ('point of lay'), age at 60% HD, body weight at 60% HD, time (weeks) from the birds' arrival in the laying shed ('transfer') until the flock reached 60% HD, and weight gain from transfer to 60% HD. There were

missing data for some of these factors as farmers do not consistently weigh birds at each week, and body weights at 5% or 60% HD were not always available. However, all these factors are linked, as weight will be associated with age. In all the factors considered in this group, the mean age for Control flocks was about 0.5 to 0.6 weeks younger than for Case flocks. For this reason, time from transfer to 60% HD was selected as the indicator variable for this set of factors. It is not clear whether this association represents a cause or an effect. Arguably flocks coming into lay slightly later may be more likely to experience SLD or conversely, flocks being affected by early infection with SLD may have their onset of lay delayed. It is not possible to determine which way this works from the data available. What can be said, however, is that if a flock is somewhat later into lay (say, point of lay at 20 weeks rather than 19 weeks of age) it may be more at risk of a subsequent SLD outbreak.

3.4.3 Stocking density

The confounding between stocking density and nest space allowance is interesting. While higher numbers of birds per nest space area was a risk factor for SLD, higher numbers of birds per m² of available floor space was protective (odds ratio < 1.0). The breed manual recommends 7–9 birds/m² of useable space in free range houses (HLB 2021). The stocking density range observed in this study had a mean of 9.92 birds/m² with a range of 7.44 to 11.92 birds/m² of useable floor space. There was no correlation between nest space and shed stocking density (correlation coefficient, $r = -0.05$, $P = 0.766$). However, nest space (birds/m²) was strongly negatively correlated with the ratio of total nest space (m²) in the shed to the total available floor space (m²) ($r = -0.92$, $P < 0.0001$). From this it is obvious that where nest space makes up a greater proportion of the shed area, there will be a lower number of birds per m² of nest space, but an increase in the number of birds per m² of available floor space. This would explain the confounding between the two measures. Statistically the measure of fit (deviance) of the model of stocking density as a risk factor for SLD showed that this parameter did not fit the data well, and thus nest space was more significant and is the risk factor identified here for SLD.

3.5 Conclusions

In layer sheds that have a full slat coverage of the floor area, a further key determinant of the risk for SLD occurrence in the flock is having a natural ventilation system (i.e. an open sided shed with curtains or shutters and only stirring fans inside the shed). Tunnel ventilation systems may be protective against SLD.

In fully slatted sheds with a natural ventilation system, increasing the number of birds per square metre of nest space results in an increased risk of SLD occurrence. Based on the available data for this survey, every increase of one extra bird per m² nest space increases the risk of SLD by 1.172 times (i.e. by 17.2%). The highest recommended nest space allocation developed from this survey data would be 112 birds/m² of nest space for colony nest systems.

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5 Plain English Summary

Project Title:	An analytical epidemiological study of Spotty Liver Disease
Australian Eggs Limited Project No	1BS004
Researchers Involved	P.J. Groves, Y.K. Gao, M. Singh, W. Muir
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Objectives	To identify key determinants for Spotty Liver Disease (SLD).
Background	<i>Campylobacter hepaticus</i> is the recently identified cause of SLD. Little is known of its epidemiology in egg layer free range and barn flocks. Filling some of the knowledge gaps will allow improved control of this troublesome disease.
Research	Three field surveys were conducted across Australia to create a database capable of epidemiological analyses to identify risk factors for SLD.
Outcomes	Three major risk factors and one potential predictor factor for SLD were identified. The first was that the presence of a scratch area in free range or barn sheds is a major risk factor for SLD. Within sheds that do not have a scratch area, tunnel ventilation was identified as protective against SLD, while in naturally ventilated sheds, higher numbers of birds per square metre of nest space was a risk factor. Flocks being slightly later into lay may be at a greater risk of subsequent SLD.
Implications	Scratch areas are a sufficient cause for SLD if the <i>C. hepaticus</i> is present. Tunnel ventilation may provide some protection against an SLD outbreak by better temperature control. Stocking birds at below 112 birds per m ² of nest space may decrease risk of SLD in naturally ventilated sheds. Flocks coming into lay a week late should be monitored for signs of SLD.
Key Words	Spotty Liver Disease, <i>Campylobacter hepaticus</i> , scratch area, ventilation, nest space.
Publications	Gao et al. (2021). Epidemiology of Spotty Liver Disease. Proceedings Australasian Veterinary Poultry Association scientific meeting. May, online. Groves et al. (2021). Spotty Liver Disease and risk factors. Proceedings Australasian Veterinary Poultry Association scientific meeting. February, Sydney.

Groves et al. (2021). An epidemiological study of Spotty Liver Disease (*Campylobacter hepaticus*) in free range layer hens. Proceedings of the Australia New Zealand College of Veterinary Scientists. July, Gold Coast.

