MANAGEMENT STRATEGIES TO AID IN THE CONTROL OF PROLIFERATIVE ENTEROPATHY

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By

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1.0 Executive Summary

Proliferative enteropathy (PE, or ileitis) is a common production limiting disease in Australia, causing reduced ADG, reduced FCE, and diarrhoea in pigs of all ages. Clinical disease affects up to 35% of grower and finisher pigs, and causes reductions in FCE of up to 50% (Gogolewski *et al.*, 1991). Sub-clinical disease is probably present on 100% of Australian pig herds, causing reductions in ADG between 9% and 42%, and reductions in FCE between 6% and 37% (McOrist *et al.*, 1997; Paradis *et al.*, 2005).

A recent survey of Australian pig practitioners indicated that antibiotics were still routinely prescribed in grower and finisher pig diets for the control of PE. Practitioners wanted to find non-antibiotic means to control PE, including vaccination and management practices to reduce the spread of disease. While vaccination had worked well in some herds, a number of practitioners had experienced difficulties controlling PE with vaccination of pigs housed in ecosheds, and had needed to re-introduce antibiotics post vaccination. The aim of this project was to better understand the spread of disease and the survival of the causative bacteria, *Lawsonia intracellularis*, in order to develop management strategies to reduce the number of *L.intracellularis* in the environment and to aid in the control of PE.

This research project demonstrated that *L.intracellularis* can survive in faeces in empty pens for at least 2 weeks on concrete slatted flooring. Sufficient numbers of *L.intracellularis* survived to transmit infection to naïve pigs introduced into dirty pens 2 weeks later. When the same pens were cleaned and disinfected with Virkon S, *L.intracellularis* was not able to survive in sufficient numbers to transmit infection to naïve pigs introduced into these cleaned pens. This is the first demonstration of the efficacy of cleaning and disinfection alone to prevent the transmission of *L.intracellularis* infection to naïve pigs. While cleaning and disinfection practices are common in farrowing crates and as part of disease eradication procedures; they are seldom used without antibiotic medication or

vaccination. This study provides pork producers and veterinarians with a better understanding of cleaning practices that can reduce the survival of *L.intracellularis* in the piggery. The cleaning strategies can be implemented between batches of pigs on a semi regular basis to reduce the bacterial 'load' of *L.intracellularis,* or as part of a disease eradication program to reduce production losses caused by ileitis.

Previous studies had tested the efficacy of disinfectants in *in-vitro* cultures of *L.intracellularis*, in the absence of organic matter. The relevance of these studies to field use of disinfectants is questionable as a wide range of disinfectants are inactivated by organic matter. The experimental design developed as part of this project can be used by chemical manufacturers to evaluate the efficacy of other disinfectants to reduce the spread of ileitis in piggeries.

The efficacy of cleaning and disinfection procedures to reduce the spread of ileitis in ecosheds with straw or rice hull bedding was also studied as part of this project. Seven herds were chosen where pigs were naïve to *L.intracellularis* prior to entering the ecoshed, to ensure that the major source of *L.intracellularis* came from the ecoshed environment. Piggeries were selected with a wide range of cleaning and disinfection practices between batches of pigs. Individual pigs were monitored by serology over 20 weeks and two subsequent batches of pigs from each herd were monitored to evaluate the effect of cleaning practices between batches of pigs on the prevalence of *L.intracellularis* infection and the severity of ileitis.

A number of significant problems plagued Australia pork producers during the period of this study, and these impacted on our ability to obtain results from our field research. The increasing cost of pork production, due to the drought and pig meat imports led to the closure of two pig herds mid trial. The drought also led to the reduced supply of bedding material for ecosheds, encouraging the recycling of bedding material and the increased use of antibiotic medication to compensate for poor hygiene in sheds. Our results were frequently confounded by changes in antibiotic medication mid trial, which lead to significant changes in disease prevalence. We were unable to determine whether changes in the disease level

between batches of pigs were due to cleaning practices or the introduction of new antibiotics. Medication changes were primarily made to control outbreaks of other diseases, but the trial was also affected by medication changes due to the introduction of new withholding periods for antibiotics in finisher pigs (export slaughter intervals).

As a consequence of the above changes, we developed an alternative study design to evaluate 3 different cleaning protocols to reduce the survival of *L.intracellularis* in ecosheds and improve the control of ileitis (including growth performance). The cleaning protocols were to be tested in a single batch of pigs housed in 9 separate ecosheds. An outbreak of the haemorrhagic form of PE caused significant mortalities in finisher pigs mid trial. In order to prevent mortalities in adult pigs, the veterinarian removed antibiotics from the weaner pigs to induce immunity at an earlier age. Our trial design was compromised, as pigs were exposed to *L.intracellularis* prior to entry to the ecosheds. The number of animals needed to demonstrate a significant difference in the prevalence of disease between cleaning treatments was dramatically increased, making the trial no longer viable with the available budget.

The trial design that we have developed can be used in other ecosheds to test the efficacy of cleaning treatments to reduce the survival of *L.intracellularis* and the severity of ileitis. We will continue to work with pork producers to identify management practices, including improved hygiene, which will reduce the load of *L.intracellularis* in all types of piggeries as part of new project to develop a semi-quantitative real time diagnostic assay for ileitis.

The third objective of this project was to determine whether *L.intracellularis* can persist in pigs following recovery from disease, and be a source of infection to naïve in-contact pigs. We discovered that recovered pigs were not a source of *L.intracellularis* infection to naïve pigs, even if the pigs were stressed by immuno-suppression. This information is directly useful to pork producers and veterinarians to aid in the design of biosecurity practices and PE eradication strategies on farm.

Our study suggested that antibiotic treatment of PE affected pigs aided recovery and prevented transmission of *L.intracellularis* infection from recovered to naïve in-contact pigs. However, we are not able to say that antibiotic medication is necessary in PE eradication protocols to completely eliminate *L.intracellularis* from pigs.

It is interesting that we were able to detect antigen reactive with an *L.intracellularis* specific antibody bound within membranes in the cytoplasm of intestinal cells from recovered pigs. Previous studies indicated that *L.intracellularis* was present in the tonsillar crypts of severely affected pigs, but our study is the first to demonstrate that *L.intracellularis* antigen may persist in intestinal tissue long after pigs have recovered from disease. Further studies are required to determine whether this antigen is from viable bacteria, and under different circumstances could lead to re-shedding of *L.intracellularis* in recovered pigs.

An opportunity to expand our studies to investigate the role of rodents in the transmission of *L.intracellularis* to naïve pigs has led to future Pork CRC studies. Rodents have been identified as potential external vectors for a number of bacterial and viral pathogens of pigs, including *L. intracellularis*, *Brachyspira pilosicoli*, *B.hyodysenteriae*, *Leptospira sp.* and parvovirus. Monitoring the dispersal of rodents between piggeries in relatively close proximity may be important in assessing the risk of transmission of these pathogens to naïve pigs. While it is likely that rodents play only a minor role in the transmission of bacteria such as *L. intracellularis* to pigs, under certain circumstances they may act as an important reservoir or point source of infection for susceptible pigs, especially if rodents travel from neighbouring farms and transmit diseases to naïve populations of pigs.

