# On farm epidemiology of major enteric diseases

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Research partner: University of Nottingham

Sponsor: BPEX

Duration: 2006 – 2009

## Aims and Objectives

To achieve a better understanding of the transmission of diseases by vectors on pig farms by studying their role in the epidemiology and control of key diseases – particularly *Lawsonia* and *Brachyspira* and PCV 2 agents.

## Methods

A set of 15 case study farms was constructed in the British Isles to include the following:- mixed-age farms, breeder only farms, recently depopulated farms, empty farms and various other scenarios. Most farms were *Lawsonia* and PCV-2 positive, but farms that are positive and negative for *Brachyspira hyodysentariae* were targeted.

Identification and access to the various farms involved was mostly achieved via direct application to both smaller and major British farm groups and their advisers, particularly Midlands farms, Easey farms and Bowes.

At each farm, numerous longitudinal vector samples and population-stratified faecal samples were collected over the summers of 2007 and 2008. Insects and invertebrates were collected via aerial sticky traps, motorised vacuum devices, floor and pitfall traps and sweep nets and identified by the fully trained post-doc entomologist scientist appointed to this project at the University of Nottingham, Dr. Ruth Blunt.

Considerable effort was taken to extract DNA from many samples of representative vectors. The GI tract of larger insects was dissected so that gut-specific carriage could be investigated. The tough chitin coat of some insects was felt to be a possible PCR inhibitor – various extraction methods and kits were tested. Routine PCR techniques were established for *Brachyspira* and *Lawsonia* and PCV 2 infections in samples from both pig faeces and potential insect vectors – the dominant and common insects on each farm.

Specific collaborations were established so that PCR-positive *Lawsonia* DNA from pigs, flies, and cockroaches on several farms were transported to a

Lawsonia reference laboratory in the USA. Each PCR product was cloned and sub-fractionated via the specialised sub-typing variable number tandem repeat (VNTR) technique, which is capable of distinguishing isolates of Lawsonia from each other.

Specific collaborations were also established so that PCR-positive PCV 2 DNA from pigs and flies on several farms were transported to a PCV 2 reference laboratory in the UK. Each PCR product was cloned and sub-sequenced, which is capable of distinguishing isolates of PCV 2 from different sub-species that differ in pathogenicity (2a compared to 2b).

We also established in-house fly and cockroach culture facilities and performed challenge exposure projects within this overall on-farm project. Both insect species were challenged with *Brachyspira hyodysenteriae* and some retained infection in their intestine for 3 days (cockroaches).

**Results**

Within the wide range of insect species and groups identified on each farm and in each production phase within each farm, prominent groups of flies, beetles and other insects have been tabulated, see Table 1. The findings of numerous Dipteroid flies on most farms and a heavy cockroach infestation on 2 farms were of note. The range and levels of Musca and Blatta insects is shown in chart form for 1 farm in 2007 and 2008, indicating similar levels and patterns occurring over those 2 years, see Figures 1 and 3. Figures 2 and 4 also illustrate the weather patterns in those years (wet summers). We analysed the patterns of insects around the various areas of a farm site, see Figure 5, with fewer numbers in the cooler finisher areas compared to the warmer weaner and farrowing accommodation.

The oriental cockroach, *Blatta orientalis* was detected in considerable numbers on 2 farms. One of these farms (Fa-6) was also positive for clinical swine dysentery and *B hyodysenteriae* (by faecal PCR) in its pigs. *B hyodysenteriae* PCR products were also detected in the faeces of several pigs on 3 other swine dysentery-positive farms (with no cockroaches). No clear Brachyspira positive fly or other vectors were noted on these 3 farms. However, PCR products indicative of *Brachyspira hyodysenteriae* were also detected in 30 % of cockroaches, caught in association with the SD pig-positive farm, see Figure 6. This agent was not detected in cockroaches on the other cockroach-positive but SD-negative farm.

Lawsonia-specific PCR products were routinely detected in weaner-grower pig faeces on 13/15 farms. On these farms, 12 were positive for numerous *Musca domestica*; total DNA extracted from the *Musca* collected on 5 farms where this was the dominant fly type, was also positive for Lawsonia DNA PCR reactions. All sets of adults, pupae and larvae of the *Syrphidae* hover fly *Eristalis* sp, collected from the only two farms positive for this fly, also had positive Lawsonia DNA reactions, as did liquid feed sample materials on one

farm in Suffolk. Drosophila fruit flies were not found to be positive. Sub-typing of the Lawsonia DNA obtained from pigs and from insects by variable number tandem repeat analysis indicated that the same VNTR isolate type occurred within the positive farm pigs and the dominant pig-associated dipteroid fly stages on the same farm. Where the dominant Dipteroid fly species was likely to contact pigs and pig faeces, the Lawsonia and Brachyspira counts in these vectors were highest.

