

## Epidemiological investigations on *Lawsonia intracellularis* infections in pig herds

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### Summary

**Objective:** A serological screening study was carried out to determine the prevalence of *Lawsonia (L.) intracellularis* infections in German pig herds. Additionally a cross-sectional study of eight farrow-to-finish herds which was designed to investigate the dynamics of seroconversion. In addition, bacteriological tests were carried out on faecal and intestinal samples from herds with diarrhoea problems to compare the prevalence of *L. intracellularis* with that of *Brachyspira* spp., *Salmonella* spp. and *Escherichia (E.) coli*. **Material and methods:** An indirect immunofluorescence technique was used to test blood samples from the herd screening study (n = 7546 from 694 herds) and the cross-sectional study (n = 936 from 8 herds) for antibodies to *L. intracellularis* and to detect *L. intracellularis* in the faecal and intestinal samples (n = 826 from 403 herds). The faecal and intestinal samples were tested for *Brachyspira* spp., *Salmonella* spp. and *E. coli* using culture techniques. **Results:** Antibodies to *L. intracellularis* were detected in 43.2% of the blood samples tested and in 81.3% of the herds tested. Herds with gilts and sows and fatter-only herds had a higher incidence of seropositivity than piglet-rearing herds, irrespective of whether the piglets were fattened on site or not. The incidence of seropositivity in herds with clinical symptoms of porcine proliferative enteropathy (PPE) was similar to that in herds free from clinical disease or with clinical symptoms of other diseases. The cross-sectional study showed that seroconversion occurred in some animals as early as 10 weeks but that most animals seroconverted between 13 and 16 weeks. Bacteriological testing of faecal and intestinal samples demonstrated that, in herds with diarrhoea problems, haemolytic *E. coli* and *L. intracellularis* were much more common (occurring in 48.4% and 33.7% of herds) than *Brachyspira (B.) hyodysenteriae* (21.1%), *Salmonella* spp. (17.3%) and *B. pilosicoli* (2.5%). Mixed infections of *L. intracellularis* with *B. hyodysenteriae*, *B. pilosicoli* or *Salmonella* spp. were rare, occurring in 3.5%, 1.0% and 5.6% of herds respectively. **Conclusions and clinical relevance:** The results indicate that infection with *L. intracellularis* is very widespread in Germany and that it occurs both in herds with diarrhoea and unthriftiness problems and in herds without clinical symptoms. Specific diagnostic measures are therefore required to exclude other diarrhoeal pathogens.

### Key words

*Lawsonia intracellularis* - porcine proliferative enteropathy - prevalence - serology - *Brachyspira hyodysenteriae*

## Introduction

The aetiology of porcine proliferative enteropathy (PPE), also referred to as porcine intestinal adenomatosis (PIA) or ileitis, remained unclear for many years, although the lesions were first described in a case report on intestinal adenoma in swine in 1931 [2] and continued to be observed sporadically thereafter. An acute form of the disease, known as porcine haemorrhagic enteropathy (PHE), has been recognised since the early 1970s [32]. The identity of the pathogen was not resolved until just over a decade ago when it was successfully co-cultured with a rat enterocyte cell line and the disease was reproduced using a pure culture of the agent. The organism was assigned to a new genus - *Lawsonia (L.) intracellularis* - and formally described in 1995 [23, 27, 29].

Identification of the pathogen in faecal or intestinal material as part of routine diagnostic testing is currently carried out using the polymerase chain reaction (PCR) or an indirect immunofluorescence test (IFT) [17, 26, 28, 37]. Specific identification of *L. intracellularis* in histological preparations is achieved by immunohisto-

chemical methods (immunoperoxidase and immunofluorescence) and in-situ hybridisation; Warthin-Starry silver staining and the modified Ziehl-Neelsen stain are used as non-specific staining methods [9, 21, 24, 40].

Herd screening is carried out by detecting antibodies to *L. intracellularis*. Antibody detection in serum can be performed using an indirect IFT, an immunoperoxidase monolayer assay (IPMA) or an enzyme-linked immunosorbent assay (ELISA) [11, 16, 19, 20, 22].