The potential for disease transmission from rodents to pigs will be studied by an Honours student using molecular biology techniques to identify the genetic profiles of rodents trapped on different pig farms. The aim of the project is to determine whether genetic variation between populations of rats can be used to demonstrate

the dispersal of rats between relatively close piggeries. If rodents from pig farms 3 km apart have the same genetic profile, then we can conclude that rodents are able to travel 3 km between piggeries and interbreed. However, if the genetic profile of rodents caught on 2 separate farms is different, then we can say that rodents do not travel the distance between the two farms, ie. they do not interbreed and thus form 2 genetically distinct populations of rodents.

In summary, this project has identified a number of potential sources of *L.intracellularis* in the piggery environment. The finding that *L.intracellularis* can survive in contaminated pens for at least two weeks and infect naïve pigs is probably the most important new information for pork producers and veterinarians. The development of cleaning and disinfection practices to reduce the survival of *L.intracellularis* will aid in the control of PE. Although we were not able to identify cleaning practices for ecosheds, we have developed a protocol for future studies. The future development of a semi-quantitative real time diagnostic assay for PE will enable on-farm evaluation of management practices including cleaning and disinfection, antibiotic medication and vaccination. Our studies have demonstrated that carrier pigs are an unlikely source of *L.intracellularis* infection, the presence of membrane bound antigen requires further investigation.

2.0 Background to Research

Proliferative enteropathy (PE) or ileitis is a production limiting disease causing reduced weight gains, reduced FCE, increased variation in days to slaughter and diarrhoea in pigs of all ages, costing up to A$7/pig in production losses and treatment costs (Holyoake *et al.*, 1996). The prevalence of PE has been estimated at 56% of Australian herds, but the prevalence of *Lawsonia intracellularis* infection (the causative agent) and subclinical disease may be as high as estimates in Europe, where more than 94% of herds are infected.

In Australian pig herds, PE is primarily controlled with antibiotics in grower and finisher diets. Strategic medication protocols have been developed to reduce production losses associated with PE, and to induce immunity to disease throughout the pig's life (Collins, 2003). Concern about the potential for antibiotic residues in export pig meat, and the emergence of antibiotic resistant bacteria have encouraged the development of non-antibiotic means to control PE.

A live, oral, avirulent *L.intracellularis* vaccine has been developed in the US (Enterisol Ileitis) for the control of PE, and was introduced into Australia in 2007. Vaccinated pigs demonstrate a significant reduction in intestinal lesions, shorter duration of faecal shedding of *L.intracellularis*, and improved weight gains relative to non-vaccinated pigs following a virulent *L.intracellularis* challenge (Kroll *et al.*, 2005). Uptake of the vaccine in Australian herds has been affected by the requirement to remove all antibiotics for 7 days during the vaccination period. In some herds this has caused significant problems with post weaning diarrhoea. Practitioners have also experienced vaccine failures in ecoshed housed pigs, which has forced them to re-introduce medication to help control PE.

Australian and overseas attempts to eradicate PE have focussed on either partial or total depopulation and repopulation of herds, requiring high levels of medication for all new and existing pigs (Flø *et al.*, 2000; Johansen *et al.*, 2002; Collins, 2003). Almost all of these eradications have failed within one year, with PE reoccurring, seen as scouring in weaner/grower pigs or as the haemorrhagic form of disease, proliferative haemorrhagic enteropathy, PHE, in finisher or breeding pigs. These

eradication strategies create naïve herds with pigs highly susceptible to *L.intracellularis*. Outbreaks of PHE and death may occur if unprotected adult pigs are exposed to significant levels of *L.intracellularis* (2 to 5% mortality). Inducing protective immunity to PE in pigs, either by vaccination or strategic medication will protect pigs from outbreaks of PHE, and can reduce the duration of bacterial shedding, and hence the bacterial "load" in the environment and the pig . However, before PE eradication protocols can be performed with confidence, further studies are needed to investigate persistent infection sources of *L. intracellularis*. Of particular concern is the ability of *L. intracellularis* to survive in the environment and be maintained by carrier pigs.

Evidence from natural and experimental trials demonstrates that *L.intracellularis* is transmitted between in-contact pigs via the faecal/oral route and this is likely to be the main route of transmission between pigs. The transmission of *L. intracellularis* via faeces has been demonstrated in outbreaks of PHE and in experimental challenge trials where pigs infected with *L. intracellularis* have infected naive in-contact sentinel pigs (Love *et al.*, 1977; Collins *et al.*, 2000; Jordan *et al.*, 2004). Estimates of bacterial numbers shed in the faeces of challenged pigs vary from 10^4 to 10^8 *L. intracellularis* per gram of faeces (Smith and McOrist, 1997; Collins *et al.*, 2000). However, transmission has also been demonstrated when the infective dose is low, and without direct contact between pigs (Jordan *et al.*, 2004), and challenge trials have demonstrated that as few as 10^5 *L. intracellularis* can cause colonisation of naive pigs (Collins and Love, 2007).

A number of questionnaires and serological surveys have identified housing or flooring types as risk factors for increased prevalence of *L. intracellularis* infection or disease (Bane *et al.*, 1997; Smith *et al.*, 1998; Bronsvoort *et al.*, 2001; Stege *et al.*, 2001; Collins, 2003). The retention or accumulation of manure in ecosheds is believed to increase the bacterial level of *L.intracellularis* in the environment and therefore increase the prevalence and severity of ileitis in ecoshed housed pigs, although the serological surveys have not always supported this. Confounding factors associated with pig housing such as differences in maximum and minimum temperatures, access to external vectors of disease, the bias towards all-in-all-out

production flows in ecosheds, the group size and the health status of the pigs may all help explain the variability in survey results with respect to identifying ecosheds as risk factors for ileitis.

The transmission of *L. intracellularis* from sows to piglets has yet to be convincingly demonstrated, and it may be more common that sows protect their piglets from infection until weaned from sow's milk. Piglets challenged with *L. intracellularis* while ingesting milk from seronegative dams did not appear to be protected and most piglets (84% and 100%) developed long lasting antibodies to *L. intracellularis* within 3 weeks of challenge (Pozo *et al.*, 2000; Barna and Bilkei, 2003). Piglets from seropositive dams challenged pre-weaning were either protected from *L. intracellularis* (Pozo *et al.*, 2000) or fewer piglets seroconverted (0% and 32%) and antibodies decayed more rapidly (Barna and Bilkei, 2003).

In endemically infected herds with continuous production, shedding of *L. intracellularis* has been demonstrated in a small number of pre-weaned piglets (Møller *et al.*, 1998). However, in these trials the PCR was not able to differentiate between passage of *L. intracellularis* through the gut or infection in the piglets, as supporting serological and pathology data was not collected.

Direct contact between infected and naïve pigs may not be necessary for the transmission of *L.intracellularis*. Previous experimental challenge studies demonstrated the survival of *L. intracellularis* in faeces stored for 2 weeks at temperatures between 5 and 15°C, and colonisation of the intestine of naive pigs inoculated orally with this infected pig faeces (Collins *et al.*, 2000). The environmental conditions favouring the survival of *L. intracellularis* in conventional pens and ecosheds are yet to be established, but contaminated faeces on boots, machinery, equipment, flooring and pen dividers could be important in the transmission of *L. intracellularis*.

