

***Lawsonia intracellularis* infections in pigs**

Terminated and ongoing studies

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Gastro-intestinal disorders among growing and finishing pigs can be caused by several intestinal bacterial infections. In yearlier days the most prevalent pathogen was *Brachyspira hyodystenteriae* causing dystentery, but to day several more or less pathogenic bacteria have been identified: *Lawsonia intracellularis*, *Serpulina intermedia*, *Brachyspira pilosicoli* and *E.coli*.

After *Lawsonia intracellularis* (L.i) was recognised as the central aetiological cause of porcine proliferative enteropathy (or porcine intestinal adenomatosis) a lot of research in the veterinary field has been devoted to this pathogen in Denmark. In the recent years several studies have been initiated in cooperation between The National Committee for Pig Production, DANISH BACON & MEAT COUNCIL (Landsudvalget for Svin, DANSKE SLAGTERIER) and Danish Veterinary Laboratory (Statens Veterinære Serumlaboratorium). This paper will give a short status on these studies, of which some are terminated and some just started. All together 5 studies will be mentioned:

- The prevalence of L.i in Danish finishing herds
- Risk factors for excretion of L.i.
- Infection dynamics of L.i on herd level
- Diagnostic tools for L.i.
- Prevention of disease caused by L.i infections

The prevalence of L.i. in Danish finishing herds

In the years 1998/99 a cross-sectional prevalence study was performed in Danish finishing herds (Stege et al, 2000). A total of 79 randomly selected herds were visited by a technician and from each herd 20 faecal samples were collected from individual pigs weighing 30-50 kg. At the time of the visit none of the herds had any recognised disease problems with diarrhoea. In total 1580 faecal samples were collected and examined by polymerase chain reaction (PCR) and by culture (isolation of all relevant pathogens were attempted).

L.i was found in 74 herds (93,7 %) with at mean with-in herds prevalence of 30% (min: 5% & max.: 100%). In 25 herds (32%) L.i. was the only pathogen to be isolated. In 51 of the herds (65%) between 2 to 5 different intestinal pathogens were detected.

Risk factors for excretion of L.i.

In the same herds as mentioned above a study to identify risk factors for infection was performed (Stege et al, 2001). The technicians visiting the 79 herds collected information on feed and management procedures. A questionnaire included information on 29 dichotomous and 3 continuous variables. Based on a multi-factorial logistic regression model it was concluded that:

- Consistent batch production (opposite to continuous production) was associated to a reduced prevalence of L.i (OR = 0.43)

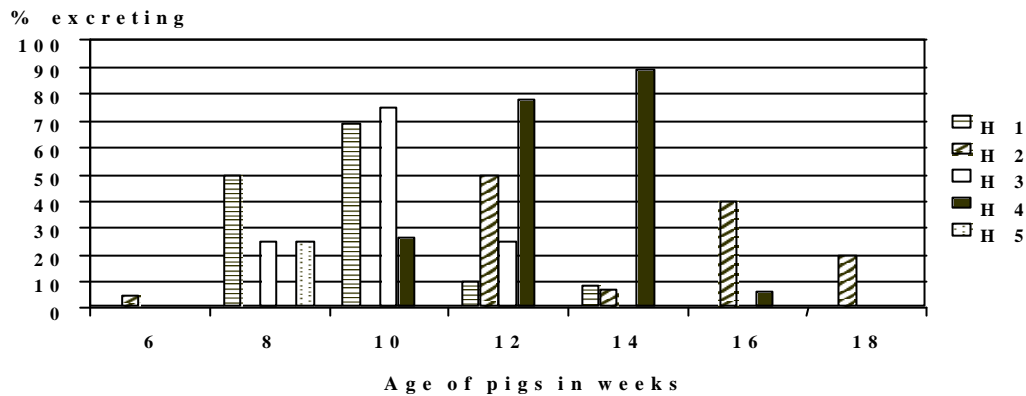
- Home mixed feed (opposite to purchased feed) was associated to a reduced prevalence of L.i (OR = 0.6)

Infection dynamics of L.i on herd level

A longitudinal study has just been performed in 5 chronically infected farrow-to-finish herds aiming at getting insight in the infection dynamic of L.i on herd level. In each herd a cohort of 20 pigs were followed from weaning (4 weeks of age) to slaughter (appr. 7-100 kg b.w.). With 14 days interval blood and faecal samples were collected from all pigs and at the same time they were weighed. At the time of slaughter the intestinal tract were examined for pathological lesions that could be related to a L.i infection. Faecal samples were tested for L.i. by PCR and the blood samples were tested in an immuno-diffusion test (Ileitest®).

Preliminary results show that most pigs excrete L.i. in the period 4-14 weeks after weaning. Out of 100 pigs 66 excreted L.i., typically for 4-6 weeks (Fig 1).

Fig. Excretion of Lawsonia intracellularis (H = herd)



Further analysis of data are under way to clarify if there is any correlation between:

- seroconversion and excretion of L.i in faeces
- weight gain of the pigs and seroconversion or excretion of L.i in faeces
- pathological lesions at slaughter and seroconversion or excretion of L.i in faeces

Diagnostic tools for L.i.