*L. intracellularis* infections in swine must be considered to be worldwide in distribution. The prevalence data quoted by various authors differ according to the testing method used. Lesions of PPE are rarely detectable in slaughter pigs, although pathological findings have been observed in necropsy material from 10 - 20% of herds submitting samples. The organism can be detected in 25 - 75% of herds with clinical symptoms of disease and in 7 - 40% of herds without clinical symptoms. In one Danish study, as many as 94% of clinically normal herds that were tested were found to be infected. In various studies worldwide, *Lawsonia* antibodies were detected in 25 - 100% of herds tested; the rates of detection were usually higher for antibodies than for the organism itself. Reviews of relevant studies are given in references [4] and [30].

IgG antibodies can be detected serologically about two weeks after experimental infection. Faecal shedding of *Lawsonia* can be detected as early as one week after infection and can persist for up to 12 weeks despite antibody formation [12]. In a cross-sectional study carried out in American herds, the interval between earliest detection of faecal shedding and seroconversion ranged from two to eight weeks [14].

The aim of the prevalence study described in this paper was to obtain, by means of serological screening tests, data on the prevalence of *L. intracellularis* infections in German pig herds, taking account of herd type, age group and stock type and examining potential correlations with different management and husbandry conditions. A cross-sectional study monitoring antibody formation across various age-groups in eight farrow-to-finish herds was also performed to determine the dynamics of the infection, with a view to developing appropriate prophylactic and therapeutic measures. In addition, bacteriological tests were carried out on faecal and intestinal samples from herds with diarrhoea problems to compare the prevalence of *L. intracellularis* with that of *Brachyspira* spp. and *Salmonella* spp. and to estimate the incidence of single and mixed infections.

## Material and methods

### *Herd screening*

For the herd screening study, a total of 7546 serum samples from 694 herds spread throughout the whole of Germany were tested over the period 2002 - 2004. The opportunity to take part in this voluntary screening programme was offered to interested herds via their vets. Each herd was asked to submit at least 10 samples if possible; in fact, an average of 10.9 samples per herd (range 2 - 42) were submitted for testing. It was left to the vets' discretion, based on the herd type and the presence or otherwise of clinical symptoms, to determine how many samples were to be taken from each age group or stock type.

A herd was classified as positive if antibodies to *L. intracellularis* were detected in one or more samples. The vets were asked to fill in a questionnaire giving details of herd type, herd size, number of purchase sources for bought-in weaners, housing type (perforated, semi-perforated or solid floors), management system (continuous flow, all-in/all-out on a herd basis, all-in/all-out for individual sections), clinical symptoms and any antibiotic treatment administered. For statistical analysis purposes, herds were divided into two groups (Group 1, with negative or questionable results, and Group 2, with at least one positive result). Comparisons between the groups in respect of the parameters included in the questionnaire were then carried out using either the  $\chi^2$  test (for qualitative parameters) or, following calculation of the mean and standard deviation, a hierarchical analysis of variance (for quantitative parameters).

### *Cross-sectional study*

For the cross-sectional study, eight farrow-to-finish herds with a history of *L. intracellularis* infections confirmed by direct PCR detection were selected. Four of the herds were from north Germany and four were from south Germany. In each herd, 117 blood samples were taken for serological testing at a single time point; of these, six were from gilts, six from second-parity sows, six from third-parity sows and 99 from weaners and fatteners at various ages (eleven each at 4, 6, 8, 10, 13, 16, 19, 22 and 25 weeks). None of the eight herds, which were tested in March 2002, was part of the prevalence study.

### *Serological testing*

Serological testing of the blood samples for antibodies to *L. intracellularis* was carried out using an indirect immunofluorescence test (IFT; IleiTest<sup>®</sup>, Elanco, Bad Homburg) [20]. For this procedure, the antigen concentrate (a formalin-inactivated whole-cell suspension) was diluted 1:30 by the addition of PBS. 5 µl of the dilution was pipetted into each of the wells of a slide and, after drying in air, the slide was fixed in acetone at -20°C. All the serum samples, including the positive and negative controls, were diluted with PBS buffer pH 7.2; in accordance with the manufacturer's instructions, only the 1:30 dilution was tested. 5 µl of the diluted serum sample was pipetted into each well and the slides were incubated overnight at 4°C in a moist chamber in a refrigerator. Serum from a gnotobiotic piglet was used as the negative control, whilst serum which was taken from a pig with clinical symptoms and histopathological evidence of *L. intracellularis* infection confirmed by PCR and which also showed a positive reaction at a dilution of 1:480 was used as the positive control.