This study indicates that the Dipteroid flies associated with pigs and pig bedding feed troughs and flooring, such as *Musca domestica* and *Eristalis* spp had the most likely potential to carry and transmit *Lawsonia intracellularis*. In contrast, Drosophila fruit flies, which do not have significant pig or pig faeces interactions on-farm were not found to be positive for Lawsonia. The infection of Dipteroid fly insects in different stages of growth also indicates that some transmission between farms may be possible, including to nearby farms in start-up situations, by infected adult flies.

PCV 2 virus was located in many weaner pig faeces as expected on most farms, especially prior to the onset of PCV vaccination programs in 2008-2009, see Figure 7. Sequencing of these PCV 2 isolates is continuing. PCV 2 was routinely detected in pig faeces' samples from several farms as expected, at levels of around 25 to 30 % of samples from grower pigs. On a cockroach positive farm, PCR results also tested positive for PCV2 for 10% cockroach samples.

The insect challenge studies indicated that the 2 key potential vectors (Musca and Blatta) may hold *B hyodysenteriae* in their gastrointestinal tracts for periods of 1 to 3 days. We determined that these insects could not only retain these bacteria in their GI tracts, but also excrete viable culturable *B hyodysenteriae* for these periods, via their faeces.

**Conclusions and transfer of findings to the pig community**

These studies suggest that a program aimed at reducing infection levels or post-eradication re-infection with bacterial and viral agents should include not only appropriate biosecurity and pig medication/vaccination, such as Pleuromutilins and piglet PCV vaccine application, but also anti-insect population control measures, targeted at cockroaches and flies in association with pigs.

Promulgation of results has been and will be via the KT team at BPEX, the scientific and farmer press, focusing on key issues such as how eradication or medication programs can be enhanced by new knowledge about key vectors and transmitted sub-types may move around pig farms and interrupt eradication programs.

