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## Monitoring of in breeding herd gilts

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5 **Monitoring of *Lawsonia intracellularis* in breeding herd gilts.**

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## Abstract

In modern pig production, proliferative enteropathy is a common cause of diarrhoea and poor growth in young animals. This study aimed to determine the possible spread of *L. intracellularis* through the sale of replacement gilts and the possibility to protect the herds by adequate biosecurity measures. This was achieved by repeated sampling of 50 gilts in an infected multiplying herd, from the last day in the farrowing pen and until sale. Further, 60 gilts sold from this herd were tested during their stay in quarantine in a recipient herd. To confirm freedom from infection, 100 growing pigs in the recipient herd were also tested. Individual faecal (n=748) and blood (n=728) samples were analysed by PCR and ELISA, respectively. Transmission of *L. intracellularis* from the sows to their offspring was not demonstrated. However, the possible transmission between herds by replacement gilts was demonstrated. Peak shedding occurred at 12 and 15 weeks of age, and single animals were also PCR-positive at 24 to 36 weeks of age in the multiplying herd and in the quarantine in the recipient herd. Further, the possible occurrence of chronically infected carrier animals was suggested. Although *L. intracellularis* is widely spread, it appears possible to avoid the transmission between herds by employing adequate bio-security measures. Thus, it would be advisable to establish herd profiles in breeding herds to avoid the selling of infected animals as well as to establish the health status of the recipient herd. Further, the health status of the recipient herds should be known.

**Keywords:** *Lawsonia intracellularis*; biosecurity; epidemiology; pig; PCR; serology

55  
56 **1. Introduction**

57 Proliferative enteropathy, manifested as diarrhoea and poor performance in growing pigs, or as  
58 acute haemorrhagic enteropathy with high mortalities in gilts and finisher pigs, is widespread in  
59 pig production (Guedes et al., 2002b; Jacobson et al., 2003b). The causative organism, *L.*  
60 *intracellularis*, has been demonstrated globally with herd prevalences ranging from 48 to 100%  
61 ( Lee et al., 2001; Jacobson et al., 2005). The microbe was first described in 1993 (Gebhart et  
62 al., 1993) and still, features of the disease such as the transmission within and between herds,  
63 remain to be fully elucidated (Lawson and Gebhart, 2000; McOrist et al., 2003). The microbe  
64 may be viable for 14 days outside the host (Collins et al., 2000) and rodents are thought to only  
65 play a minor role in the spread (McOrist et al., 2003). Thus, pig-to-pig transmission is  
66 considered as the main route of infection (Jordan et al., 2004) and the possibility that single  
67 gilts transmit the microbe to their offspring has occasionally been suggested (Jensen et al.,  
68 2005; Kroll et al., 2005). A few infected piglets will carry the bacterium to the grower-finisher  
69 facilities where the main shedding and spread will occur (Bronsvort et al., 2001; Jensen et al.,  
70 2005). Within these units, the infection may persist due to poor sanitation measures and short  
71 empty-time space between batches ( Smith et al., 1998; McOrist et al., 1999). Experimentally,  
72 shedding has been detected 1-12 weeks post inoculation, whereas antibodies have been  
73 detected in sera from 2-13 weeks ( Smith and McOrist, 1997; Guedes and Gebhart, 2003).  
74 However, only a few studies have addressed the sequential shedding (Guedes et al., 2002b;  
75 Stege et al., 2004; Jensen et al., 2005) or seroconversion in conventional herds (Just et al.,  
76 2001; Marsteller et al., 2003). In these, peak shedding occurred between 10-16 weeks of age  
77 and persisted for 2-6 weeks. Seroconversion was seen from 10-24 weeks of age, although  
78 serum antibodies only persisted for 1-6 weeks in single animals. In the spread between herds,  
79 the replacement animals are considered to be of major importance (Smith et al., 1998; McOrist

80 et al., 2003). However, the actual infection status of breeding animals close to the introduction  
81 into new herds has rarely been investigated (Friendship et al., 2005).