Groves et al. (2022). Epidemiology of Spotty Liver Disease – starting from scratch. Proceedings Western Poultry Disease Conference, Vancouver, Canada

Gao et al. (2022). Spotty Liver Disease and risk factors – starting from scratch. Poultry Information Exchange. May, Gold Coast

Appendix 1 – SLD Rearing Survey

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*SLD Rearing Survey
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1. Personal Info

SLD Trial ID

Date

Participant name

Contact number

Email

Farm owner

Farm manager

Other

Any comments

2. Property Info

Company name

Farm name

Farm address

Property Identification Number (PIC)

Farm size

Number of sheds

Is the site multi-aged?

☐ Yes ☐ No

If multi-age on site, how? E.g. age differences

Do you rear and lay on the same property?

☐ Yes ☐ No

Other domestic animals on property?

☐ Yes ☐ No

What domestic animals are they

Water Sources

Water source for drinking

☐ Town water ☐ Bore water ☐ Dam water ☐ Rain water ☐ Other

Water source for cooling

☐ Town water ☐ Bore water ☐ Dam water ☐ Rain water ☐ Other

Water source for washing	
☐ Town water ☐ Bore water ☐ Dam water ☐ Rain water ☐ Other	
Water source for irrigation	
☐ Town water ☐ Bore water ☐ Dam water ☐ Rain water ☐ Other	
Any run off into surface water?	
Any water treatment(s)?	
Any regular water testing(s)	
(What and frequency)	
Other	
Any comments	

3A. Shed Interior

Trial Shed

Trial shed number

Shed type

☐ Cage ☐ Barn ☐ Aviary ☐ Free Range ☐ Other

Other shed type (please specify and describe)

Number of bays

Shed Dimensions

Shed width (m)

Shed length (m)

Shed Floor

Shed surface area (m2)

Floor Type

☐ Concrete floor ☐ Clay floor ☐ Slats only ☐ Concrete with slats ☐ Clay with slats**Slats**

Slat width (m)

Slat length (m)

Slatted area (m2)

Slat coverage (%)

Platforms

Platforms in the shed ☐ Yes ☐ No

Platform design
(e.g. where, material, shape)

Number of platforms in shed

Platform width (m)

Platform length (m)

Platform surface area (m²)

Total platform areas in shed

Total Useable Area

Total useable area (m²)

Perches

Perches in the shed ☐ Yes ☐ No

Perch design
(e.g. where, material, shape)

Perch length per perch (m)

Number of perches in shed

Total perch length in shed (m)

Aviary Shed

Number of aviary unit(s) _____

Aviary unit design _____

Litter

Litter in the shed

☐ Yes ☐ No (faeces only)

Litter material type _____

Litter depth (cm) _____

Litter quality

☐ Dry ☐ Wet**Other**

Any comments _____

3B. Shed Feeding System

Feeding System	
Feeding system type	☐ Pan feeders ☐ Chain feeders ☐ Other
Pan brand	_____
Pan model	_____
Pan shape	☐ Round ☐ Eclipse
Pan diameter (cm)	_____
Feeding position size	_____
	(Width (cm))
Feeding positions per pan	_____
	(How many per pan)
Number of feeder lines in the shed	_____
Number of feeder pans per line	_____
Total number of feeder pans in the shed	_____
Total number of feeding positions in the shed	_____
Distance between pans (cm)	_____
	(Rim to rim)
Pan height off floor (cm)	_____
	(Height at rim)
Feeder line cable	☐ With cable ☐ Without cable ☐ Both (Prevent pans from swinging)

Chain Feeders Only	
Chain feeder brand	
Chain feeder model	
Number of chain feeders in the shed	
Length of each chain feeder line (m)	
Total length of chain feeder in the shed (m)	
Other feeder type (please specify and describe)	

3C. Shed Drinking System

Drinking System	
Drinking system type	☐ Nipple drinkers ☐ Other
Nipple Drinkers only	
Drinker model	
Drinker brand	
Number of drinker lines in the shed	
Number of drinker nipples per line	
Total number of drinker nipples per shed	
Distance between drinker nipples (cm)	
Drinker height off floor (cm)	 (Height at drinker nipple)
Drip cups	☐ With cups ☐ Without cups ☐ Both
Other drinker type (please specify and describe)	

3D. Range Info

Pop Hole

Position of pop holes

☐ One side only ☐ Both sides
☐ Other

Other pop hole positions (please specify and describe)

Total number of pop holes

Size of pop holes

(Width (cm))

Range Setup

Range design

Range size

(Please specify unites)

Vegetation on range

☐ Grass ☐ Shrubs ☐ Trees ☐ No vegetation (mainly dirt floor)

Mowing frequency

Shade on range

Feed access on range

☐ Yes ☐ No

Drinking water on range

☐ Yes ☐ No

Water on Range	
Water body on range	☐ Yes ☐ No
Access to water body on range	☐ Yes ☐ No
Ditches/holes/drains when it rains	
Other Animals	
Predators on range	☐ Yes ☐ No
What predators are they	
Wildlife on range	☐ Yes ☐ No
What wildlife are they	
Other domestic animals on range	☐ Yes ☐ No
What domestic animals are they	