External vectors such as rodents, birds, and insects have also been suggested as potential sources of *L. intracellularis* to naïve pigs, but it is likely they play only a minor role in transmission of *L. intracellularis* to pigs (Collins *et al.*, 1999).

However, the role of rodents in *L. intracellularis* transmission may become important if rodents or other external vectors move from "dirty" herds to clean herds re-populated with naïve pigs.

The possibility that *L. intracellularis* can persist in pigs after recovery from disease and cessation of faecal shedding of *L. intracellularis*, requires investigation as eradication of PE from pig herds necessitates elimination of *L. intracellularis* from the pig. While intermittent shedding of *L. intracellularis* will not cause disease in the infected animal, this could be a source of *L. intracellularis* infection and disease to in-contact naïve pigs. *L. intracellularis* have been detected by PCR in the tonsils and mesenteric lymph nodes of severely affected pigs following either natural or experimental challenge, coinciding with the presence of intestinal lesions (Jensen *et al.*, 2000; Kroll *et al.*, 2005). The detection of *L. intracellularis* in tonsils and mesenteric lymph nodes post resolution of intestinal lesions still needs to be investigated to determine whether a reservoir of *L. intracellularis* persists in the host.

This project aims to improve our understanding of *L.intracellularis* survival and transmission in piggeries, in order to develop management practices to better control ileitis.

3.0 Objectives

1. Determine if management strategies such as AIAO production, the type of housing, cleaning and disinfection of pens can significantly reduce the bacterial "load" of *L.intracellularis* in the piggery environment.

2. Determine whether *L.intracellularis* can survive in pens previously occupied by pigs shedding *L.intracellularis* in their faeces, and transmit infection to naïve pigs introduced into these pens following various levels of cleaning and disinfection.

3. Determine whether *L.intracellularis* can survive in ecosheds and be a source of infection to naïve pigs, following various levels of cleaning and disinfection between batches of pigs.

4. Determine whether *L.intracellularis* can be completely eliminated from the pig.

5. Determine if previously infected pigs can start to re-shed *L.intracellularis* under stressful conditions, and become a potential source of *L.intracellularis* infection to in-contact naïve animals.

4.0 Survival of *L.intracellularis* in faeces and the environment (conventional housing), with and without disinfection of pens

4.1 Methods

Twelve Large White x Landrace finisher pigs shedding *L. intracellularis* in their faeces were used to contaminate conventional pens over a 3 week period. The pigs were subclinically affected, but polymerase chain reaction (PCR) monitoring detected faecal shedding of *L. intracellularis* in all of the pigs over this period. The infected pigs were removed, and the pens were left vacant for 2 weeks. During this period, half of the contaminated pens were power hosed and disinfected with Virkon S, and left to dry for 3 days. The other pens were left empty and uncleaned. Two groups of naïve weaner pigs were introduced into these pens; one group (n=10) was introduced into the uncleaned environment, and another group (n=12) into the cleaned environment. Faeces and blood were collected weekly from individual pigs to detect faecal shedding of *L. intracellularis* by PCR and serum IgG antibodies to *L. intracellularis*. Pigs were necropsied 56 days after introduction, and ileum and colon tissue was collected for PCR detection of *L. intracellularis*. Minimum and maximum temperatures, recorded daily throughout this period, ranged from an average overnight minimum of 9°C to an average daily maximum of 18°C.

4.2 Results

No evidence of *L. intracellularis* transmission from the environment was demonstrated by faecal PCR or serology in the 12 pigs introduced into the cleaned environment for up to 56 days after exposure to the cleaned environment (Table 4.1). Likewise, *L. intracellularis* DNA was not detected in the ileum or colon of any of these pigs at necropsy.

Clinical signs of ileitis were not evident in any pigs, whether housed in clean or dirty pens. Transmission of *L. intracellularis* infection was demonstrated in naive pigs introduced into the uncleaned pens, with faecal shedding of *L. intracellularis*

detected in 2 of 10 pigs between 21 and 35 days post exposure to the dirty environment (Table 4.1). Antibodies to *L. intracellularis* were detected in 8 of 10 of the same pigs between 28 and 56 days post exposure (Table 4.1). *L. intracellularis* DNA was not detected in the ileum or colon of these pigs necropsied 56 days after entering the uncleaned pens (results not shown). This was not unexpected because faecal shedding of *L. intracellularis* had ceased to be detected in the pigs housed in the dirty environment three weeks earlier. Pigs are able to recover from PE, with intestinal lesions resolving 2 to 4 weeks after clinical signs of disease are evident.

Table 4.1 Summary of *L. intracellularis* infection monitoring of pigs over 7 weeks after introduction into cleaned or uncleaned pens (dirty) following contamination with *L. intracellularis*

Pen Hygiene	Proportion of PCR positive pigs (# days post introduction to pens)								
	0	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**
Clean	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0/12
Dirty	0/10	0/10	0/10	1/10	1/10	1/10	0/10	0/10	0/10
	Proportion of seropositive pigs (# days post introduction to pens)								
	0	**7**	**14**	**21**	**28**	**34**	**42**	**49**	**56**
Clean	0/12	NT	NT	0/12	0/12	0/12	0/12	0/12	0/12
Dirty	0/10	NT	NT	0/10	1/10	6/10	7/10	7/10	5/10

4.3 Discussion

L. intracellularis is able to survive in faeces in uncleaned conventional pens for at least 2 weeks at temperatures ranging between 9° and 18°C, and colonize naïve pigs. Power hosing and disinfection of conventional pens contaminated with faeces containing *L. intracellularis* was able to significantly reduce the survival and transmission of *L. intracellularis* to naïve pigs introduced into that environment ($P<0.05$).

Transmission of *L. intracellularis* infection by the ingestion of infectious faeces was not demonstrated in all of the pigs housed in the dirty pen. This may be dose related, although we were unable to calculate the number of viable *L. intracellularis* per gram of faeces or the quantity of faeces ingested by pigs. Previous research demonstrated that pigs can excrete between 5 x 10^4 and 7 x 10^8 *L.intracellularis* per gram of faeces (Smith and McOrist, 1997) and pigs dosed with as few as 10^5 *L.intracellularis* can shed detectable numbers of *L. intracellularis* in their faeces and develop a serum IgG response (Collins and Love, 2007).

Transmission of *L. intracellularis* infection appeared to be better detected by serology than PCR, probably due to the relatively long duration of the immune response relative to the shorter duration of faecal shedding. The absence of *L. intracellularis* DNA in the ileum or colon of these pigs at necropsy was not unexpected because faecal shedding of *L. intracellularis* had ceased to be detected in the pigs housed in the dirty environment three weeks earlier. Pigs are able to recover from PE, with intestinal lesions resolving 2 to 4 weeks after clinical signs of disease are evident (Roberts *et al.*, 1979).