82  
83 Hence, the aim of this study was to determine the possible spread of *L. intracellularis* through  
84 the purchase of infected breeding herd gilts. Shedding and the development of circulatory  
85 antibodies were determined during rearing and at the time of transfer from a breeding herd  
86 selling gilts at various ages. Further, the possibility to protect the recipient herd from the  
87 infection by adequate biosecurity measures was investigated by repeated sampling of gilts sold  
88 from the breeding herd during their stay in quarantine in a recipient herd.

89

## 90 **2. Materials and methods**

91 The study was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden.

92

### 93 *2.1. Herds*

94 Pigs from the breeding herd had tested positive for *L. intracellularis* by necropsy and by PCR  
95 on faecal samples 3 months prior to the start of the study. Batches of 18 sows farrowed at 4  
96 weeks interval. Piglets were weaned at five weeks of age and remained in the farrowing pen for  
97 one additional week before they were transferred to the grower facilities in a separate building.  
98 Between each batch, all units were washed, disinfected and left empty for one week. Litters  
99 were not mixed. The pigs remained in the growing unit for 4-6 weeks and thereafter, gilts were  
100 moved to an un-insulated barn and mixed in two large pens with deep straw litter bedding.  
101 Within this unit, gilts were moved and mixed 3-4 times. On average, 10-15% of the gilts were  
102 sold as pre-pubertal gilts (*i.e.* ~12-14 weeks old), 10-15% were sold as young gilts (~22 weeks  
103 of age), 10-15% as gilts for mating (~30 weeks of age) and 55-70% as pregnant gilts (~42  
104 weeks of age).

105  
106 The recipient herd was a 160-sow, piglet-producing herd where *L. intracellularis* had never  
107 been demonstrated (Jacobson et al., 2003b). Usually, six young gilts and six gilts for mating  
108 were bought every second month from the multiplying herd and introduced into the quarantine,  
109 located at a one-km distance from the main herd. Separate equipment, clothing and footwear  
110 were used. The gilts remained here until they were ready for mating (three to nine weeks). In  
111 the main herd, the sows farrowed in batches of 30 sows into nursery units that had previously  
112 been washed with hot water under high pressure followed by disinfection with Virkon S  
113 (Pharmacia & Upjohn Animal Health AB, Helsingborg, Sweden). Piglets were weaned at 5  
114 weeks of age and remained in the nursery for 1-2 additional weeks, before they were moved to  
115 the grower facilities, mixed and sorted according to weight. The piglets were sold at 75 days of  
116 age (~30 kg b. w.). The post-weaning mortality was 0.4 to 1.5% and the average piglet growth  
117 rate was 500 g/day.

118

## 119 2.2. Animals and experimental design

120 In the breeding herd, 50 individually ear-tagged, crossbred Yorkshire × Swedish Landrace gilts  
121 (1-3 per litter), born within one week and in one farrowing unit, were included in the study.  
122 Faecal and blood samples were collected every 3rd week from the last day in the farrowing pen  
123 (*i.e.* one week post weaning) and until sale. The pigs were sold to eight different herds.

124

125 The recipient herd only bought gilts from the breeding herd. All of the 60 gilts (22-35 weeks  
126 old) bought during one year were included in the study. Sampling was performed at arrival,  
127 after 3 weeks stay in quarantine, on one occasion also after 6 weeks stay in quarantine, and  
128 before the gilts were transferred to the main herd. To further confirm the infection status of the  
129 herd, samples were obtained from 20 growing pigs in the age of 10-12 weeks on five occasions

130 during a 1 ½-year period (in total 100 pigs). With one exception, individual rectal faecal  
131 samples and blood samples were collected at each occasion.

132

### 133 2.3. Analysis

134 Faecal samples were stored at -80°C until analysis by nested PCR as previously described  
135 (Jacobson et al., 2004). All reactions were performed in 25-µL reaction volume and 1 µL of an  
136 internal control (mimic) was added to each tube (Jacobson et al., 2003a). Positive and negative  
137 controls were included in each run. Inhibited samples were re-analysed by PCR once or twice,  
138 and if results were still not obtained, faecal DNA was extracted by a commercial kit (QIAamp®  
139 DNA Stool Mini Kit, Qiagen Inc., Valencia, California, USA) and analysed by conventional  
140 PCR (Jacobson et al., 2004).