4A. Bird History

Bird Source	
Breed	
Hatchery	
Date of hatch	
Date of delivery to rearing farm	
Age of birds at visit (weeks)	
Number of birds delivered	
Number of birds on hand	
Intended laying site and shed	
Distance from laying farm (km)	
Intended delivery age to laying farm (weeks)	
Bird History	
Beak trimming	☐ None ☐ Once ☐ Twice
Beak trimming age(s)	
Rearing vaccination program	
	(Age, strain, how, %)
Was there any known issues with vaccination this batch?	
Any monitoring tests available for this batch e.g. serology	

Standard worming program	
Attending vet and frequency	
Other	
Any comments	

4B. Bird Info

Bird Weight

Last body weight available (kg)

How old was the flock at last weighing (weeks)

Bird weight at end of rearing (kg)

(Will this be available to us for this flock?)

Feeder Space - Pan

Feed space at delivery - Pan (birds per pan)

Feed space at visit - Pan (birds per pan)

Feeding position ratio at delivery - Pan (birds per feeding position)

Feeding position ratio at visit - Pan (birds per feeding position)

Feeder Space - Chain

Feed space at delivery - Chain (cm per bird)

Feed space at visit - Chain (cm per bird)

Drinker Space

Drinker space at delivery (birds per nipple)

Drinker space at visit (birds per nipple)

Perch Space

Perch space at delivery (cm per bird)

Perch space at visit (cm per bird)

Density - Floor

Stocking density at delivery - Floor (bird per m2)

Stocking density at visit - Floor (bird per m2)

Density - Useable Space

Stocking density at delivery - Useable Space (bird per m2)

Stocking density at visit - Useable Space (bird per m2)

Free Range Density

Free range density at delivery (m2 per 1000 bird)

Free range density at visit (m2 per 1000 bird)

Range Access

First let out age (wk)

Range let out time

(In 24 hr format)

Range close time

(In 24 hr format)

Range access per day (hr)

What percentage of flock uses the range (%)

What percentage of the range is used by the birds (%)

Does allowed range access vary with season and weather

☐ Yes ☐ No

How?

4C. Bird Feed And Water

Feed Source	
Feedmill	_____
Nutritionist	_____
Feed specifications available?	_____
Ration(s) since placement	_____
Age(s) of ration change (weeks)	_____
Feed type for each ration (e.g. crumble, mash, pellet)	_____
Feeding program	☐ Ad lib ☐ Restricted feeding
Feed management practice	☐ Varied feed run times ☐ Midnight runs ☐ N/A ☐ Other
Feed management practice - other	_____
Feed Additives	
Feed additives?	☐ Probiotics ☐ Prebiotics ☐ Essential oils ☐ Organic acids ☐ Oregano ☐ Other ☐ N/A
Feed additives - other	_____
Feed additives - please specify	_____

Feed Intake

Feed allocation per bird for each ration (g/bird/day)

Total quantity of each ration ordered (l)

Actual feed intake per bird for each ration at end of rearing (g/bird)

(Will this info be made available?)

Change(s) in daily feed intake, when?

(e.g. interruptions, sudden increase or decrease)**Water Intake**

Water measurement available?

☐ Yes ☐ No

Water intake rate/ pattern

Change(s) in daily water intake, when?

(e.g. any interruptions, sudden increase or decrease)

Any water additives given this batch?

Other

Any comments

4D. Bird Lighting

Light schedule (from day old)

Change(s) in light schedule, when?

(e.g. any interruptions)

Type of light units

Dimmable light

Colour of light

Light intensity (lux)

How many rows of lights in the shed

Number of light units per row

Total number of light units in the shed

Other

Any comments

4E. Bird Environment Condition

Ventilation

Shed ventilation system

☐ Natural ventilation ☐ Tunnel ventilation
☐ Other

Shed ventilation system - other

Any additional cooling system(s)

Any known ventilation issues this batch?

Environment Conditions

Any temperature and humidity monitoring system available?

Targeted temperature range at time of visit (C)

Maximum temperature reached in summer inside the shed (C)

Maximum temperature reached in summer outside the shed (C)

Targeted humidity range (%)

Maximum humidity reached in summer inside the shed (%)

Maximum humidity reached in summer outside the shed (%)

Ammonia

Is ammonia an issue in the shed?

Ammonia reading, if available

Other

Any comments

4F. Bird Health

Bird Health

Total mortality at time of visit (%)

Main cause(s) of mortality so far

Disease history (for this flock, shed and farm)

Antimicrobial history (for this flock, shed and farm)

Any known antimicrobial resistance(s) on this farm?

Any pecking in the flock at time of visit

Pecking% (if applicable)

Other medication history

(E.g. worming, anti-coccidials)

Enteric Health

Has the flock experienced any diarrhoea event(s) (abnormal droppings)?

Has the flock had coccidiosis? Were they treated?

Has the flock had roundworm infestation? Were they treated?

Has the flock had tapeworm infestation?

Has the flock had bacterial enteritis? E.g. Gray gut? Were they treated?

Other Issues

Has the flock experienced a peritonitis problem?