This is the first demonstration of the efficacy of cleaning and disinfection alone to prevent the transmission of *L.intracellularis* infection to naïve pigs. Cleaning and disinfection practices are common in farrowing crates to prevent the transmission of *E.coli* from the environment to suckling piglets, and as part of disease eradication procedures following depopulation of herds. However, cleaning and disinfection of pens are seldom used without antibiotic medication or vaccination. This study provides pork producers and veterinarians with a better understanding of cleaning practices that can reduce the survival of *L.intracellularis* in the piggery. The cleaning strategies can be implemented between batches of pigs on a semi regular basis to reduce the bacterial 'load' of *L.intracellularis,* or as part of a disease eradication program to reduce production losses caused by ileitis.

5.0 Survival of *L.intracellularis* in ecosheds

5.1 Methods

The survival of *L. intracellularis* in ecosheds was assessed in field studies by serological monitoring of consecutive batches of weaned pigs introduced into a range of cleaned and/or disinfected ecosheds over time. Herds were selected on the basis that weaner or grower pigs were naïve to *L. intracellularis* prior to entry to the ecosheds, and that pigs were exposed to *L. intracellularis* within the ecoshed environment. Ecosheds were chosen with a range of cleaning procedures between batches of pigs, including no cleaning, removal of bedding material only (partial or complete), power hosing and disinfection.
Producers willing and able to monitor growth performance of consecutive batches of pigs were preferentially chosen so that the growth performance could be associated with the prevalence of *L. intracellularis* infection after cleaning and/or disinfection.

Forty-five pigs were randomly selected within 2 weeks of arrival at each ecoshed, and eartagged for longitudinal serological monitoring of *L. intracellularis* infection. Blood was collected from these pigs (about 10% of pigs in individual sheds or 10% of weekly production) at monthly intervals and tested for serum IgG antibodies to *L. intracellularis*. Two additional groups of 45 pigs were selected in the two subsequent batches of pigs for longitudinal serological monitoring of *L. intracellularis* infection over their lifetime.

5.2 Results

Farm #1 was a high health status herd with strict quarantine measures for people and machinery coming onto the farm. Strict quarantine measures were also instituted between different age groups of pigs within the farm, with significant distances between the breeding herd and the weaner pigs (>10km) and the grower/finisher pigs (20km). Weekly batches of 150 pigs were weaned into

cleaned ecosheds at 25 days of age and remained there until 10 weeks of age. The sample size for serological monitoring of *L. intracellularis* infection was calculated to detect a 10% prevalence of disease in the sample population.

The prevalence of *L. intracellularis* infection in weaner/grower pigs was very low due to a combination of strict quarantine practices on the farm, and the antibiotic medication used throughout production to control ileitis (Table 5.1). The very low prevalence of *L. intracellularis* infection and the short interval that pigs were housed in ecosheds made this herd unsuitable to continue studies on survival of *L. intracellularis* in ecosheds, as very large numbers of animals would need to be sampled to detect significant differences in *L. intracellularis* prevalence in successive batches of pigs following routine cleaning and disinfection procedures.

Tale 5.1 Summary of *L. intracellularis* infection results (serum IgG response) from longitudinal monitoring of pigs housed in ecosheds

Farm #	Date sampled	Entry to ecoshed		Exit from ecoshed	
		Age	% ileitis	Age	% ileitis
1	Nov 06	4 wks	0%	11 weeks	4%
2a	Dec 06	9 wks	0%	15 wks	12.5%
2b	March 07	9 wks	7.5%	16 wks	57.5%
2c	April 07	10 wks	0%	15 wks	0%
3	May 07	10 wks	0%	19 weeks	0%
4	Sept 07	9 wks	0%	14 wks	87%
5	Oct 07	8 wks	0%	12 weeks	70%
6	Nov 07	10 wks	0%	14 wks	70%
7	Dec 07	9 wks	0%	18 weeks	80%

Farm # 2 was part of a multi-site production piggery, with very poor hygiene in the grower ecosheds and minimal quarantine restrictions both within and between farms. Batches of 180 pigs were weaned into ecosheds with partial replacement of straw between batches. Frequent mixing of pigs occurred between batches of pigs and each batch of pigs was moved two more times to different ecosheds. The difficulty in obtaining straw (due to the drought) meant that no cleaning or

replacing of straw had occurred in some ecosheds for subsequent batches of pigs. Even with the extremely poor hygiene at this farm, the prevalence of *L. intracellularis* infection was suppressed due to the continuous supply of antibiotics in feed (Table 5.1).

In January 2007, changes were made to regulations for withholding periods for antibiotic use prior to slaughter, termed export to slaughter interval (ESI). This encouraged the removal of certain antibiotics from grower/finisher feeds, due to their extended ESI (eg olaquindox) for at least 28 days prior to slaughter. These changes affected the timing and prevalence of *L.intracellularis* infection and the associated level of disease.

Following the change in medication 6 weeks earlier, we re-sampled the older pigs and sampled a new batch of growers. The prevalence of *L. intracellularis* infection increased substantially in grower pigs following the change in medication (March 07, Table 5.1). Five weeks later, blood was collected from the second batch of grower pigs and a third batch of grower pigs.

Serology results indicated that there had been a substantial reduction in the proportion of infected pigs between March and April 2007 (57.5% and 0% respectively). Discussion with the consulting veterinarian identified that high doses of tetracyclines had been used in the grower pigs in March and April 2007 due to an outbreak of diarrhoea. At this point, we decided not to continue with this herd, as we were unable to separate the effects of antibiotic medication and hygiene on the control of ileitis.

We identified a third herd where the consulting veterinarian believed that antibiotic medication would not prevent detection of *L. intracellularis* infection. However, we were not able to detect seroconversion in any pigs, and discussion with the producer after our visit confirmed that a mistake had been made in the medication protocol, and the medication used would prevent *L. intracellularis* infection.

Preliminary serological sampling was initiated on two new herds with significant ileitis problems in ecosheds; although the vaccine was being used (farms 4 and 5). Unfortunately, both of these herds were closed down after preliminary screening, so longitudinal monitoring of consecutive batches of pigs could not be performed.

Two new farms with large numbers of ecosheds (rammed earth floors and concrete floors) were identified to evaluate cleaning methods to reduce the survival of *L. intracellularis* in ecosheds (farms 6 and 7). Preliminary serological monitoring of both of these grow out units indicated that pigs were infected with *L. intracellularis* after being moved into ecosheds at 9 to 10 weeks of age (Table 5.1). A significant increase in the prevalence of *L. intracellularis* infection was observed on farm 7 after pigs reached 15 weeks of age, which coincided with an increase in bacterial numbers in pig faeces. The large size of these facilities encouraged us to find alternative ways to evaluate the effect of cleaning and disinfection on control of ileitis, eliminating the problems we experienced previously with changes in antibiotic medication over time. We decided to evaluate cleaning strategies that would reduce the survival of *L. intracellularis* between batches of pigs (clean bedding and lime) as well as during the finisher phase (addition of extra straw).