141

142 Blood samples without additives were centrifuged and sera were stored at -20°C until analysis  
143 by blocking ELISA according to the instructions given by the manufacturer (Svanova Biotech,  
144 Uppsala, Sweden, and Bioscreen GmbH, Münster, Germany). Validation of this method has  
145 not been published in a scientifically scrutinised paper, but the stated sensitivity was 96.5% and  
146 the specificity, 98.7% (Svanova biotech, unpublished results). The resulting OD value was read  
147 at 450 nm and converted to the calculated percent inhibition (PI). Values above PI 35 were  
148 regarded as positive.

149

## 150 3. Results

### 151 3.1. Sampling and analysis

152 In total, 492 faecal samples and 484 sera were obtained from the breeding herd (Table 1). Six  
153 animals were sold as prepubertal gilts (88-94 days old), three pigs were sold as young gilts (24  
154 weeks of age), and 23 animals as pregnant gilts. Three pigs were culled at 40 weeks of age



155 because of poor growth. In addition, seven animals died or were culled for other reasons. The  
156 study was terminated 8 months after the first sampling (in total 14 sampling occasions), when  
157 only eight animals remained. From the recipient herd, 256 faecal samples and 244 sera were  
158 obtained from in total 60 gilts and 100 growing pigs.

159  
160 Following the first PCR analysis, a total of 214 samples (29%) were inhibited. Of these, 32  
161 (4%) remained inhibited following the second analysis and 4 samples were prepared by the  
162 commercial kit before being re-analysed.

163  
164 *3.2. Faecal shedding of L. intracellularis in the breeding herd*

165 In total, 49 of 50 pigs shed *L. intracellularis* at least once and no shedding was detected on the  
166 first sampling occasion (in the farrowing unit). The results are shown in Table 1. In 25 pigs,  
167 shedding was detected on one occasion only. In 11 pigs, shedding was detected on two  
168 consecutive samplings, in 2 pigs, shedding was detected on three consecutive samplings and in  
169 one pig, shedding was detected on four consecutive samplings. In 10 pigs, shedding occurred  
170 non-consecutively (Table 2). One of these pigs shed the microbe on two consecutive  
171 samplings, followed by seven negative samplings, and then shed the microbe again at 36 weeks  
172 of age (Table 2).

173  
174 *3.3. Clinical signs in the breeding herd*

175 During the study, 21 pigs (42%) exhibited clinical signs that might have been related to  
176 proliferative enteropathy, with diagnoses such as runt or “small” pigs, unthriftiness, and/or  
177 diarrhoea. Ten of fourteen pigs suffering from unthriftiness or diarrhoea were simultaneously  
178 PCR-positive for *L. intracellularis*. Fourteen pigs, seven of which also had suffered from  
179 diarrhoea, were noted as being “runt” or “small” at various time points during the study.

180

181 *3.4. Development of detectable antibodies to L. intracellularis in the breeding herd*

182 The results are shown in Table 1 and Figs. 1-2. Most pigs (83-85%) were seropositive at 12 and  
183 at 15 weeks of age (average PI 55 and 50, respectively). Thereafter, the number of seropositive  
184 animals, as well as the average PI values (Fig. 2), gradually decreased. On the 7<sup>th</sup> sampling  
185 occasion, at 24 weeks of age, the average PI was 27 and only 18% of the animals were  
186 seropositive. A second, lower “peak” occurred on the 10<sup>th</sup> to 12<sup>th</sup> sampling occasion (59 and  
187 52% of the animals, average PI 40 and 34, respectively).