Has the flock experienced external parasite infestation? Was this treated? E.g. red mites, lice

Any other diseases occurred in this flock? E.g. ILT, IB etc

Has the flock experienced any wildlife invasion?

Has the flock experience any smothering problems?

Any other known cause(s) to mortality not mentioned above? E.g. Ventilation issues

Other

Any comments

5. Biosecurity

Biosecurity	
Farm specific biosecurity plan available	☐ Yes ☐ No
Vehicle disinfection equipment available	☐ Yes ☐ No
Bird (pullet) crate disinfection	☐ Yes ☐ No
Any equipment sharing with another farm?	
Any personnel sharing with another farm?	
Any contractor sharing with another farm?	
Quarantine period between farms of same operation (days)	
Quarantine period required from another operation (days)	
Any known vector connection with other SLD positive farms?	 (E.g. Vehicle, personnel)
Shed manure disposal practice	
Dead bird disposal practice	
Shed resting time prior to this placement	
Shed cleaning and sanitisation at the end of last batch	
What was the rodent burden like in this shed (and range)?	

What is the rodent control program?

What was the fly burden like in this shed?

What is the pest control program e.g. beetles and flies

Other

Any comments

Appendix 2 – SLD Laying Survey

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*SLD Laying Survey
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1. Company Info

SLD Trial ID	
Date	
Company name	
Farm name	
Farm owner	
Farm manager	
Participant name	
Farm address	
Postal address	
Contact number	
Email	
Company Info e.g. no. of rearing and/or laying farms; bird numbers; production types	
Other	
Any comments	

2. Property Info

Property Identification Number (PIC)

Property size (production area)

Total number of sheds on the farm

Total number of birds on the farm

Water Sources

Water source for drinking

☐ Town water ☐ Bore water ☐ Dam water ☐ Rain water ☐ Other

Water source for cooling

☐ Town water ☐ Bore water ☐ Dam water ☐ Rain water ☐ Other

Water source for washing

☐ Town water ☐ Bore water ☐ Dam water ☐ Rain water ☐ Other

Water source for irrigation

☐ Town water ☐ Bore water ☐ Dam water ☐ Rain water ☐ Other

Any run off into surface water?

Any water treatment(s)?

Any regular water testing(s)

(What and frequency)

Other

Any comments

3A. Shed Interior

Trial Shed

Trial shed number

Shed type

- ☐ Free range ☐ Barn
☐ Other

Other shed type (please specify and describe)

Shed Dimensions

Number of bays

Shed width (m)

Shed length (m)

Shed height (m)

Shed Floor

Shed surface area (m2)

Floor Type

- ☐ Concrete floor only ☐ Clay floor only ☐ Slats only ☐ Concrete with slats ☐ Clay with slats

Slat coverage (%)

Platforms

Platforms in the shed

- ☐ Yes ☐ No

Platform design

Number of platforms in shed

Platform width (m)

Platform length (m)

Platform surface area (m2)

Total platform areas in shed

Do birds use platform regularly? Any issue with getting birds onto platforms?

Perches

Perches in the shed

☐ Yes ☐ No

Perch design

Perch length per perch (m)

Number of perches in shed

Total perch length in shed (m)

Perch space per bird (if known)

Do birds use perches regularly? Any issue with getting birds onto perches?

Nest Boxes	
Nest box type	
Nest box brand	
Nest box model	
Nest box design	
Rows of nest boxes in shed	
Number of nest boxes per row	
Total nest boxes in shed	
Nest box width (m)	
Nest box length (m)	
Nest box height (m)	
Nest box size (m2)	
Total nest box area in shed (m2)	
Nesting space per bird (if known)	
Any issue with getting birds to use nestboxes?	

Total Useable Floor Area

Total useable floor area (m2)

Useable floor area per bird (if known)

Litter

Litter in the shed

☐ Yes ☐ No (faeces only)

Litter material type

Litter depth (mm)

Litter quality

☐ Dry ☐ Wet

3B. Shed Feeding System

Feeding System	
Feeding system type	☐ Pan feeders ☐ Chain feeders ☐ Other
Pan brand	_____
Pan model	_____
Pan shape	☐ Round ☐ Eclipse
Pan diameter (cm)	_____
Feeding position size	_____
	(Width (cm))
Feeding positions per pan	_____
	(How many per pan)
Number of feeder lines in the shed	_____
Number of feeder pans per line	_____
Total number of feeder pans in the shed	_____
Distance between pans (cm)	_____
	(Rim to rim)
Feeder line cable	☐ With cable (electrified) ☐ With cable (non-electrified) ☐ Without cable
Chain Feeders Only	
Chain feeder brand	_____
Chain feeder model	_____
Chain feeder design	_____

Number of chain feeders in the shed

Length of each chain feeder line

Total length of chain feeder in the shed

Other Feeding System

Other feeder type (please specify and describe)

Feeder Accessibility

What percent of feeding spaces are accessible to birds at time of feeding? e.g. 100%, 75%, 50% or less.