**Figure 1. Insects caught per fortnight in 2007 on farm Fa2.**

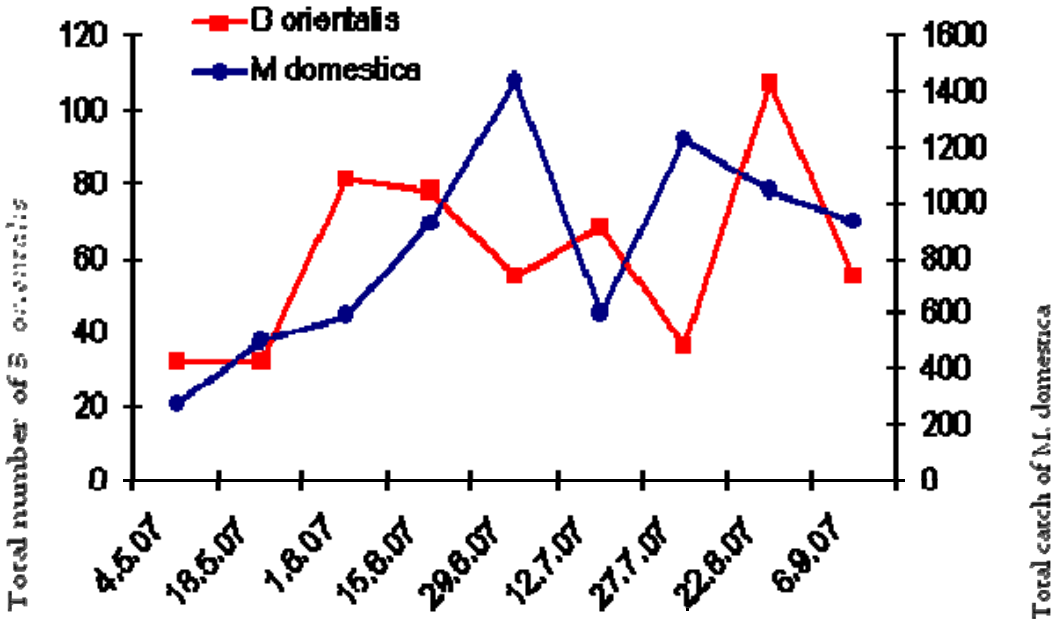


**Figure 2. Average temperature and rainfall for the UK in 2007 compared with monthly averages for the last 10 years.**

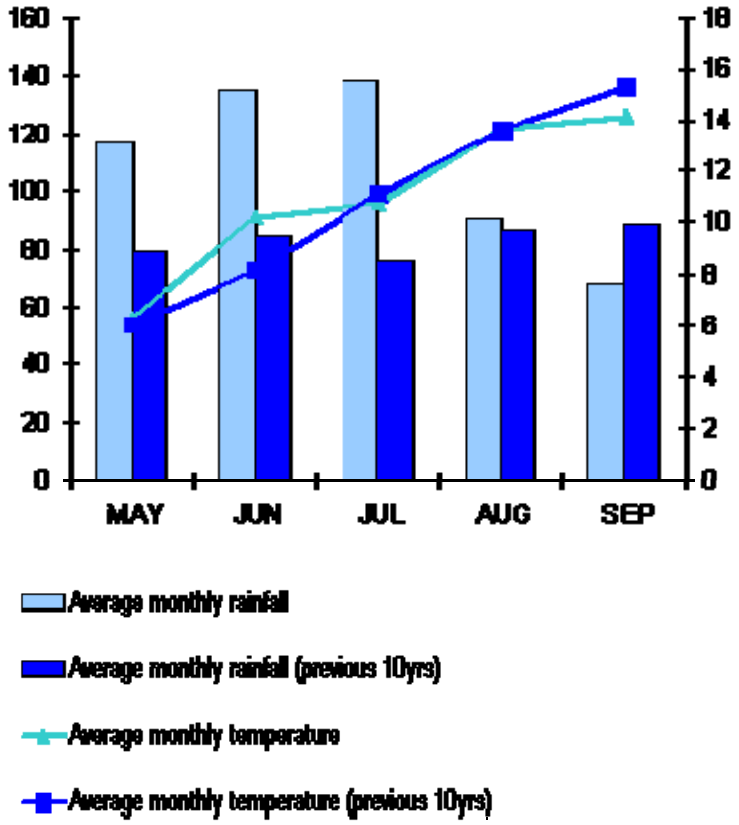

**Figure 3. Insects caught per fortnight in 2008 on farm Fa2.**

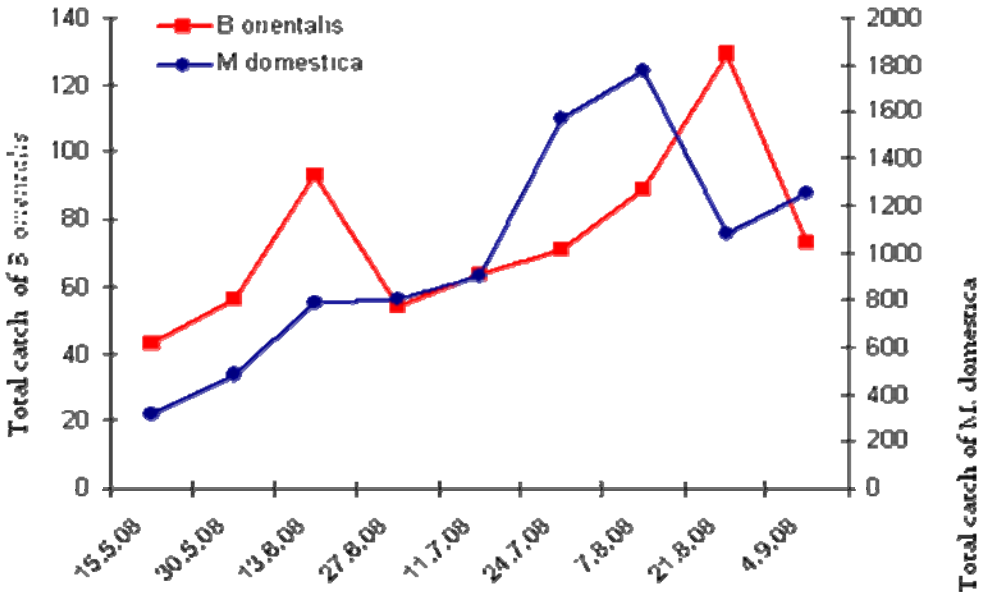


**Figure 4. Average temperature and rainfall for the UK in 2008 compared with monthly averages for the last 10 years.**

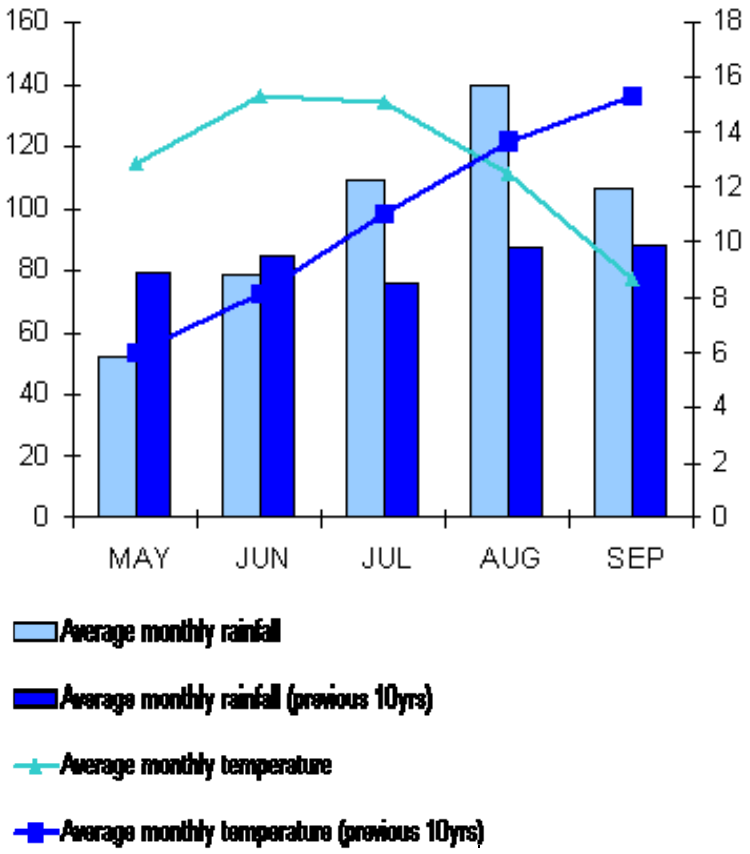