188 In total, 36% of the animals were seropositive on the first demonstration of shedding. Further,  
189 50% of the animals seroconverted three weeks after the first positive faecal sampling. Five pigs  
190 (13%) were seronegative despite that the microbe had been demonstrated in faeces three weeks  
191 earlier and one pig never seroconverted despite that *L. intracellularis* was demonstrated in  
192 faeces on three consecutive samplings. In 15 pigs, positive titres were only demonstrated for  
193 three weeks following shedding. However, in two pigs, detectable levels of antibodies were  
194 demonstrated for 30-33 weeks following the demonstration of *L. intracellularis*. Five pigs were  
195 seropositive on the first positive faecal sampling occasion; thereafter detectable levels of  
196 antibodies were below the cut-off value for 3-9 weeks, followed by the demonstration of the  
197 microbe in faeces. One pig only was PCR-negative on all sampling occasions (n=13), and with  
198 the exception of the sampling at 33 weeks of age when a weak positive titre (PI 46) was  
199 demonstrated, this pig was also seronegative throughout.

200

201 *3.5. Faecal shedding of L. intracellularis in the recipient herd*

202 Altogether, samples from 60 gilts and 100 growers were obtained on 21 sampling occasions.  
203 All growing pigs and three groups of gilts were negative with respect to faecal PCR. In the last  
204 two groups of gilts, one gilt per group (27 and 32 weeks old, respectively) were PCR-positive

205 at arrival. Both these animals were negative on the consecutive samplings, but on the next  
206 sampling occasion three other gilts within these two groups were positive. Two of these latter  
207 gilts remained in quarantine for six weeks and samples were collected a third time. At that  
208 time, both gilts were negative by PCR. The third gilt stayed in quarantine for three weeks and  
209 was PCR-positive the day before being moved to the main herd. Piglets from her first litter  
210 were mixed with other pigs and sampled at 12 weeks of age (in total 20 animals). All samples  
211 were negative.

212

### 213 *3.6. Clinical signs in the recipient herd*

214 On three sampling occasions, altogether eight growing pigs had diarrhoea. Furthermore, four  
215 gilts in quarantine had “loose stools”. None of these animals were positive for *L. intracellularis*  
216 by PCR.

217

### 218 *3.7. Development of detectable antibodies to *L. intracellularis* in the recipient herd*

219 In the recipient herd, 58 of 60 gilts were seropositive (PI>35) on all sampling occasions. Two  
220 gilts had PI values of respective 32 and 34 at arrival, and on the consecutive samplings the PI  
221 had increased to 85 and 88, respectively. The pigs that were PCR positive had PI values  
222 ranging from 40-82 on all occasions. All growing pigs were seronegative on all sampling  
223 occasions.

224

## 225 **4. Discussion**

226 We were not able to demonstrate any transmission from the sows to their offspring in the  
227 breeding herd. All piglets included in the study were negative in the farrowing unit, both with  
228 respect to the presence of *L. intracellularis* in faeces and detectable antibodies in sera. Instead,  
229 the major spread of *L. intracellularis* occurred in the grower facilities and during the first three

230 weeks in the deep straw litter bedding pens. Probably, once the microbe had gained entrance  
231 into the herd, the short time that the pens were left empty between each batch facilitated the  
232 persistence of the infection within the grower units and the deep straw litter bedding pens.

233  
234 However, a possible transmission between herds by breeding herd gilts was clearly  
235 demonstrated: The six pigs sold at 13 weeks of age were all PCR positive on the previous  
236 sampling occasion and could thus have carried the microbe into the new herd. Furthermore,  
237 despite that most animals shed the microbe at 9-12 weeks of age, shedding was also detected at  
238 24 and 36 weeks of age (Table 2). This was also illustrated by the demonstration of *L.*  
239 *intracellularis* in faeces from two 27- and 32-week-old gilts at arrival to the quarantine in the  
240 recipient herd. Although *L. intracellularis* is widely spread, there are still a number of herds  
241 that may not suffer from the infection. Considering the large economical consequences of the  
242 disease, it would be desirable to minimize the risk of introducing the infection into these herds.  
243 The better understanding of the infection status of the breeding animals and the recipient herds  
244 for the establishment of adequate quarantine periods may prohibit outbreaks of the acute form  
245 of the disease (Friendship et al., 2005). Therefore, it might be advisable to establish herd  
246 profiles to avoid selling infected animals. Such profiles should identify the age category where  
247 the main shedding occurs and the pattern of shedding in particular herds. Alternatively, animals  
248 “at risk” may be kept in quarantine for a prolonged period in the recipient herd. A further  
249 possibility would be to eradicate the microbe from single herds (Harm Nielsen, 2004;  
250 Conradsen, 2006), although the risk of re-introducing this endemically spread microbe must be  
251 considered (Johansen et al., 2002).