What are the reason(s) if less than 100% feed accessibility?

Other

Any comments

3C. Shed Drinking System

Drinking System	
Drinking system type	☐ Nipple drinkers ☐ Other
Nipple Drinkers only	
Drinker model	
Drinker brand	
Number of drinker lines in the shed	
Number of drinker nipples per line	
Total number of drinker nipples per shed	
Distance between drinker nipples (cm)	
Drip cups	☐ With cups ☐ Without cups ☐ Both
Other Drinker Type	
Other drinker type (please specify and describe)	
Drinker Accessibility	
What percent of drinkers are accessible to birds at time of drinking? e.g. 100%, 75%, 50% or less.	
What are the reason(s) if less than 100% feed accessibility?	

Other

Any comments

3D. Distance Between Structures

From Feed	
Feed line to Feed line (max)	
	(Rim to rim)
Feed line to Feed line (min)	
	(Rim to rim)
Feed line to drinker line (max)	
Feed line to drinker line (min)	
Feed line to nest boxes (max)	
Feed line to nest boxes (min)	
Feed line to pop holes (max)	
Feed line to pop holes (min)	
Feed line to perch (max)	
Feed line to perch (min)	
Feed line to platform (max)	
Feed line to platform (min)	
From Drinker	
Drinker line to drinker line (max)	
Drinker line to drinker line (min)	
Drinker line to nest boxes (max)	

Drinker line to nest boxes (min)

Drinker line to pop holes (max)

Drinker line to pop holes (min)

Drinker line to perch (max)

Drinker line to perch (min)

Drinker line to platform (max)

Drinker line to platform (min)

From Nest Boxes

Nest boxes to pop holes (max)

Nest boxes to pop holes (min)

Nest boxes to perch (max)

Nest boxes to perch (min)

Nest boxes to platform (max)

Nest boxes to platform (min)

3E. Range Design and Access

Pop Hole

Position of pop holes

☐ One side only ☐ Both sides
☐ Other

Other pop hole positions (please specify and describe)

Pop hole design

Total number of pop holes

Height of pop holes (cm)

Width of pop holes (cm)

Range Boundary

Fencing type

Can this flock access/mingle with other birds from a different flock/range?

Range Setup

Range design

Range size

(Please specify unites)

Vegetation on range

☐ Grass ☐ Shrubs ☐ Trees ☐ No vegetation (mainly dirt)

Other vegetations

Mowing frequency

Irrigation frequency

Shade on range

Feed access on range

☐ Yes ☐ No

If feed on range, please describe.

Drinking water on range

☐ Yes ☐ No

If drinking water in range, please describe.

Other Water Body on Range

Are there any water bodies on range e.g. dam, pits normally, or when rains?

Can birds access to these water bodies on range?

Are there any ditches/holes/drains when it rains?

Animals on Range

Predators on range

☐ Yes ☐ No

What predators are they

Wildlife on range

☐ Yes ☐ No

What wildlife are they

Domestic animals on range e.g. dogs

☐ Yes ☐ No

What domestic animals are they?

Range Access

First let out age (wk)

Range let out pattern (open and close)

(In 24 hr format)

Range access per day (hr)

What percentage of flock uses the range (%)

What percentage of the range is used by the birds (%)

Does allowed range access vary with season and weather, and how?

Any interruption(s) to range let out this batch? When and why?

Other

Any Comments

4A. Bird History

Bird Source	
Breed	
Hatchery	
Date of hatch	
Rearing site and shed	
Distance from rearing farm	
Date transferred	
Age transferred (wk)	
Number of birds transferred	
Number of birds on hand	
Bird History - General	
Laying vaccination program	
	(Age, strain, how, %)
Any monitoring tests e.g. serology	
Any worming treatment in lay	

Other

Any comments

4B. Bird Space and Density

Free Range Density

Free range density (per bird)

Feeder Space

Feeder space (per bird)

Drinker Space

Drinker space (per bird)

Perch Space

Perch space (per bird)

Nest Box Space

Nest box space (per bird)

(bird/m2 or bird/hole)

Stocking Density

Stocking density at delivery

Stocking density at visit

Other

Any Comments

4C. Bird Feed and Water

Feed Source	
Nutritionist	
Feedmill	
Feed specifications available?	
Ration(s) since arrival	☐ Prelay ☐ Layer ☐ Other
Please specify other ration type.	
Age(s) of ration change	
Feed type (e.g. mash, pellet)	
Feeding Practice	
Feeding program	☐ Ad lib ☐ Restricted feeding
Please describe feeding program.	
Additional feed management practice	☐ Varied feed run times ☐ Midnight runs ☐ N/A ☐ Other
Please describe.	
Do the pans or chains ever run empty?	☐ Yes ☐ No
How long would the pans or chains be empty for?	
How often is this practice done?	

Feed Additives

Feed additives?

☐ Probiotics ☐ Prebiotics ☐ Eubiotics ☐ Organic acids ☐ Oregano ☐ Other ☐ N/A

Please specify:

Feed additives specifically for Spotty Liver Disease?