**Figure 5. Total *M. domestica* caught at each 2 week sample interval in each Fa-2 farm area: farrowing houses, weaner accommodation and finisher pens.**

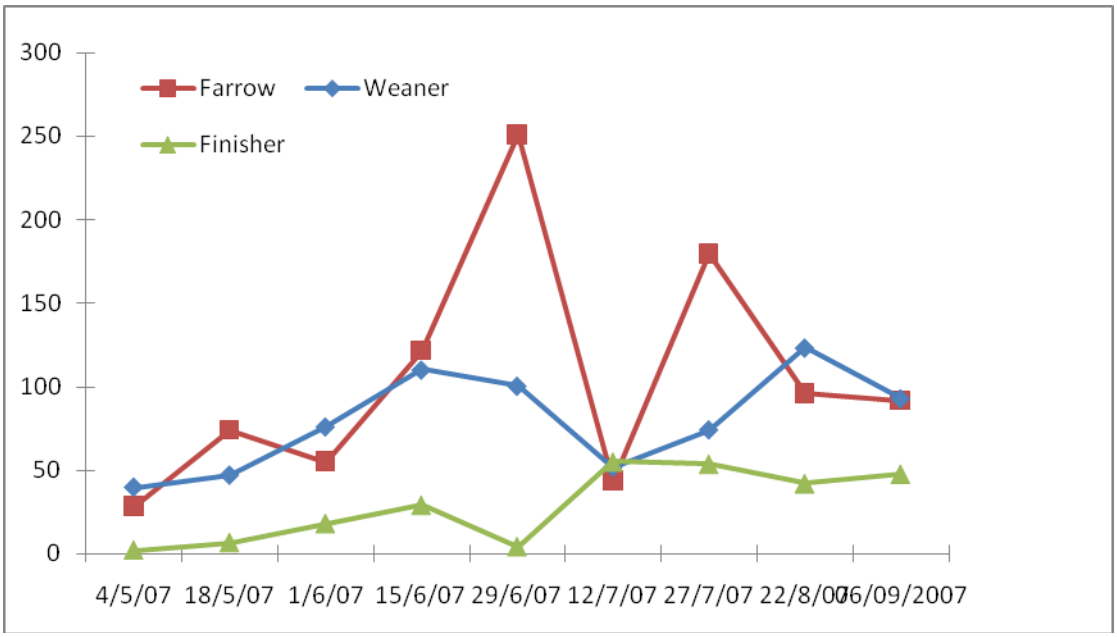


**Figure 6. Samples from pig faeces (left section) positive for Brachyspira hyodysenteriae and right section positive for B hyo from samples processed from the digestive tracts of cockroaches on the same farm (Fa-6).**