A variation to this project was requested in January 2008, which required additional funding. The new trial design aimed to evaluate the standard cleaning practice at each facility with 2 other treatments to identify cleaning practices that reduce *L. intracellularis* survival in subsequent batches of pigs (3 replicates per treatment).
Prior to the commencement of the trial, faeces and blood was collected from ecosheds containing 17-18 week old pigs to demonstrate *L. intracellularis* infection in each shed. Infected sheds were to be randomly allocated to 3 replicates of each of the 3 cleaning treatments. The standard cleaning practice of partial removal of bedding and manure and pressure cleaning of the feed pad area was to be the control cleaning. Two other treatments were to be investigated; the addition of lime on the floor after removal of the bedding, and the addition extra straw when pigs were 15 weeks of age (coinciding with the build up of manure).

The survival of *L. intracellularis* in ecosheds was to be assessed in the new batch of pigs by longitudinal serology monitoring. Pigs were to be bled at 9 weeks of age, on entry to the ecoshed, while they were still naïve to Lawsonia to ensure that *L. intracellularis* infection comes from the ecoshed environment. The same pigs were to be re-bled 4 to 5 weeks later and again on exit from the ecoshed. The prevalence of *L. intracellularis* infected pigs was to be compared between cleaning treatment groups. Batches of pigs were to be weighed on entry and exit from the ecoshed to determine the effect of *L. intracellularis* prevalence and survival on growth performance.

An alternative project design was also developed which was considerably less costly than the protocol above, as suggested by the Pork CRC Board. Changing the primary parameter for evaluating the control of ileitis to the percentage of pigs at or above the prime weight at 21 weeks of age would allow a significantly reduced project cost, as compensation would not be required for the early slaughter of pigs.

The effect of cleaning treatments on ileitis prevalence and severity could be determined by measuring the variation in the proportion of pigs at prime weight and correlating this with evidence of ileitis such as the mean serum IgG antibody titre in pigs at slaughter, and the proportion of PCR positive pools of faeces collected from sheds on 3 occasions.

Unfortunately, an outbreak of the haemorrhagic form of ileitis occurred in finisher pigs in March 2008. This led to the removal of antibiotics from weaner pig diets, to ensure all pigs were exposed to *L. intracellularis* at an early age and were thus immune to disease as adults. The change in antibiotic medication meant that pigs entering the ecosheds were no longer naïve to *L.intracellularis*. The variation in the proportion of pigs at prime weight at slaughter between treatments could be explained by a lot more factors than just hygiene effects. In order to use this trial design (epidemiological in nature) to determine the effect of hygiene on the proportion of pigs at prime weight at slaughter, we would need to look at more

pigs, in more sheds and on more farms. Unfortunately, this is not possible given budget constraints.

5.3 Discussion and where to next?

Despite considerable efforts, we have not been able to identify cleaning and disinfection practices for ecosheds that reduce the survival of *L. intracellularis* between batches of pigs. The main obstacle in our longitudinal field studies was the frequent changes to management practices including antibiotic medication, which masked the effects of cleaning practices on *L. intracellularis* survival in ecosheds.

The frequent modification of antibiotic medication led to substantial variability in the prevalence of *L. intracellularis* infection between batches of pigs, making it difficult to separate the effects of antibiotics and cleaning practices on *L. intracellularis* survival in ecosheds. The presence of other diseases and changes to export slaughter intervals necessitated the medication changes that occurred mid trial.

To overcome this issue, we investigated herds where ileitis was being controlled by vaccination. Unfortunately, all the herds we investigated eventually required the use of antibiotics in addition to vaccination to control ileitis. Once again, we were faced with variability in the prevalence of *L. intracellularis* infection within the herd due to changes in antibiotic medication.

Following extensive discussions with our biometrician, we have developed two new strategies to identify cleaning strategies to reduce survival and transmission of *L. intracellularis* in ecosheds. The trial designs that we have developed can be used in other ecosheds to test the efficacy of cleaning treatments to reduce the survival of *L.intracellularis* and the severity of ileitis in pigs housed in ecosheds.

Our studies on the survival and transmission of *L. intracellularis* in ecosheds and conventional sheds have identified the need for a semi-quantitative real time diagnostic assay to estimate the bacterial 'load' of *L. intracellularis* in the

environment, and provide rapid feedback to producers on the efficacy of PE control strategies. We will continue to work with pork producers to identify cleaning practices that will reduce the load of *L.intracellularis* in ecosheds and conventional pens as part of new Pork CRC project 'Development of a semi-quantitative real time diagnostic assay for ileitis.'

6.0 Carrier pigs as a potential source of *L. intracellularis* infection

6.1 Natural recovery from ileitis

6.1.1 Methods

Two groups of 4 naïve weaner pigs (Groups 1A and 1B) were orally inoculated with *L. intracellularis* and allowed to naturally recover. Pigs were monitored for 10 weeks by an indirect fluorescent antibody test (IFAT) and polymerase chain reaction (PCR) to detect serum IgG antibodies to *L. intracellularis* and faecal shedding of *L. intracellularis* respectively.

After faecal shedding of *L. intracellularis* ceased, these previously infected (and recovered) pigs were cleaned of all organic matter, then moved to a cleaned and disinfected pen. Four of the recovered pigs (Group 1A) were treated with dexamethasone (DMX), a corticosteroid used to suppress their immune system, and put in contact with 9 naïve grower pigs (Group 3A). The remaining 4 pigs, Group 1B, were put in contact with a second group of 9 naïve growers, Group 3B (Table 6.1).

Table 6.1 Experimental design

Group #	# pigs	+ *L. intracellularis* [a]	+ OTC[b]	+ DMX[c]	In-contact with pigs
1A	4	Yes	No	Yes	3A
1B	4	Yes	No	No	3B
2A	10	Yes	Yes	Yes	
2B	10	Yes	Yes	No	
3A	9	No	No	No	1A
3B	9	No	No	No	1B

[a] Pigs exposed to *L. intracellularis*, either by oral inoculation (Groups 1A and 1B) or transmission of infection from the environment(Groups 2A and 2B)

[b] Pigs treated with oxytetracycline after clinical signs of PE observed

[c] Pigs treated with dexamethasone to suppress immune response after recovery from PE

The recovered Group 1A and 1B pigs were maintained in contact with the naive Group 3A and 3B pigs for a period of 4 weeks following dexamethasone treatment, with faeces collected twice weekly to test for shedding of *L. intracellularis*. Blood was also collected every two weeks to test for serum IgG antibodies to *L. intracellularis.* Four weeks after contact with the naïve Group 3A and 3B pigs, the recovered Group 1A and 1B pigs were euthanased and tissue was collected from the jejunum, ileum, colon, tonsils, and mesenteric lymph nodes (MLN) for PCR and immunohistochemistry detection of *L. intracellularis*.

The naïve pigs, in-contact with the recovered pigs (Groups 3A and 3B), continued to be monitored for transmission of *L. intracellularis* infection by faecal PCR and serology over a 7 week period (4 weeks in contact with the recovered pigs and an additional 3 weeks). At the end of this period, the Group 3A and 3B pigs were orally challenged with a high dose of *L. intracellularis* to determine if they were still naïve to *L. intracellularis*.

6.1.2 Results

L.intracellularis infection was demonstrated in all Group 1 pigs (1A and 1B) following oral challenge. Faecal shedding of *L.intracellularis* was consistently detected by PCR between 10 and 42 days post oral challenge. The immune response to *L.intracellularis* infection persisted from 21 to 77 days post challenge (Figure 6.1). Clinical signs of ileitis were mild in the majority of pigs with soft faeces observed in only 3 pigs over a 7 day period.