252  
253 It seemed plausible, that the microbe would be most easily detected in the grower facilities in  
254 the recipient herd. However, despite repeated sampling (in total 100 piglets), *L. intracellularis*

255 was not demonstrated. It is possible, that the low incidence of disease in the age category of  
256 gilts bought, combined with the prolonged quarantine period for particularly the youngest of  
257 these animals, have protected the herd from infection. Other possible explanations include poor  
258 sensitivity of the diagnostic methods used. However, the PCR has previously been shown to  
259 demonstrate as few as  $10^2$ - $10^3$  organisms/g faeces and the use of an internal control enabled the  
260 demonstration of suboptimal reactions caused by the presence of inhibitory factors.  
261 Furthermore, serum antibodies to *L. intracellularis* were not demonstrated in the growers. In  
262 addition, some as yet unknown factors related to protection may be present in the herd.

263  
264 The interval between the shedding in faeces and demonstration of antibodies in sera generally  
265 varied from 0-6 weeks. This is largely consistent with other reports, where serum antibodies  
266 were detected 1-3 weeks after infection (Guedes and Gebhart, 2003; Stege et al., 2004; Collins  
267 and Love, 2007). However, in two pigs, positive antibody titres were not demonstrated until 21  
268 and 24 weeks post onset of shedding, and one pig never seroconverted. Similar results have  
269 been noted by others and it is possible that an infection limited to the enterocytes may not elicit  
270 a systemic immune response (Guedes et al., 2002b). Thus, the detection of antibodies may  
271 correlate with the presence of profound intestinal lesions rather than with exposure (Guedes et  
272 al., 2002a). Hence, single subclinically infected animals may not be detected.

273  
274 The results in Fig. 1 might indicate the development of protective immunity to *L.*  
275 *intracellularis*. However, 15 pigs shed the microbe in faeces consecutively for 6-9 weeks or  
276 intermittently for 15-24 weeks, despite being seropositive. Furthermore, 15 pigs remained  
277 seropositive (PI>35) for 3 weeks only. Hence, the systemic antibody response does not seem to  
278 be entirely correlated to protection, as also discussed by Guedes *et al.* (Guedes et al., 2002b).  
279 Further, the results may also be caused by constant re-exposure to the microbe.

280

281 The second shedding at 24 weeks of age might indicate re-infection. Alternatively, some pigs  
282 were latently infected, or shed the microbe in amounts too low to be detected by PCR, and thus  
283 were asymptomatic, chronic carriers (Guedes and Gebhart, 2003). This may explain the  
284 detection of positive antibody titres in some pigs during 30-33 weeks. Hence, the three pigs  
285 that were culled because of poor growth at 42 weeks of age might have been chronically  
286 infected. These pigs shed the microbe at 9-15 weeks of age, and in one case also at 36 weeks of  
287 age, and antibodies to *L. intracellularis* were demonstrated on almost all sampling occasions.

288

289 In the multiplying herd, diarrhoea and unthriftiness was correlated to shedding of *L.*  
290 *intracellularis* in 20% of the pigs. Furthermore, after 40-weeks rearing period, three pigs (6%)  
291 were culled because of poor growth. Thus, it seems reasonable to believe that losses in this  
292 herd were substantial, although figures on average growth rate were not available. On the  
293 contrary, the recipient herd that was negative with respect to *L. intracellularis* had a good  
294 average performance.

295

296 In the breeding herd, one pig only remained PCR-negative throughout the study. The low levels  
297 of serum antibodies demonstrated on one single occasion might have been a false positive  
298 reaction and this single pig appears to have escaped the infection. Alternatively, this pig might  
299 have contracted the infection subclinically for a short period only.