On the following day, the slides were washed four times with PBS for 5 min each time and then dried at 37°C in an incubator. After drying, 5 µl of fluorescein isothiocyanate (FITC)-conjugated goat anti-swine IgG (Dianova, Hamburg), diluted 1:10 with PBS, was added to each well and the slides were incubated at 37°C for a further 30 min. Washing and drying of the slides were then repeated as described above. The slides were mounted with glycerin, examined under a fluorescence microscope ( $\lambda = 495$  nm) and assessed as follows: Brilliant fluorescence at margins = positive; weak fluorescence at margins = questionable; no fluorescence = negative. The test has been validated by Knittel et al. [20] using sera from both experimental and field infections; cross-reactions with *Brachyspira* (*B.*) *hyodysenteriae*, *B. pilosicoli*, *B. innocens*, *Salmonella* (*S.*) *typhimurium*, *S. choleraesuis*, *Campylobacter mucosalis* and *Campylobacter hyointestinalis* were not observed. Questionable results, which can be obtained as a result of non-specific reactions or in animals which show rising or falling titres below the threshold titre of 1:30, were included with the negative results in the prevalence study but were reported separately in the cross-sectional study in order to enable any increase or decrease in the number of questionable samples at specific time points to be documented.

### *Testing of faecal and intestinal samples*

Over the period 2002 - 2003, a total of 826 faecal and intestinal samples from 403 herds were tested for the presence of *L. intracellularis* using an indirect IFT; the intestinal samples were from necropsy material. A test for *Brachyspira* spp. was carried out using cultural and biochemical techniques in 812 of the samples (399 herds), whilst 300 of the samples (139 herds) were tested for *Salmonella* spp. and 165 of the samples (91 herds) were tested for haemolytic *E. coli*. All samples were submitted for routine diagnostic testing as a result of diarrhoea problems and were not taken as part of the prevalence study. Samples from female animals were mainly from gilts which had shown signs of PHE.

The IFT was carried out on smeared slides which were fixed in acetone at -20°C for 15 min, overlaid with a 1:1000 dilution of monoclonal mouse hybridoma antibody [26] and incubated at 37°C in a moist chamber for 60 min. After washing twice for 10 min with PBS and drying at 37°C, the slides were incubated with FITC-conjugated goat anti-mouse IgG (Dianova, Hamburg) (diluted 1:400 with PBS) at 37°C in a moist chamber for 30 min. Washing and drying were then repeated as described above before the slides were mounted with glycerin and examined under a fluorescence microscope.

Samples were cultured for *Brachyspira* spp. on appropriate selective culture media in an anaerobic atmosphere (Oxoid AnaeroGen System; Oxoid, Wesel), usually at 42°C. Differentiation of *Brachyspira* isolates was carried out on the basis of haemolysis pattern, indole production, hippurate hydrolysis and  $\alpha$ -galactosidase and  $\alpha$ - and  $\beta$ -glucosidase activity [8].

*Salmonella* spp. and *E. coli* were isolated using conventional culture techniques [3].

## Results

### Herd screening

The herd screening results showed that antibodies to *L. intracellularis* were present in 43.2% of the blood samples tested and that 564 out of 694 herds (81.3%) contained at least one seropositive animal. The incidence of herd seropositivity differed both between different *Länder* and different herd types (see Tables 1 and 2).

As regards geographical distribution, the lowest incidence of herd seropositivity was found in Hesse (66.6%) and the highest in Saxony (100%); as regards herd type, over 80% of herds with gilts and sows (including farrow-to-finish herds) and over 80% of fattener-only herds had at least one seropositive animal, whilst the respective figures for piglet-rearing herds were substantially lower (18.2% for herds which only reared piglets and 38.5% for herds which also had a fattening operation). When considering these results, it should be borne in mind that not all age groups or stock types were tested in every herd. A breakdown of results by age and stock type is given in Table 3. This shows, for instance, that, of the 208 herds with sows or gilts where these animals were actually sampled and tested, 96.6% contained at least one seropositive sow or gilt and that, of the 297 herds where fatteners over 50 kg were sampled and tested, 88.9% contained at least one such animal that was seropositive.

Figure 1 shows the percentage of individual animals testing positive, again broken down by age group/stock type. The incidence of seropositivity was found to be highest in gilts, sows, boars and fatteners over 50 kg and lowest in weaners.