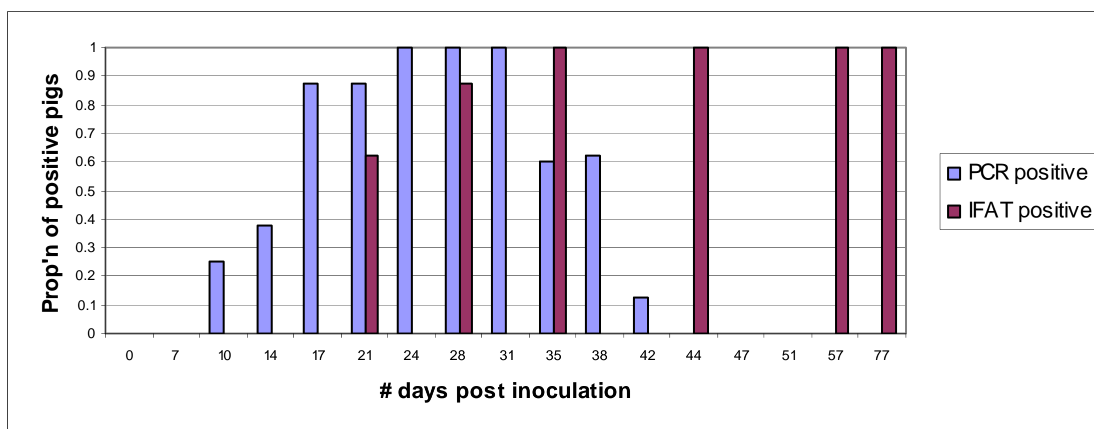

Figure 6.1. Monitoring of *L. intracellularis* infection in all 8 Group 1 pigs orally inoculated with *L.intracellularis* by faecal PCR and serum IgG antibodies to *L.intracellularis* (IFAT).

All pigs recovered from *L.intracellularis* infection, with no detection of faecal shedding of *L.intracellularis* between 44 and 77 days post challenge (Figure 6.1).

Dexamethasone treatment of Group 1A pigs did not lead to detectable re-shedding of *L. intracellularis* in pig faeces' in the following 4 weeks (results not shown). Serum IgG antibodies continued to be detected in all pigs with a steady decline in titre in the 4 weeks following dexamethasone treatment (Figure 6.2).

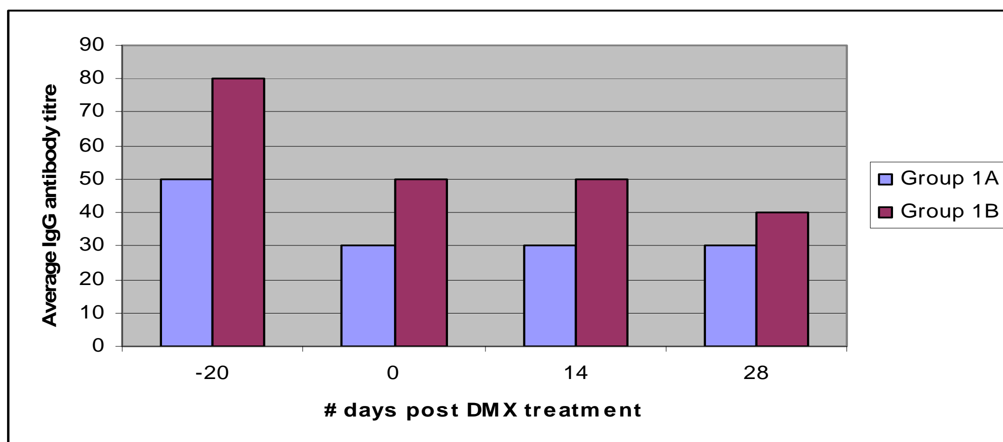

Figure 6.2. Average serum IgG antibody titre in Group 1A (dexamethasone treated) and 1B (control) pigs from 20 days pre-DMX treatment to 28 days post treatment (titres expressed as reciprocals of sera dilutions).

L. intracellularis DNA was detected by PCR in all types of intestinal tissues from Group 1 pigs at necropsy (Table 6.2). The mesenteric lymph nodes were the tissue where *L. intracellularis* were most frequently detected in recovered Group 1 pigs. There were no significant differences in the proportion of PCR positive tissues in dexamethasone treated pigs (Group 1A) compared with control pigs (Group 1B).

L. intracellularis antigen was also detected in intestinal tissues from recovered pigs by immunohistochemistry, which is the gold standard assay for diagnosis of PE. *L. intracellularis* antigen was detected in the central cytoplasm of epithelial crypt

cells enclosed within a membrane. This differs from the characteristic lesion for PE where *L.intracellularis* bacteria are found free in the apical cytoplasm of crypt cells. The *L. intracellularis* antigen also appeared to be degraded and intact individual *L. intracellularis* bacteria could not be visualised. The tissue sections were sent to another laboratory in the US for confirmation of antigen reacting to *L. intracellularis*.

Table 6.2. Proportion of recovered pigs positive for *L. intracellularis* by PCR or immunohistochemistry in individual intestinal tissues

Tissue	Test	Proportion of positive pigs	
		Group 1A (DMX[a])	Group 1B
Jejunum	402bp PCR	0.25	0.25
	IHC	0.50	0.25
Ileum	402bp PCR	0.50	0.25
	IHC	0.50	0.25
Colon	402bp PCR	0.25	0
	IHC	0	0.25
MLN	402bp PCR	0.50	0.75
	IHC	0.25	0
Tonsils	402bp PCR	0.50	0.25
	IHC	0.50	0.25

[a]Pigs treated with dexamethasone to suppress immune response after recovery from PE

All of the Group 3 pigs remained naïve to *L. intracellularis* over the 4 week period after contact with the recovered Group 1 pigs. Neither faecal shedding of *L.intracellularis* nor antibodies to *L.intracellularis* was detected in any of the naïve Group 3 pigs while in contact with the recovered pigs for 4 weeks or for an additional 3 weeks following contact (results not shown).

Following oral challenge with a high dose of *L. intracellularis* 7 weeks after contact with the recovered pigs, all of the Group 3 pigs (regardless of dexamethasone

treatment) shed *L. intracellularis* in their faeces between 10 and 17 days post inoculation and developed serum IgG antibodies to *L. intracellularis* (Figure 6.3). Severe diarrhoea was observed in the faeces of 2 Group 3B pigs from 13 to 21 days post inoculation, with blood observed at 15-17 days. Less severe diarrhoea was observed in another 2 Group 3B pigs over the same period. Mild diarrhoea was observed in the faeces of 4 Group 3A pigs over the same period.

No significant differences were observed between treatment groups in the prevalence or duration of diarrhoea and faecal shedding of *L. intracellularis*. The prevalence or average antibody titre to *L. intracellularis* was not significantly different between Group 3A and 3B pigs at 20 days post inoculation (results not shown).