300

## 301 **5. Conclusion**

302 Although *L. intracellularis* is widely spread, it appears possible to avoid the transmission  
303 between herds by employing adequate bio-security measures. Therefore, it would be advisable  
304 to establish herd profiles in breeding herds to avoid the selling of infected animals as well as to

305 establish the health status of the recipient herd. In addition, the possible occurrence of single  
306 animals acting as asymptomatic, chronic carriers was suggested.

307

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311

### 312 **Conflict of interest statement**

313 None of the authors of this paper has a financial or personal relationship with other people or  
314 organisations that could inappropriately influence or bias the content of the paper.

315

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393

394 **Legend to the figures**

395

396 **Figure 1.** The development of positive (PI >35) serum antibody titres demonstrated by an  
397 ELISA (■) and shedding (▲) of *Lawsonia intracellularis* in faeces detected by PCR in 29  
398 gilts from a breeding herd. The pigs remained in the herd for at least 10 months and were  
399 consecutively sampled every third week from the last day in the farrowing pen and until sale.

400

401 **Figure 2.** The average serum antibody levels (PI) to *Lawsonia intracellularis* demonstrated by  
402 an ELISA, given as a mean + SD, in gilts from a herd known to be infected with *L.*  
403 *intracellularis*. The gilts were sampled from 6 weeks of age and every third week thereafter  
404 until sale. Samples below PI 35 were regarded as negative.

405

406

407

Figure 1.

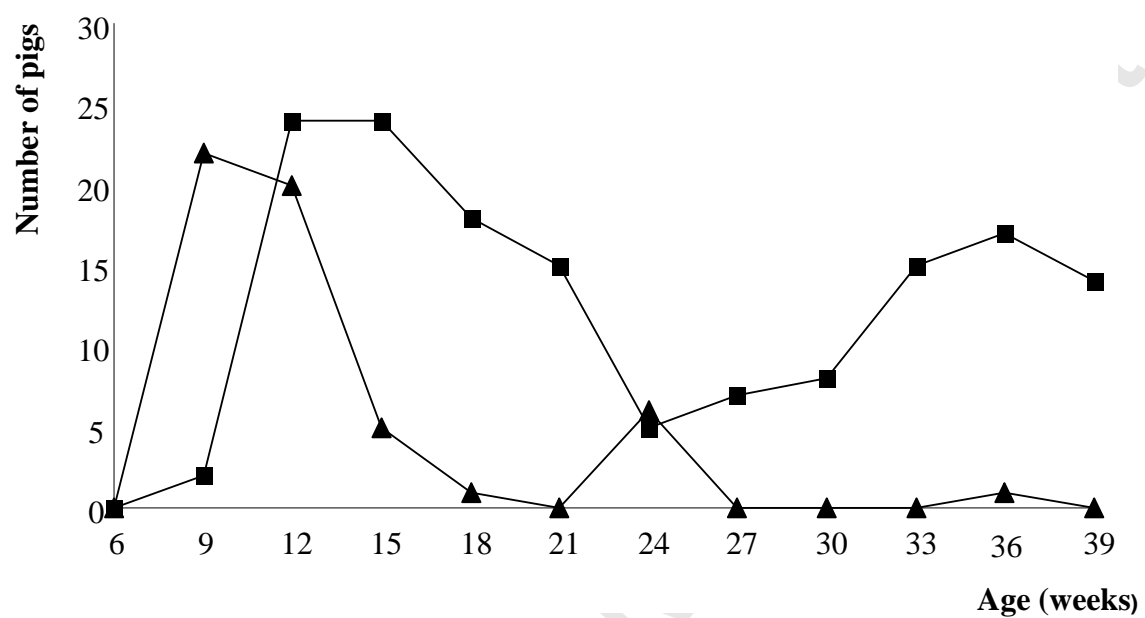
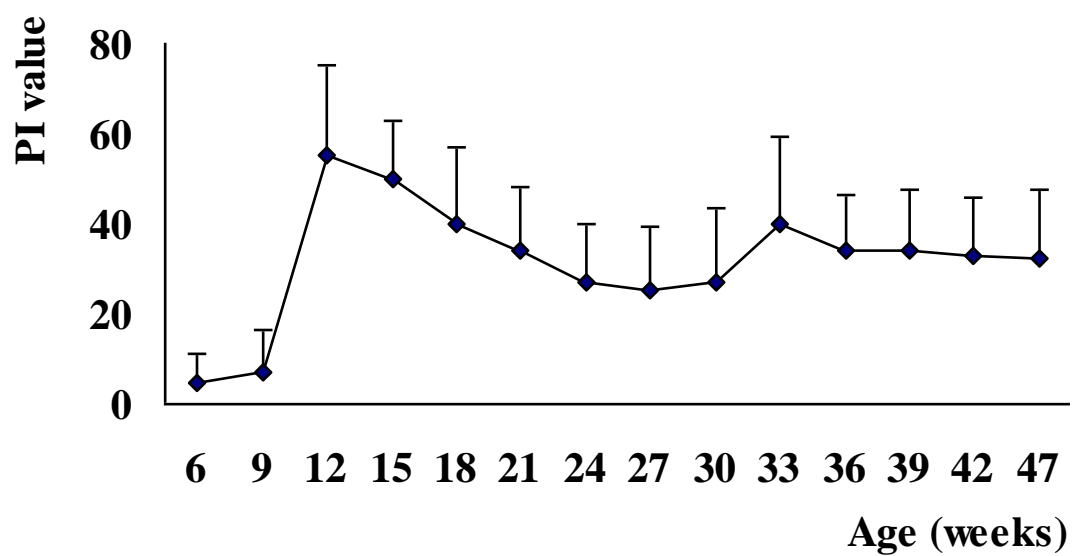