Most herds submitting samples reported a history of diarrhoea, which in some cases was associated with unthriftiness (see Table 4). Of 546 herds reporting known symptoms of *Lawsonia* infection (diarrhoea, unthriftiness and sudden death associated with bloody diarrhoea (PHE)), 80.8% contained at least one seropositive animal. In the 90 herds which were free of clinical symptoms or which reported a history of other diseases (such as pneumonia, meningitis or enterotoxaemia), the figure proved to be slightly higher at 85.6% ( $p > 0.05$ ).

**Table 1:** Results of serological testing for antibodies to *L. intracellularis* in 694 herds from 12 German *Länder*, broken down by *Land*

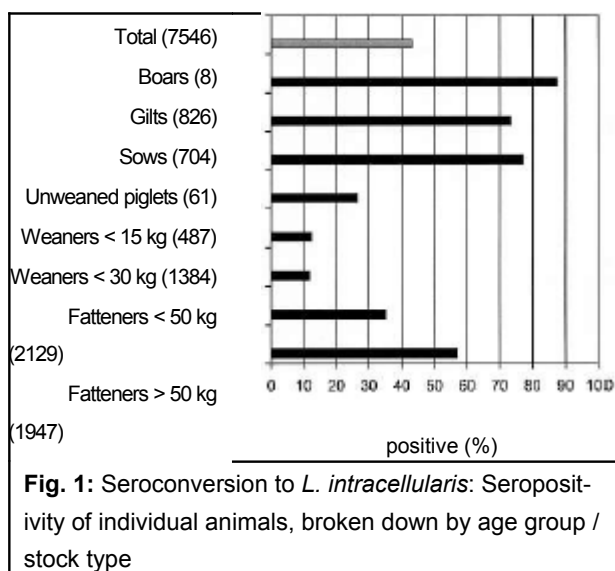
<i>Land</i>	No. of herds tested	% of herds seropositive
Baden-Württemberg	119	79.0
Bavaria	198	81.3
Brandenburg	12	83.3
Hesse	9	66.6
Lower Saxony	18	77.8
Mecklenburg-Western Pomerania	130	80.8
North Rhine-Westphalia	141	85.1
Rhineland-Palatinate	4	75.0
Saxony	4	100.0
Saxony-Anhalt	21	71.4
Schleswig-Holstein	25	80.0
Thuringia	13	92.3
Total	694	81.3

**Table 2:** Results of serological testing for antibodies to *L. intracellularis* in 694 herds from 12 German *Länder*, broken down by herd type

Herd type	No. of herds tested	% of herds seropositive
Multiplier herd (breeding sows with or without rearing)	73	90.4
Gilt rearing	16	93.8
Piglet producer	154	79.9
Piglet producer with fattening	157	89.2
Piglet rearing	22	18.2
Piglet rearing with fattening	13	38.5
Fattening	259	81.5
Total	694	81.3

**Table 3:** Seroconversion to *L. intracellularis*: Herd seropositivity broken down by age group and stock type

Age group / stock type sampled and tested	No. of herds in which this age group / stock type was tested	% of herds classified seropositive, where this age group / stock type was seropositive	% of herds classified seropositive, where this age group / stock type was questionable or seronegative and another age group / stock type was seropositive	% of herds classified as questionable or seronegative (on the basis of results from all age groups / stock types)	Average number of samples from this age group / stock type (n plus range)
Gilts and sows	208	96.6	0.5	2.9	7.4 (1 - 40)
Boars	8	87.5	12.5	0	1.0
Unweaned piglets	22	45.4	36.4	18.2	2.8 (1 - 12)
Weaners < 15 kg	115	22.6	51.3	26.1	4.2 (1 - 15)
Weaners < 30 kg	252	29.4	39.7	30.9	5.5 (1 - 30)
Fatteners < 50 kg	350	64.3	18.9	16.8	6.1 (1 - 24)
Fatteners > 50 kg	297	88.9	2.7	8.4	6.6 (1 - 40)



**Table 4:** Results of serological testing for antibodies to *L. intracellularis*, broken down by herd symptom history

Symptoms	No. of herds tested	% of herds seropositive
Diarrhoea	306	83.7
Unthriftiness	45	80.0
Diarrhoea + unthriftiness	190	76.3
Bloody diarrhoea + sudden death (PHE)	5	80.0
Other symptoms	56	80.4
No symptoms	34	94.1
No data given	58	79.3
Total	694	81.3