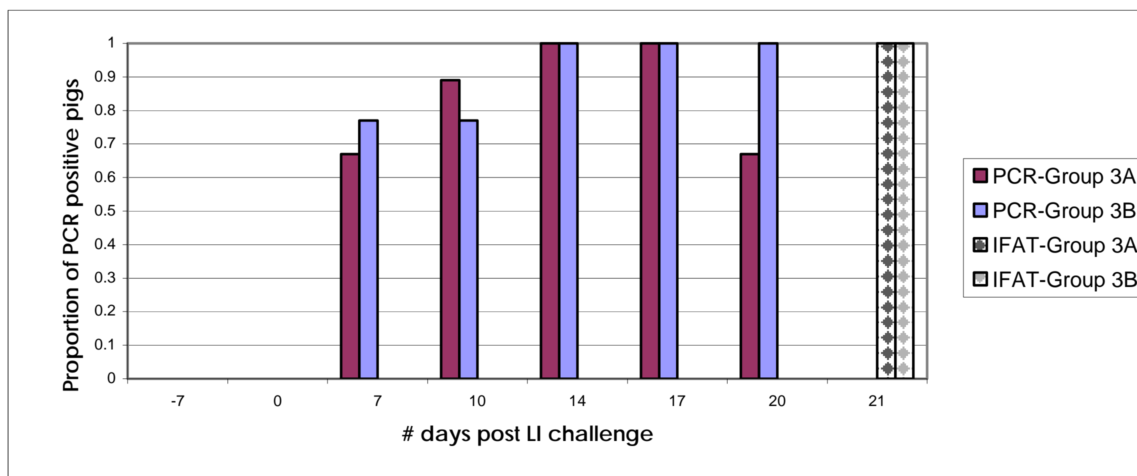

Figure 6.3 Prevalence of *L. intracellularis* infection in Group 3A and 3B pigs, as demonstrated by faecal PCR and serum IgG IFAT following oral inoculation with *L.intracellularis* 7 weeks after contact with naturally recovered Group 1A pigs (treated with dexamethasone) or Group 1B pigs (controls).

6.1.3 Implications and Recommendations

Our results suggest that pigs do not continue to intermittently shed detectable numbers of *L.intracellularis* in their faeces following natural recovery from ileitis (following a single oral challenge with *L. intracellularis*), and do not transmit infection to naïve in-contact pigs. Naive pigs in contact with recovered pigs

remained naïve, as demonstrated by their susceptibility to infection when challenged with *L. intracellularis* 7 weeks after contact with the recovered pigs. Pigs previously exposed to *L. intracellularis* are immune to re-infection, even when exposed to low numbers of bacteria, given sufficient time (Collins and Love, 2007).

However, we are unable to state that under different conditions, ie. higher infection pressure in a less hygienic environment or ecoshed, that intermittent faecal shedding of *L. intracellularis* and transmission of infection from recovered pigs to naïve in-contact pigs would not occur. In this study the challenged pigs had 11 weeks to recover following initial exposure to *L. intracellularis* before they were brought into contact with naïve pigs. Such a long recovery period is unlikely to be common in commercial piggeries and may only happen during disease eradication programs.

The detection of *L. intracellularis* in intestinal tissues from recovered pigs by PCR and immunohistochemistry appears to contradict our other evidence that recovered pigs are not a source of *L. intracellularis* to naïve pigs. However, the *L.intracellularis* antigen appeared to be fragmented and enclosed within a membrane in the central cytoplasm of the crypt cells. The PCR assay is able to detect DNA from live and dead organisms so it is possible that the PCR was detecting dead bacteria in intestinal tissues. In previous studies where *L. intracellularis* were detected in the tonsillar crypts of severely affected animals by PCR and immunohistochemistry, no attempt was made to determine if affected pigs could transmit infection to naïve in-contact pigs (Jensen *et al.*, 2000).

If *L. intracellularis* can persist in intestinal tissues of recovered pigs, suppression of the immune system with dexamethasone may allow dormant intracellular bacteria such as *L. intracellularis* to start replicating and be shed in the faeces of pigs. Placing a group of naïve pigs in contact with recovered pigs following immune suppression will allow detection of transmission of infection from the recovered pigs to the naïve pigs.

Corticosteroid treatment of recovered pigs also failed to demonstrate intermittent faecal shedding of *L. intracellularis* or transmission of *L. intracellularis* from recovered pigs to naïve in-contact pigs. Oral challenge of the in-contact pigs (7 weeks after exposure to recovered pigs) indicated that they were still naïve to *L. intracellularis*. This evidence suggests that recovered and corticosteroid treated pigs do not appear to be a significant source of *L. intracellularis* infection to naïve pigs.

The corticosteroid dexamethasone has been used by other researchers to suppress the immune system of pigs and enhance colonization of *L. intracellularis* in experimentally challenged pigs (Jordan *et al.*, 2004; Winkelman *et al.*, 1997). However, Joens *et al.*,(1997) demonstrated that dexamethasone treatment of pigs inoculated with *L. intracellularis* had no effect on the development of proliferative enteropathy lesions. We have no direct measure of immune suppression following corticosteroid treatment of pigs in our study, and have relied upon detection of re-shedding of *L. intracellularis* in faeces as an indicator of re-activation of bacterial replication.

Virus re-activation following a period of viral latency has been demonstrated in pigs treated with the same dose of dexamethasone. Pigs infected with either PRRS or pseudorabies virus (PRV) were able to transmit infection to in-contact naïve pigs following a period of viral latency (Albina *et al.* 1994; Ferrari *et al.*, 1998). However, in some pigs dexamethasone treatment did not lead to viral re-activation or transmission of infection to naïve in-contact pigs, although PRV was detected in ganglia by PCR. This study supports the suggestion that PCR may detect non-viable organisms, and may not be a useful assay for the demonstration of carrier pigs.

If immune suppression had occurred before faecal shedding of *L. intracellularis* ceased in our trial, it is possible that recovering pigs may have continued to intermittently shed *L. intracellularis*, and be a source of infection to naïve in-contact pigs. Stresses in commercial production such as frequent mixing or movement of pigs could lead to intermittent shedding of *L. intracellularis* and

transmission of infection to naïve in-contact pigs and this requires further investigation.

6.2 Antibiotic medicated recovery from ileitis

6.2.1 Methods

Two groups of 10 naïve grower pigs (Groups 2A and 2B) were exposed to *L. intracellularis* from the environment. Treatment with oxytetracycline (12mg/kg bodyweight single IM injection and 12mg/kg bodyweight in feed over 4 days) began when clinical signs of ileitis were evident in these pigs 44 days after the start of the trial (Table 61). Blood and faeces were collected weekly to monitor *L. intracellularis* infection.

When faecal shedding of *L. intracellularis* had ceased (41 days after clinical signs first appeared), one group of 10 pigs (Group 2A) were treated with dexamethasone and the other group (Group 2B) were not treated. Faeces were collected twice weekly for evidence of re-shedding of *L. intracellularis* and blood was collected fortnightly to determine the titre of serum IgG antibodies to *L. intracellularis*.

Four weeks after the Group 2A pigs were treated with dexamethasone, the recovered Group 2A and 2B pigs were euthanased and tissue was collected from the jejunum, ileum, colon, tonsils, and mesenteric lymph nodes (MLN) for PCR detection of *L. intracellularis*.