Figure 2.



Accepted

**Table 1.** The shedding of *Lawsonia intracellularis* in faeces and development of detectable circulating antibodies to the microbe in a group of pigs from a breeding herd. The gilts were sampled every third week from the last day in the farrowing pen and until sale.

Age at sampling	Pigs sampled	PCR –positive, n (%)	ELISA-positive n (%)
6 weeks*	50	0 (0)	0 (0)
9 weeks*	50	30 (60)	2 (4)
12 weeks	47	32 (68)	39 (83)
15 weeks	40	9 (23)	34 (85)
18 weeks*	39	2 (5)	24 (62)
21 weeks*	38	2 (5)	18 (47)
24 weeks	33	7 (21)	6 (18)
27 weeks*	33	0 (0)	8 (24)
30 weeks*	32	0 (0)	10 (31)
33 weeks*	32	0 (0)	18 (56)
36 weeks*	32	1 (3)	19 (59)
39 weeks*	33	0 (0)	17 (52)
42 weeks*	25	0 (0)	11 (44)
45 weeks	8	0 (0)	3 (38)

\*All or some pigs were moved during the time period elapsing to the next sampling occasion

**Table 2.** The various patterns of shedding of *Lawsonia intracellularis* in faecal samples from 50 pigs weaned at 5 weeks of age. Samples were collected the last day in the farrowing pen (one week post weaning), and every third week thereafter until sale (altogether 14 sampling occasions). The number of pigs that exhibits each particular pattern of shedding is given. Positive samples are indicated as **+**. Shedding at consecutive sampling occasions is shadowed.

Number of pigs	Age (weeks)													
	6	9	12	15	18	21	24	27	30	33	36	39	42	45
<b>PCR positive once</b>														
n = 11		+												
n = 13			+											
n = 1						+								
Total 48 %														
<b>PCR positive twice</b>														
n = 8		+	+											
n = 3			+	+										
n = 1		+		+										
n = 1		+				+								
n = 3		+					+							
Total 32 %														
<b>PCR positive three times</b>														
n = 1		+	+	+										
n = 1			+	+	+									
n = 2		+	+					+						
n = 1		+	+									+		
Total 10 %														
<b>PCR positive four times</b>														
n = 1		+	+	+	+									
n = 2		+	+	+				+						
Total 6 %														
<b>PCR negative</b>														
n = 1														
Total 2%														