**Table 5:** Comparison of average herd sizes in herds that were questionable/seronegative (-) or seropositive (+) for *L. intracellularis*

Herd type	Serological status	No. of herds tested	Average herd size ( $\bar{x} \pm$ s.d.)	p > 0.05
Multiplier herd (breeding sows with or without rearing)	-	6	176.7 $\pm$ 80.7	p > 0.05
	+	51	351.1 $\pm$ 913.9	
Piglet producer	-	29	237.2 $\pm$ 356.7	p > 0.05
	+	114	338.6 $\pm$ 625.7	
Piglet producer with fattening	-	16	586.6 $\pm$ 1474.7	p > 0.05
	+	124	256.5 $\pm$ 598.2	
Piglet rearing	-	17	2052.4 $\pm$ 1370.5	p > 0.05
	+	3	1466.7 $\pm$ 1361.4	
Fattening	-	45	2414.0 $\pm$ 4441.0	p > 0.05
	+	190	1618.3 $\pm$ 2153.1	

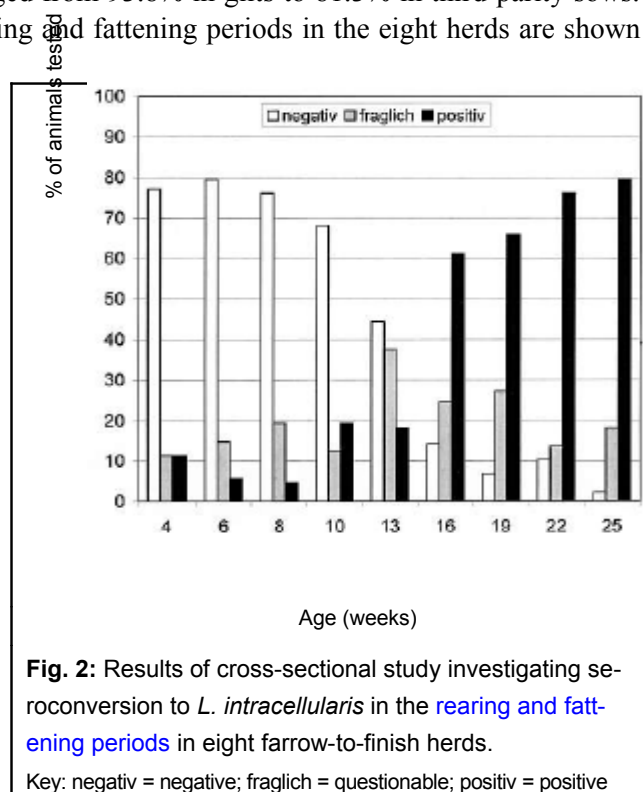
A comparison of herd sizes showed that there were no statistically significant differences in average herd size between questionable/seronegative (Group 1) and seropositive (Group 2) herds for any individual herd type (see Table 5). There were also no significant differences in the number of purchase sources for bought-

in weaners between questionable/seronegative and seropositive fattening herds (average number of sources was  $3.1 \pm 6.5$  for Group 1 and  $4.5 \pm 8.7$  for Group 2;  $p > 0.05$ ). Fattening herds which purchased their weaners from a single source did not differ in serological status from those which purchased their weaners from multiple sources. Prior antibiotic treatment and housing type (perforated, semi-perforated or solid floors) also had no effect on whether a herd was questionable/seronegative or seropositive ( $\chi^2$  test;  $p > 0.05$ ).

Only fattening herds were compared in respect of the effect of the management system on serological status. Of the 76 herds using an all-in/all-out system on a herd basis, 14 were Group 1 and 62 were Group 2, whilst of the 168 herds using a continuous flow system or an all-in/all-out system for individual sections, 30 were Group 1 and 138 were Group 2, so the hypothesis that herds using an all-in/all-out system on a herd basis would have a lower incidence of negative or questionable results than those using either of the other systems was not confirmed ( $\chi^2$  test;  $p > 0.05$ ).