A smaller pilot study on the use of pooled faecal samples has been performed in one herd. The aim was to get an impression on the sensitivity of pooled samples as compared to samples from individual pigs to point out infected pens. From 99 different pens each housing 10 weaners (25 kg b.w.) faeces were sampled from 5 pigs. The 5 samples as well as a pool of the 5 samples were tested for L.i by PCR.

Based on this small study pooled samples seems to have a lower sensitivity to detect infected pens than testing several pigs in the pen.

Prevention of disease caused by L.i infections

By 2001 a 3-year study has been started aiming at reducing diarrhoea caused by L.i and antibiotic resistance. The title of the project is: "Development of production methods for reducing diarrhoea that needs antibiotic treatment and for reducing antibiotic resistance in grower and finisher pigs". It is a 11,5 mill. D.kr. project and it has been financially supported by the Directorate for Food, Fisheries and Agro Business. The project consists of 6 parts:

1. Prevention of diarrhoea caused by L.i by change of the feed

- a. In chronically infected herds intervention studies are performed testing home *mixed feed* against *purchased feed* (phase one)
- b. In chronically infected herds intervention studies are performed testing *an optimal feeding regime* against *the normal feeding* of the herd (phase two). The optimal feeding regime will be based on the results from phase one and from ongoing basic studies on different feed components ability to reduce or eliminate L.i infections from pigs (study by the Danish Veterinary Laboratory and the Danish Agricultural Research Institute: "Alternatives to antibiotic growth promoters in animal production").

Status: One out of 6 herds are included.

2. Prevention of diarrhoea caused by L.i by improving the internal bio-security of the farm.

In chronically infected herds intervention studies are performed testing the benefit of:

- a. Consistent batch production (all in – all out production) without mingling of the pigs during the stay in the unit
- b. Cleaning, disinfecting of the units and the use of a down period to ensure drying out of the environment between the batches
- c. Use of special pens for diseased pigs and use of separate boots and tools for the different units.

Status: No herds have been included. To be tested in 3 herds.

3. Eradication of L.i on herd level

By use of relevant antibiotics it is tested whether it is possible to eradicate L.i from sow herds at establishment or from existing sow herds.

At establishment of a new herd the new stock is medicated for two periods of typically 14 days. Between the two medication periods the gilts/sow are carefully cleaned and disinfected, especially the hoofs. After the cleaning the animals are moved to clean and disinfected units of the farm before the second medication period takes place.

In existing herds an eradication procedure as for eradication of *Mycoplasma hyopneumoniae* (enzootic pneumonia) are used (partial depopulation or the Swiss method) (Bækbo 2001). All pigs younger than 10 months are removed from the farm and the remaining animals are medicated as described for the herds at establishment.

After the medication periods all herds are monitored for 2 years by testing 20 faecal samples and 20 blood samples 6 times with 4 months interval.

Status: Nine newly established herds and one existing herds are monitored.

4. Development of a system for monitoring antibiotic resistance on herd level

The aim of this activity is to estimate the variability of antibiotic resistance:

- Over time in the same pig
- Between pigs in the same pen
- Between pigs in different pens
- Between different herds

- and to relate it to the use of antibiotic in the pigs/herds.

Based on the knowledge of the dynamics of the antibiotic resistance a monitoring program for Danish pig herds will be suggested.

The antibiotic resistance will be estimated by testing faecal samples for enterococci and *E.coli* and looking for resistance against markrolides and tetracyclins (enterococci) and tetracycline and quinolones (*E.coli*). A part from this one *E.coli* and one *E.faecium* isolate will be identified from all samples and tested for resistance in the same procedure as is used in the national surveillance program in Denmark.

Status: Is started up in one out of 3 herds.

5. Molecular methods to characterising of the gut flora

The aim of this part is to develop molecular methods to identify and quantify the gut flora and to use these methods to characterise a “healthy” gut flora. The methods to be used to “fingerprint” and profiling the flora are all implementing the 16S-part of the ribosomal RNA and using Terminal-Restriction Fragment Length Polymorphism and Denaturing Gradient Gel Electrophoresis

A more detailed information on the flora will be obtained through development of a so-called bio-chip, which includes probes from different 16S rRNA genes in the gut. The perspective of the bio-chip is to be able to identify those elements of the gut flora that is a prerequisite for L.i. to infect and cause disease.

Status: Optimising the bio-chip has been started.

(A paper on this subject is presented by Thomas Leser at the workshop: “Qualitative and quantitative characterization of the gut flora using molecular methods”)

6. Development of a serological test for L.i

The aim of this part is to develop an ELISA for detection of antibodies to L.i. One central part is to develop methods for growing L.i. in cell cultures, to be able to produce relevant amount of antigens.

Status: Optimising the growth methods have been started.

In the future new research activities will be started in the area of L.i infections partly based on the activities mentioned above. One activity of interest for both The National Committee for Pig Production and Danish Veterinary Laboratory is testing of L.i vaccines (if/when they turn up) under field conditions.

References

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