6.2.2 Results

Two groups of 10 naïve grower pigs (Groups 2A and 2B) exposed to *L. intracellularis* from the environment (44 days after the start of the trial) showed severe clinical signs of ileitis, with blood in the faeces of 2 pigs. Treatment with oxytetracycline (OTC) commenced immediately, but faecal shedding of *L.intracellularis* continued to be detected for 27 days following the initiation of antibiotic treatment (Figure 6.4). The immune response to *L.intracellularis* infection was detected at the time of clinical signs of ileitis and continued to be

detected for a further 6 weeks (Figure 6.4). All pigs recovered from ileitis, with no detection of faecal shedding of *L.intracellularis* between 34 and 41 days after initiation of antibiotic treatment (Figure 6.4).

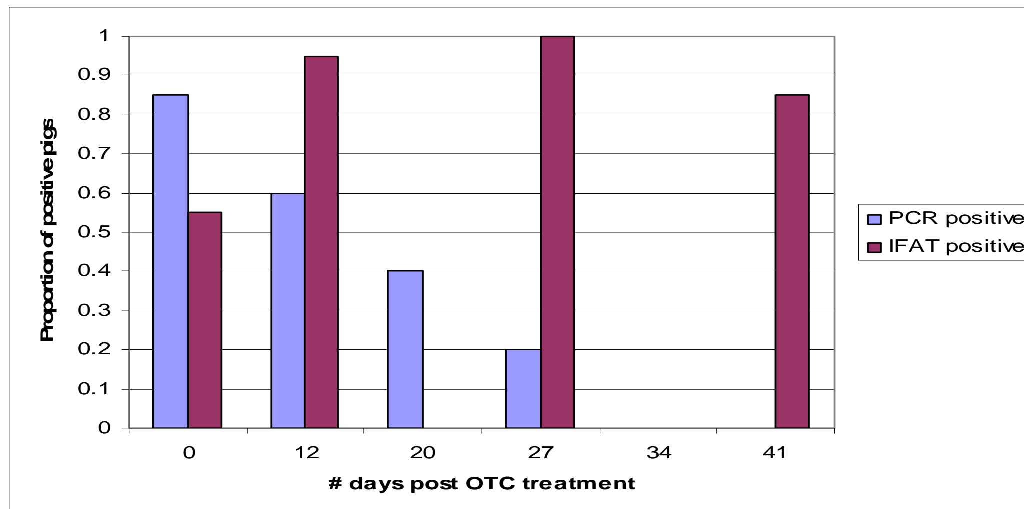

Figure 6.4. *L. intracellularis* infection in Group 2 pigs following ileitis outbreak and OTC treatment at day 0 (44 days after start of trial), monitored by faecal PCR and serum IgG IFAT.

Dexamethasone treatment of Group 2A pigs following recovery from *L. intracellularis* infection did not lead to detectable re-shedding of *L. intracellularis* in any pig's faeces in the following 4 weeks (Figure 6.5). Intermittent faecal shedding of *L. intracellularis* was not detected in control pigs (Group 2B) over the same period. Serum IgG antibodies to *L. intracellularis* continued to be detected in most pigs until necropsy, with a steady decline in titre over time. There was no significant difference in the prevalence or antibody titre to *L.intracellularis* between treatment groups following dexamethasone treatment (results not shown). *L. intracellularis* DNA was not detected in any intestinal tissues (ileum, jejunum, colon, mesenteric lymph nodes and tonsils) from Group 2A or Group 2B pigs at necropsy four weeks after dexamethasone treatment (results not shown).

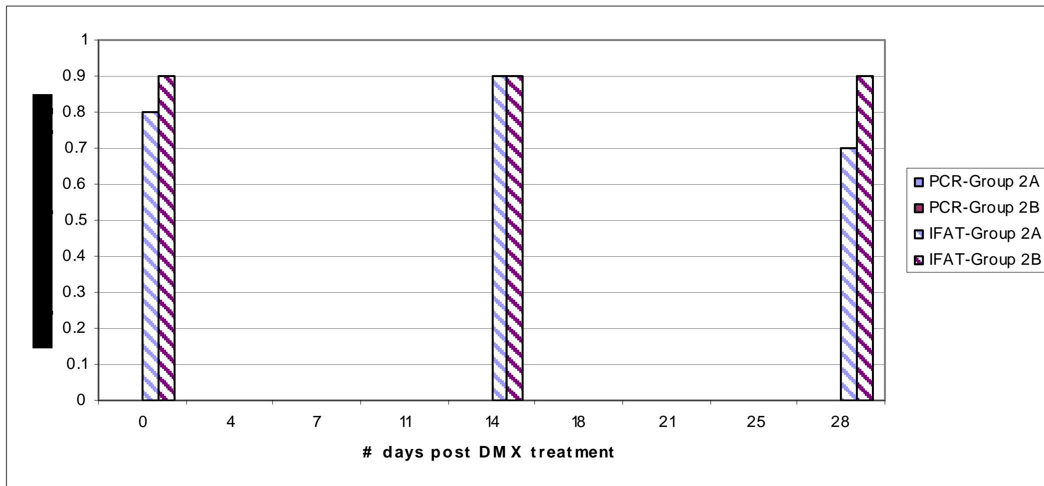

Figure 6.5. Monitoring of *L. intracellularis* infection in Group 2A (DMX) and Group 2B (control) pigs following recovery from ileitis and DMX treatment at day 0 (41 days post OTC treatment). *L. intracellularis* infection monitored by faecal PCR and serum IgG IFAT.

6.2.3 Implications and Recommendations

Treatment of pigs with oxytetracycline rapidly controlled clinical signs of ileitis, but took longer to terminate *L. intracellularis* infection. Oxytetracycline treatment of pigs with ileitis appeared to completely eliminate *L.intracellularis* from pigs. However, as the *L. intracellularis* challenge was different for our OTC treated and untreated pigs, it is not possible to say whether antibiotics are needed to prevent survival of *L. intracellularis* in recovered pigs and to prevent transmission of *L. intracellularis* to naïve in-contact pigs.

Further research is required to know whether antibiotic medication is an integral part of eliminating *L. intracellularis* from pigs, especially in disease eradication strategies.

7.0 Budget Summary

Funding allocation	2005-2006	2006-2007	2007-2008	Total
Salary	$5,572	$16,717	$28,945	$51,234
Operating	$8,161	$24,480	$7,940	$40,581
Travel	$900	$2,700	$2,200	$5,800
Total Pork CRC cash allocated:	$14,633	$43,897	$29,265	$97,615

In-kind contributions Per Year	2005-2006	2006-2007	2007-2008	Total
Research Organisation Category of in-kind staff	$24,475	$119,500	$84,475	$228,450
Total other non-staff in-kind contributions ($)		$54,300	$41,500	$95,800

8.0 Acknowledgements

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10.0 Publications Arising

Collins, A. Eradiation of proliferative enteropathy – current limitations in knowledge. *Proceedings of APV*, Melbourne 2006.

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Collins, AM and Holyoake, PK (2007) Alternative to antibiotics for the control of ileitis. Camden Pork Alliance; Elizabeth Macathur Agricultural Institute

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