## Cross-sectional study

In the eight herds which supplied samples for the cross-sectional study, antibodies to *L. intracellularis* were found in 72.2 - 100% of the sows tested (sow numbers in the individual herds were between 80 and 1900). The incidence of seropositivity in female animals ranged from 93.8% in gilts to 81.3% in third-parity sows. The aggregated serological results for pigs in the rearing and fattening periods in the eight herds are shown in Figure 2. In four-week-old piglets, antibodies were detected in only four of the herds. Overall, only 11.4% of piglets were seropositive at four weeks, and the incidence of seropositivity was even lower at six and eight weeks (5.7% and 4.5% of animals respectively); only two of the herds contained seropositive animals at six weeks and only three contained such animals at eight weeks. The overall incidence of seropositivity was higher after 10 weeks, with 19.3% of animals being seropositive and only one herd not being classified as seropositive. At 13 weeks, 18.2% of animals were seropositive and all herds contained at least one seropositive animal. There was a marked increase in the incidence of seropositive animals at 16 weeks (61%), and after this point, the incidence steadily rose to 79.6% by the 25th week, although one herd showed a rather less marked increase, with the percentage of seropositive animals at week 25 being just 36.4%.



## Testing of faecal and intestinal samples

The results of bacteriological tests carried out on faecal and intestinal samples are shown in Table 6. *L. intracellularis* was found in 33.7% of the 403 herds which submitted samples. Only haemolytic *E. coli* was more common, occurring in 48.4% of the 91 herds tested, although it should be borne in mind that pathogenicity factors for the *E. coli* strains involved were not determined. *B. hyodysenteriae* was isolated from 21.1% of the 399 herds tested, whilst only 2.5% of herds were found to be infected with *B. pilosicoli*. Of the species of *Brachyspira* that are not considered pathogenic, *B. innocens* was most common, occurring in

19.3% of herds tested. Species of *Brachyspira* which could not be further differentiated were isolated in 8.9% of the samples. *Salmonella* spp. were detected in 17.3% of the 139 herds tested.

The incidence of mixed infections involving *L. intracellularis* and another organism was relatively low; mixed infections with haemolytic *E. coli* were found in eight of the herds tested (8.8%), whilst mixed infections with *Salmonella* spp., *B. hyodysenteriae* or *B. pilosicoli* were found in eight herds (5.6%), fourteen herds (3.5%) and four herds (1.0%) respectively. Only in one herd were both *Salmonella* spp. and *B. hyodysenteriae* found in addition to *L. intracellularis*.

**Table 6:** Results of bacteriological tests carried out on faecal and intestinal samples from herds with a history of diarrhoea problems

Pathogen	Samples		Herds	
	Number	% positive	Number	% positive*
<i>L. intracellularis</i>	826	24.7	403	33.7
<i>B. hyodysenteriae</i>	812	15.9	399	21.1
<i>B. pilosicoli</i>	812	2.1	399	2.5
<i>B. intermedia</i>	812	1.7	399	2.5
<i>B. innocens</i>	812	17.5	399	19.3
<i>B. murdochii</i>	812	6.8	399	9.5
<i>Salmonella</i> spp.	300	8.3	139	17.3
Haemolytic <i>E. coli</i>	165	40.6	91	48.4

\* Herd contains at least one positive sample

*L. intracellularis* was found most commonly in herds which submitted samples from gilts / sows (n = 33; 51.5% positive) or fatteners (n = 178; 35.4% positive). By contrast, the organism was only detected in 20% of herds with diarrhoea problems in weaners (n = 50). No history for the animals sampled was given by 142 herds.

## Discussion

The results of herd-based serological screening, which showed that 81.3% of herds were seropositive, demonstrate that *L. intracellularis* is very widespread in Germany, without there being any particular concentration in individual regions. This finding is consistent with data from studies carried out by ourselves over the period 1999 - 2001 (covering 165 herds, of which 78.2% were seropositive) [37, 38] and studies by other authors from Germany (150 herds, of which 53.3% were seropositive) [31]. Seroconversion occurs in a particularly high percentage of gilts, sows and older fatteners but, as these groups were not always tested in the herds investigated, actual herd prevalence rates are probably even higher. Table 3 shows that 96.6% of herds in which gilts and sows were sampled and 88.9% of herds in which fatteners over 50 kg were sampled were positive. Similarly high prevalence rates have been found in serological studies from numerous other countries [1, 6, 30]. The serological test method employed in our study may also contribute to prevalences being underestimated since only one titre level is tested and results classified as questionable may in some cases be due to incipient antibody formation or falling titres rather than to non-specific reactions; this is clearly illustrated by the results of the cross-sectional study shown in Figure 2. The number of questionable results increases as the number of animals with maternal antibodies decreases and as the infection manifests itself between weeks 13 and 16. If there is a high incidence of questionable results during diagnostic testing, it may be advisable to carry out follow-up tests. False positive results may be obtained by inexperienced analysts because of the subjective nature of fluorescence evaluation. Cross-reactions with various other diarrhoeal pathogens have been shown by previous studies not to occur with this method [20].