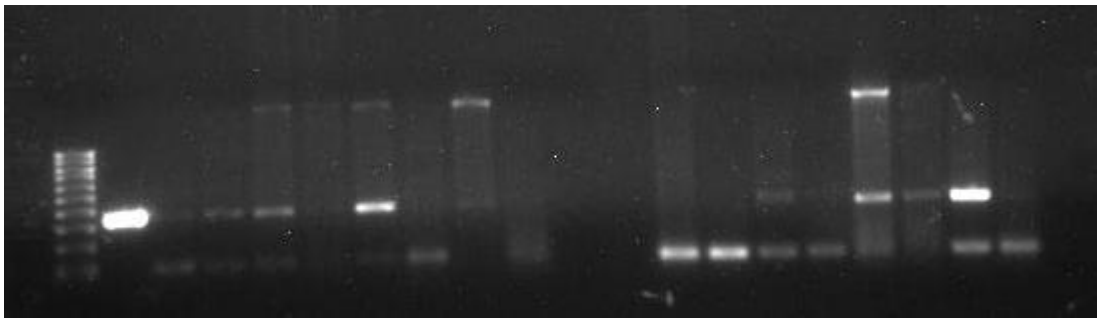

**Figure 7. Positive Lawsonia PCR results in pig faeces, pig feed materials and Eristalis fly pupae on a farm in Suffolk (Fi-2).**

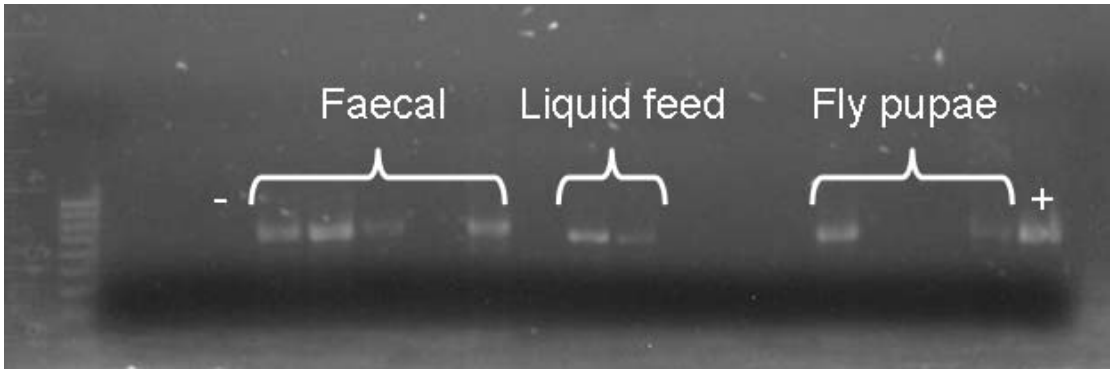


**Figure 8. Positive PCV 2 PCR results in pig faeces and Musca fly materials collected in 2007 on a farm in Nottinghamshire (Fa-1).**