☐ Probiotics ☐ Prebiotics ☐ Eubiotics ☐ Organic acids ☐ Oregano ☐ Other ☐ N/A

What are the feed additive(s) for SLD?

Feed Intake

Feed allocation per bird for each ration (g/bird/day)

Total quantity of each ration ordered (T)

Actual feed intake per bird for each ration by the end of 35wk (g/bird)

Feed intake at 5%HD

Feed intake at 60%HD

Feed intake at peak lay

Did SLD affect feed intake (If applicable)?

☐ Yes ☐ No

Feed intake before SLD occurred (if applicable).

Feed intake at time of SLD (if applicable).

Any feed change(s) or interruption(s), when?

(e.g. interruptions, sudden increase or decrease)

Water Intake	
Water measurement	☐ Yes ☐ No
Water intake rate/pattern	
Any interruptions to water intake, when?	
	(e.g. any interruptions, sudden increase or decrease)
Any water additive(s) given this batch? What and frequency.	
Any water additive(s) specifically for Spotty Liver Disease (if applicable)?	
Other	
Any comments	

4D. Bird Lighting

Light Program	
Light schedule upon arrival (from rearing)	
Light schedule for laying (L:D)	
Changes in light schedule, when?	
Type of light units	
Are lights dimmable to mimic sunlight and sunset?	
Colour of light	
Light intensity (lux)	
Other	
Any comments	

4E. Bird Environment Condition

Ventilation System

Shed ventilation system

☐ Natural ventilation ☐ Tunnel ventilation ☐ Other

Please describe:
e.g. cooling, fans etc

Any known ventilation issue(s) this batch?

Shed Environment

Any temperature and humidity monitoring system
available?

Targeted temperature at time of visit (C)

Maximum temperature reached in summer OUTSIDE the
shed (C)

Maximum temperature reached in summer INSIDE the shed
(C)

Targeted humidity range (%)

Maximum humidity reached in summer OUTSIDE the shed
(%)

Maximum humidity reached in summer INSIDE the shed
(%)

Ammonia

Is ammonia an issue in the shed?

Ammonia reading, if available

Other

Any comments

4F. Bird Weight and Egg Production

Weighing

How often are birds weighed? Method?

Egg Production and Bird Weights

Can weekly WEIGHTS, UNIFORMITY and MORTALITY information be supplied?

Body weight at arrival (kg)

Age of first egg (week)

Body weight at first egg (kg)

Age of 5%HD (week)

Body weight at 5%HD (kg)

Age of 60%HD (week)

Body weight at 60%HD (kg)

Age of peak lay (week)

What is the peak lay%

Body weight at peak lay (kg)

Body weight at onset of SLD (kg) (if applicable)

Egg Collection

Over what period in the day are hens laying?

What time(s) in the day are eggs collected?

How are eggs collected? E.g. egg belt or manual collection?

Any issues with egg collection so far? e.g. interruption, belt breakdown etc

Laying Abnormality

Any issues with egg laying so far?

Has the flock had a high level of prolapses? When?

Has the flock had a high level of double yolk eggs? When?

Has the flock had abnormal egg sizes for age e.g. small or large? When?

Has the shell quality, albumen quality and yolk colour been satisfactory since early lay?

Has there been any problem with floor eggs/eggs outside?

What's floor egg%?

Other

Any comments

4G. Bird Health

Veterinarian

Attending veterinarian and frequency

Was veterinary intervention required for this batch?
Visit or over the phone?
What was the outcome?

Any diagnostic tests performed? What are the results?

Feather Pecking

Any pecking/unusual feather loss or cannibalism been
noted so far? When?

Any pecking/unusual feather loss in the flock at time
of visit?

Pecking% (if applicable)

Feather coverage score (50 birds)

Moulting

Has the flock gone through a partial or full moult
since arrival? When?

Cause(s) of the moulting?

Diseases - General

Disease history (for this flock, shed and farm)

Total mortality at time of 35 weeks of age (%)

Total mortality at time of visit (%)

Main cause(s) of mortality so far

Antimicrobial history (for this flock, shed and farm)

Any known antimicrobial resistance(s)?

Other medication history

(E.g. worming, anti-coccidials)

Has the flock experienced any diarrhoea event(s) e.g. abnormal droppings?

Has the flock had coccidiosis? Were they treated?

Has the flock had roundworm or tapeworm infestation? Were they treated?

Has the flock had bacterial enteritis e.g. gray gut? Were they treated?

Other Health Issues

Has the flock experienced a peritonitis problem?

Has the flock experienced external parasite infestation e.g. red mites, lice? Were they treated?

Any other disease occurred during this batch? e.g. ILT, IB, Cholera, Mycoplasma etc?

Has the flock experienced any wildlife invasion this batch?

Has the flock experienced any smothering problems?