In our study, high prevalence rates were found in both multiplier herds and conventional herds. The only exceptions were herds which only reared piglets (prevalence rate 18.2%) and herds which reared piglets and also had a fattening operation (prevalence rate 38.5%). The low rates in these cases may have been due to sampling being carried out before seroconversion could take place (as Figure 1 shows, seroconversion rates are likely to be lowest in weaners). It would seem to be worth investigating whether early weaning and separate rearing in another herd might be a viable way of breaking the chain of *Lawsonia* infection, although reports from other authors suggest that medicated early weaning (MEW) and segregated early weaning (SEW) techniques in fact rarely lead to the elimination of infection [39].

It is striking that the incidence of seropositivity in herds with clinical symptoms of PPE or PHE is similar to that in herds which had completely different symptoms or no symptoms at all. This implies that latent infections with *L. intracellularis* are also widespread. In Denmark, the organism was detected by PCR in 74 out of 79 herds which had no clinical symptoms [34]. The diagnosis of diarrhoeal diseases should therefore be based not only on detecting the organism in the faeces but also on histopathological tests designed to identify the characteristic changes of PPE.

A survey of 319 British pig farms found that sow herd sizes of over 500, the use of fully perforated floors in rearing and fattening accommodation and the non-use of all-in/all-out management systems on a herd basis in fattening facilities were all risk factors for PPE. Weaning age, the type of diet, the feeding and watering system, the types of building used and the use of regular antibiotic treatment were not identified as risk factors [33]. Other authors have suggested that all-in/all-out management on a herd basis is more likely to break the chain of infection than continuous flow management or all-in/all-out management for individual sections [5]. In the studies carried out by ourselves, herd size, number of purchase sources for bought-in weaners, housing type, fattening management system and herd antibiotic treatment were all investigated as potential predisposing factors for *L. intracellularis* infection, but none were shown to have any statistically significant effect. This may be related to the very high prevalence of the infection in the herds tested, which employed a range of husbandry and management systems, and to the fact that our serological screening tests revealed a large number of latent infections which were not taken into account by other researchers.

The cross-sectional study, which was carried out in eight farrow-to-finish herds, confirmed a high incidence of seropositivity in gilts and sows and, like the herd screening tests, indicated that piglets had low levels of maternal antibodies which disappeared shortly after weaning [10, 37]. After this decline, seropositive results only started to occur again in small numbers at about 8 - 10 weeks but a continuous upward trend was seen after this. There was a very marked rise in weeks 13 - 16 and, since antibodies are likely to form about two to three weeks after infection, it can be concluded that infection occurs chiefly in the growing phase, at around 12 - 14 weeks [12]. Regrouping of animals, which is often only done for the first time when weaners are moved to fattening units, probably favours the spread of the organism within the herd at this point. This increase in the incidence of seropositivity occurred in all herds but one and was maintained until the end of the fattening period. In the one herd which did not follow this pattern, only 36% of animals had seroconverted by the end of the fattening period but, at the same time, there was a notable rise in the number of questionable results. These findings may be due to the fact that the animals in this herd were routinely given a 10-week course of tylosin after weaning whereas, in the other herds, antibiotics were not administered at all or, if they were, were given only for a short period. Long-term treatment prevents early infection with *L. intracellularis* and thus prevents antibody formation [7]. Findings from other studies have in some cases replicated ours but, in some, seroconversion has occurred either earlier, between weeks 7 and 12, or later, between weeks 15 and 19 [6, 10, 13, 14, 19, 25]. The incidence of seropositivity was much lower in outdoor-raised pigs than in their indoor-raised counterparts. The time of infection is likely to be closely related to the infection pressure within the herd, which is greater in closed systems than in herds where rearing and fattening are separated.

Serological profiling of herds is useful for estimating when infection actually occurs and thus for determining the optimum timing of medication or vaccination. Whereas vaccination should be carried out three to four weeks before the animals' first contact with the pathogen, medication must be administered before the appearance of clinical disease but preferably *after* the animals' first contact with the pathogen, so that animals can build up immunity in spite of treatment [36].