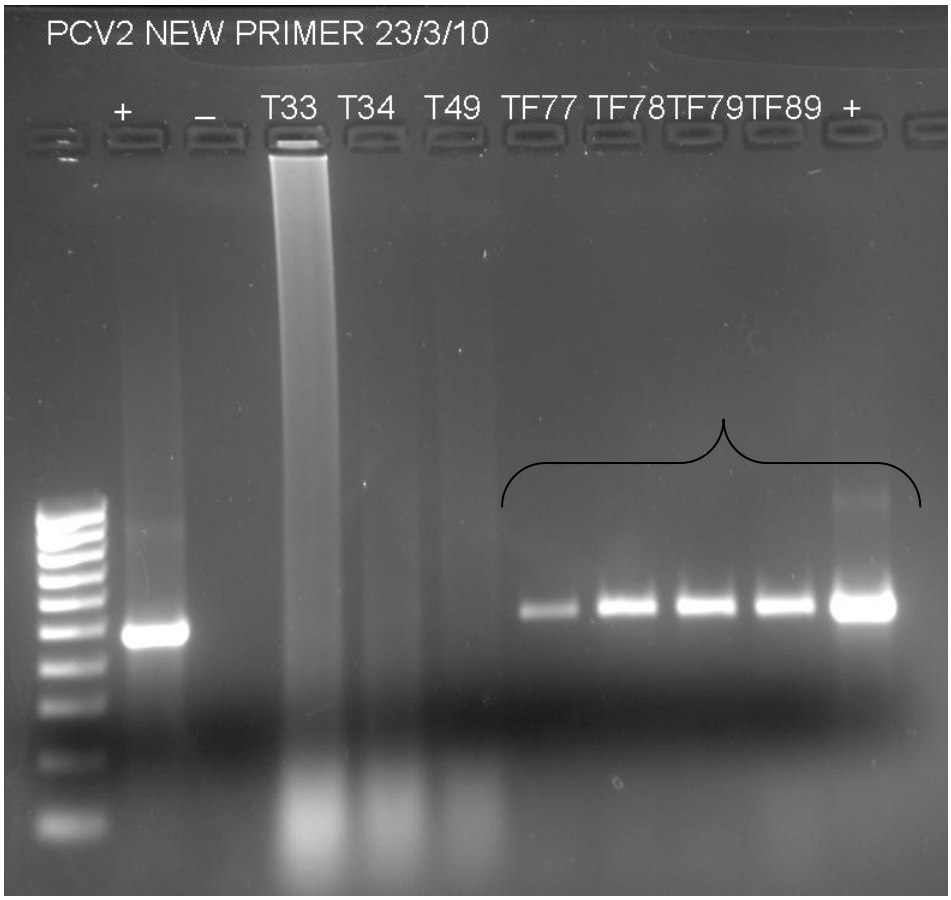

**Table 1. Classification of full sets of species found on British Isles pig farms.**

| | | |
|---|---|---|
| **Generalists** | | **Isopoda, Carabid beetles** |
| **Beneficial's** | **Predatory beetles** | **Staphylinidae** |
| | **Parasitic wasps** | **Ichneumonidae**<br>**Braconidae**<br>**Chalcidae**<br>**Petromalidae** |
| **Potential vectors** | **Diptera fly** | ***Musca domestica* (house fly)**<br>***Muscina stabulans* (false stable fly)**<br>***Ophyra spp* (garbage fly)**<br>***Stomoxys calcitrans* (stable fly)**<br>***Eristalis/Eristalinus* sp (hover flies)** |
| | **Cockroach** | ***Blatta orientalis***<br>**(oriental cockroach)** |
| **Pest of stored product** | **Beetle pests** | **Curculionoidea**<br>**Bruchidae**<br>**Tenebrionoidea** |
| | **Moths and mites** | **Mill moth and Warehouse moth** |

**Table 2. Results of pathogens detected in pigs on different UK and Irish farms.**

| Farm | Size | Straw | Location | 07 | 08 | *L. int* | *B. hyo* | *B. pil* |
|---|---|---|---|---|---|---|---|---|
| Farrow to Finish | | | | | | | | |
| Fa1 – independent | 200 | **** | Nottinghamshire | * | | 14% | | |
| Fa2 – independent | 500 | **** | Nottinghamshire, | * | * | 75% | | * |
| Fa3 | 600 | * | Yorkshire, | * | | 100% | * | |
| Fa4 | 2000 | * | County Kildare, Ireland | | * | 53% | * | |
| Fa5 | 6000 | | County Meath, Ireland | | * | 50% | * | |
| Fa6 | 800 | * | Cheshire | | * | 81% | * | |
| Breeder – Grower | | | | | | | | |
| B1 | 500 | * | Leicestershire | * | | 100% | | |
| B2a | 350 | * | East Anglia | * | | 66% | | |
| B2b | 600 | | East Anglia | * | | 1% | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Finisher** | | | | | | | | |
| **Fi1** | **650** | **** | **Lincolnshire** | * | | **30%** | | |
| **Fi2a** | **1000** | * | **Suffolk** | * | * | **66%** | | |
| **Fi2b** | **800** | | **Suffolk** | * | * | **30%** | | |