Has the flock experienced any production problems?	
Has the flock experienced any unexplained mortality or production problems not mentioned above?	
Other	
Any comments	

5. Biosecurity

Biosecurity	
Farm specific biosecurity plan available	☐ Yes ☐ No
Vehicle disinfection equipment available	☐ Yes ☐ No
Bird (pullet) crate disinfection	
Any equipment sharing with another farm?	
Any personnel sharing with another farm?	
Any contractor sharing with another farm?	
Quarantine period between farms of the same operations (days)	
Quarantine period required from another operation e.g. visitors (days)	
Any known vector connect with other SLD positive farms?	 (e.g. contractors, vehicle)
Waste Disposal	
Shed manure disposal practice	
Dead bird disposal practice	

Cleaning and Disinfection

Shed/range resting time prior to this placement

Shed/range cleaning and sanitisation at the end of last batch

Rodent burden in shed and range e.g. light, moderate, heavy?
Rodent control program?

Pest control e.g. beetles and flies?

Other

Any comments

6. Spotty Liver Disease Questions

Spotty Liver Disease History

Does this company has a history of spotty liver disease?

Does this farm has a history of spotty liver disease?

Are there any changes to the disease experience since the first outbreak? e.g. the disease pattern, severity and/or response to treatment? Any possible reasons?

Did this flock experience any spotty liver disease outbreak(s)?

☐ Yes ☐ No

SLD Outbreak (if applicable)

How many times has SLD occurred so far?

Date of outbreak(s)

Age of outbreak(s)

What were the signs that suggested that SLD was occurring? e.g. mortality rate, production loss, bird depression?

What was the mortality pattern like (before and) during the SLD outbreak? Bird numbers and/or %

How long did the SLD outbreak(s) last for?

Estimated total mortality due to SLD (number of birds or %).

Estimated total egg production losses due to SLD

Did a poultry veterinary diagnose the SLD or confirmed the diagnosis (this batch)?

☐ Yes, by visit ☐ Yes, over the phone ☐ No

How was SLD diagnosed or confirmed by the veterinarian?

☐ Clinical signs ☐ Gross Pathology ☐ Histopathology ☐ PCR ☐ Culture ☐ By treatment
☐ Not sure

SLD Treatment and Management

Did this flock receive any preventative treatment(s) for SLD? E.g. any in feed or in water treatment, or vaccination specific for SLD

How effective were they?

Did this flock receive any antibiotic treatment(s) for SLD e.g. at the time of clinical outbreak?

_____ (What drug, for how long, dose rate.)

How effective were they?

What were the signs/triggers that suggested that antibiotic treatment(s) was required? e.g. mortality rate, production loss, bird depression?

Did this flock receive any non-antibiotic treatment(s) for SLD e.g. at the time of clinical outbreak?

_____ (e.g. any in water or in feed treatment specifically for SLD)

How effective were they?

Has SLD recurred after any of therapeutic treatment(s)?
If yes, how soon?

Any other management strategies not mentioned above applied to prevent the occurrence of SLD or to minimise SLD impact?

Rainfall

Were there any rain event(s) since arrival?

☐ Yes ☐ No

When and how often did the rain occur?

Did birds access the range during and/or after rain?

Did birds drink from puddle water from the range?

☐ Yes ☐ No

Were the birds restricted from range access for any period of time during rainfall?

☐ Yes ☐ No

If restricted access, for how long and how did the birds respond to it, were they stressed?

Did SLD follow any rain event(s)? E.g. within 1-2 weeks (if applicable)

How soon did SLD follow the rain? (if applicable)

Heat Stress

Has there been any ventilation breakdown or interruption during this batch that may have led to SLD?

Were there any extreme heat event(s) since arrival? E.g. >35C

☐ Yes ☐ No

When and how often did the extreme heat occur?

How were the bird kept cool during extreme heat?

Did SLD follow any extreme heat event(s)? E.g. within 1-2 weeks (if applicable)

How soon did SLD follow the extreme heat? (if applicable)

Other environmental Stress

Any other unusual climate/weather event(s) since arrival?

☐ Yes ☐ No

If yes, what unusual climate/weather events occurred?

Did SLD follow any of these unusual weather event(s)? E.g. within 1-2 weeks (if applicable)

How soon did SLD follow the unusual climate/weather event? (if applicable)

Any Interruption(s) to Normal Routine and SLD

Were there any feed change(s) or interruption(s) linked to SLD? ☐ Yes ☐ No

If yes, how and when did the feed change(s) or interruption(s) occur?

Were there any water interruption(s) linked to SLD? ☐ Yes ☐ No

If yes, how and when did the water interruption(s) occur?

Were there any malfunction(s) to nest boxes or egg belt linked to SLD? ☐ Yes ☐ No

If yes, how and when did the egg collection malfunction(s) occur?

Were there any unintended interruptions to range access linked to SLD? ☐ Yes ☐ No

If yes, how and when did the range access interruption(s) occur?

Did SLD follow any of the above interruptions (feed, water, egg collection, range access)? E.g. within 1-2 weeks (if applicable)

Behaviour and Health

Did SLD follow any pecking or moulting? E.g. within 1-2 weeks (if applicable)

Did SLD follow any health issue or disease? E.g. within 1-2 weeks (if applicable)

Other

Any other possible trigger(s) to SLD not mentioned above (if applicable)?