The bacteriological tests carried out by ourselves show that, in herds with diarrhoea problems, *L. intracellularis* occurred much more frequently than *B. hyodysenteriae* or *Salmonella* spp. (33.7% of herds compared

with 21.1% and 17.3% respectively). Infection with haemolytic *E. coli* was more common than *L. intracellularis* infection only among weaners. *B. pilosicoli* played a minor role, being found only in 2.5% of herds, but it should be borne in mind that prevalence rates for *Brachyspira* spp. can easily be underestimated because samples are not always submitted under appropriately anaerobic conditions; incorrect transportation of samples may render the organisms impossible to culture, leading to false negative results [35].

There was a slight increase in the prevalence of *L. intracellularis* associated with diarrhoeal problems in this study compared to earlier studies carried out by our group [35, 38]. Similar prevalence rates have been found in other recent studies in German herds with diarrhoeal problems; in one, *L. intracellularis* was detected in 30.0% of herds and *B. hyodysenteriae* in 27.1% [15] whilst, in another, *L. intracellularis* was detected in 28.7% of faecal samples, compared to 11.5% of samples for *B. hyodysenteriae* and 2.1% of samples for *B. pilosicoli* [18]. The incidence of mixed infections involving *L. intracellularis* and either *B. hyodysenteriae* or *Salmonella* spp. was unexpectedly low in the current study (3.5% and 5.6% of herds respectively). The high proportion of sows and gilts in which *L. intracellularis* was found is due to the fact that most of the samples came from animals with the characteristic symptoms of PHE and had been submitted for specific diagnostic testing in connection with this.

The results indicate that infection with *L. intracellularis* is very widespread in Germany and occurs both in herds with diarrhoea and unthriftiness problems and in herds without clinical signs. Accordingly, specific diagnostic measures are required to exclude other diarrhoeal pathogens. In addition to the detection of *L. intracellularis*, histopathological tests designed to assess the effects of the infection on the intestine are required. The economic impact of the infection should be evaluated by carefully monitoring performance parameters, such as average daily weight gain and feed conversion efficiency, in subclinically infected herds; only in this way can diagnostic testing enable a specific, cost-effective and financially realistic vaccination or treatment strategy to be developed and applied. Treatment is effective for animals who are already infected but prevents antibody formation in non-infected animals, who can still become infected in spite of treatment. Vaccination must be timed correctly in order to ensure a high enough level of antibody formation prior to infection. Adequate immunity is of particular importance when gilts are introduced into infected sow herds.

## Implications for practice

The current study shows that *L. intracellularis* infections are very widespread throughout the whole of Germany and that they are of greater importance in herds with diarrhoeal problems than *B. hyodysenteriae*, *B. pilosicoli* and *Salmonella* spp. However, as infections often take a subclinical course or only result in non-specific signs such as reduced growth rate, problem herds must be subjected to a) specific diagnostic procedures (including direct identification of the organism) in order to exclude other diarrhoeal pathogens and b) histopathological testing designed to identify the characteristic changes caused by the disease. Mixed infections involving *L. intracellularis* and other diarrhoeal pathogens appear to be relatively rare, however. Building up a serological profile of the herd is useful for establishing when infection actually occurs and thus for determining the optimum timing of medication or vaccination.

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**Translator's notes on paper by Wendt et al., Tierärztl Prax 2006; 34 (G): 230-9**

Page numbers refer to German version. [C] indicates change made in translation (highlighted in blue).

p.231, right-hand column, para 1, final sentence:

Faecal and intestinal samples were examined for *E. coli* in addition to *Brachyspira* spp. and *Salmonella* spp. but no reference to *E. coli* is made.

p.233, Table 3, bottom row:

"Mastschweine (< 50 kg KM)" should read "Mastschweine (> 50 kg KM)". [C]

p.233, right-hand column, para 2, lines 1/2:

"negativ ... eingestuft" should read "fraglich/negativ ... eingestuft". [C]

p.234, Table 5, title:

"Betriebe ohne Nachweis von Antikörpern ... (-)" includes herds with questionable results, not just herds with negative results. [C]

p.234, Figure 2, title:

These figures refer to pigs in the rearing and fattening period and the title should be expanded accordingly. [C]

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