Any comments

Appendix 3 – Phase 2 Survey

Date:	
Company Name:	

	Company name					
	Laying farm name					
	Shed number					
	SAD occurrence (Y/N)					
REARING FARM						
	Date hatched					
	Breed					
	Hatchery					
	Pertches in rearing? (Y/N)					
	Ventilation in rearing shed: tunnel or natural					
LAYING FARM						
	Location of laying farm (Suburb)					
	Date transferred					
	Age transferred (wk)					
	Number of birds transferred					
	Shed type (conventional vs aviary free-range)					
Shed set up	Total usable space (m ²)					
	Stocking density (birds/m ²)					
Slat	Slat brand					
	Slat material (e.g. plastic)					
	Is the slat cleaned during the batch? (Y/N)					
	If yes, how?					
	Is the slat cleaned between batches? (Y/N)					
	If yes, how?					
Pertch	Pertches in lay (Y/N)					

	Total perch length available (m)				
	Perch space n lay (cm/bird)				
Platform	Any platform(s) in shed? (Y/N)				
	Total platform area available (m ²)				
	Platform space (birds/m ²)				
	Is the platform cleaned during batch (Y/N)?				
	if yes, how?				
	Is the platform cleaned between batches (Y/N)?				
	if yes, how?				
Feeding System	Feeder type (chain or pan)				
	Total feed chain length (m)				
	Feed space - Chain (birds per m)				
	Total number of pans (if applicable)				
	Pan brand				
	Feed space - Pan (birds per pan)				
Drinking system	Drinker type				
	Total nipples in shed				
	Drinker space - nipple (birds per nipple)				
	Drinker space - bell (birds per bell)				
	Ventilation in laying farm: tunnel or natural				
Ventilation					
Lighting	Light colour (warm or cool white)				
Nesting	Nest box brand				
	Total nest space available (m ²)				
	Nest space (birds/m ²)				
	Age of first access to nest boxes (wk)				
	Night closure (to prevent bird access) (Y/N)				

	Nest box cleaning during hatch (Y/N)				
	If yes, how?				
	Nest box cleaning between batches (Y/N)				
	If yes, how?				
Range	First let out age (wk)				
	Range size (ha)				
	Range density (birds/ha)				
	Soil type				
Feed and Water	Nutritionist				
	Feedmill				
	Number of rations from arrival to 40 weeks of age				
	Ration 1 start age (wk)				
	Ration 2 start age (wk)				
	Ration 3 start age (wk)				
	Ration 4 start age (wk)				
	Ration 5 start age (wk)				
	Any in feed additives in lay till 40 weeks of age (Y/N)				
	Feed Additive 1 (if applicable)				
	Feed Additive 2 (if applicable)				
	Feed Additive 3 (if applicable)				
	Feed Additive 4 (if applicable)				
	Any water additives in lay till 40 weeks of age (Y/N)				
	Water Additive 1 (if applicable)				
	Water Additive 2 (if applicable)				
	Water Additive 3 (if applicable)				
	Water Additive 4 (if applicable)				
Arrival	Body weight at arrival (kg)				
First Egg	Age at first egg (wk)				

5% HD	Age at 5% HD (wk)				
	Feed intake at 5% HD (g/bird/day)				
	Body weight at 5% HD				
60% HD	Age at 60% HD				
	Feed intake at 60% HD (g/bird/day)				
	Body weight at 60% HD				
Peak lay	Age at peak lay (wk)				
	Peak lay %HD				
	Feed intake at peak lay (g/bird/day)				
	Body weight at peak lay (kg)				
SD outbreak (if applicable)	Age at SD outbreak (wk)				
	Feed intake at SD (g/bird/day)				
	Body weight at SD (kg)				
	Lowest %HD during SD				
	How long did				
	Highest daily mor% during SD				
40 weeks	%HD at 40 weeks				
	Feed intake at 40wks (g/bird/day)				
	Body weight at 40wks (kg)				
	Total mortality at 40wks				
Egg production	Any problem with floor eggs? (Y/N)				
	Floor egg% at peak lay				
	Age occurred				
	Duration of floor egg issue (>2%) (days)				
Disease	Other disease other than SD (Y/N)				
	Age of disease occurrence (wk)				
	Any antibiotic treatment not for SD?				
SD SPECIFIC	Did SD occur this batch (Y/N)				
	Has SD recurred so far?				
	Age of outbreak 1 (wk)				

	How long did the first outbreak last?				
	Age of outbreak 2 (wk, if applicable)				
	How long did the second outbreak last?				
	SD diagnosis based on (select all that apply):				
	Clinical signs (depression)				
	Gross pathology (white spots)				
	Histopathology				
	PCR				
	Culture				
	By treatment				
	Not sure				
	Was this flock treated with antibiotics for SD (Y/N)				
	What antibiotic was used?				
	Other antibiotic used, please specify:				
	Range across prior to SD outbreak?				
	Any comments on SD outbreak of this flock not covered above?				