

**LAWSONIA INTRACELLULARIS DETECTION IN SWINE FECES  
FROM IMPORTANT PRODUCING REGIONS IN BRAZIL**

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ABSTRACT

Porcine proliferative enteritis is an important enteric disease that has been reported in many countries around the world. In this study, we used polymerase chain reaction (PCR) to detect *Lawsonia intracellularis* in fecal samples from 1,215 pigs with clinical signs of diarrhea, ages from 25 days to 12 months. These samples were collected in 207 swine herds from important swine producing areas in Brazil between January 1997 and December 1999. A hundred and eighty-one positive fecal samples (15%) were observed and sixty-three herds showed positive animals (30%). A similar frequency of positive animals was observed in some age groups: 25 to 42 day old, 70 to 120 day old, and 120 to 180 day old. However, pigs in the 43 to 70 day old age group were significantly less positive to *L. intracellularis* detection than others, and animals older than 180 days were significantly more affected by this agent ( $P < 0.001$ ). These results assert the importance of PPE in the major swine production regions of Brazil. The PCR showed to be a fast, sensitive and useful method in epidemiological studies.

KEY WORDS: *Lawsonia intracellularis*, pig, diarrhea, ileitis, PCR, porcine proliferative enteritis.

RESUMO

DETECÇÃO DE *LAWSONIA INTRACELLULARIS* EM AMOSTRAS DE FEZES DE SUÍNOS DE IMPORTANTES REGIÕES PRODUTORAS DO BRASIL. A enterite proliferativa suína (PPE) é uma importante doença descrita em muitos países do mundo. No presente estudo foi utilizada a reação em cadeia pela polimerase (PCR) para detectar o agente *Lawsonia intracellularis* em amostras de fezes de 1.215 suínos com sinais clínicos de diarreia e idade entre 25 dias e 12 meses. Estas amostras foram colhidas em 207 granjas de suínos de importantes regiões produtoras do Brasil entre janeiro de 1997 e dezembro de 1999. Cento e oitenta e uma amostras de fezes positivas (15%) foram observadas e sessenta e três granjas apresentaram animais positivos (30%). Uma frequência similar de animais positivos foi observada em algumas faixas etárias: 25 a 42 dias, 70 a 120 dias e 120 a 180 dias, no entanto, suínos com idades variando entre 43 e 70 dias foram significativamente menos positivos para *L. intracellularis* que entre outras idades. Animais com mais de 180 dias foram significativamente mais afetados por este agente ( $P < 0,001$ ). Estes resultados reafirmam a importância da PPE nas regiões produtoras de suínos do Brasil. A PCR demonstrou ser um método rápido, sensível e útil para estudos epidemiológicos e diagnóstico da PPE.

PALAVRAS-CHAVE: *Lawsonia intracellularis*, suínos, diarreia, ileite, PCR, enterite proliferativa suína.

INTRODUCTION

Porcine proliferative enteritis (PPE) is a major worldwide swine disease (KNITTEL et al., 1997). It is estimated that this disease costs the industry \$20/sow/year in Australia and a total of \$20 million in the United States. In endemic areas, roughly 15 to 30% of the herds are affected with a 5 to 20% infection rate in the herds (COOPER et al., 1998).

The subacute and chronic forms of PPE are associated with reduced growth rate and diarrhoea, most frequently seen in 6 to 20 week old pigs. The acute form is characterised by severe diarrhoea and death in 12 to 30 weeks old or older pigs (ROWLAND & LAWSON, 1992). The causative agent has been identified and assigned a new taxonomic genus, *Lawsonia intracellularis* gen. nov, sp nov (MCORIST et al., 1995).

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Direct culture of the agent from faecal samples is not a practical diagnostic option because of the fastidious growth requirements, as well as the presence of high numbers of contaminating organisms commonly found in the intestine of pigs. A test using polymerase chain reaction (PCR) for amplification of the 16S ribosomal DNA of *L. intracellularis* specific and sensitive for detection of the bacteria (JONES *et al.*, 1993). Using PCR amplification of a 319 base pair fragment of *L. intracellularis*, as few as  $10^3$  bacteria/g faeces were successfully detected in experimentally inoculated animals (JONES *et al.*, 1993).

The purpose of this study was to detect *L. intracellularis* in faeces of pigs with diarrhoea in the major producing regions of Brazil by using PCR.

## MATERIALS AND METHODS

### Fecal samples

A total of 1,215 fecal samples from animals with diarrhoea was collected in sterile plastic vials and sent to the Swine Pathology Laboratory at the School of Veterinary Medicine of the University of São Paulo, between January 1997 and December 1999 (total of 36 months), where they were submitted to PCR for detection of *Lawsonia intracellularis*. The age ranged from 25-day-old piglets to one-year-old breeders.

### Studied areas

Fecal samples were obtained from 207 swine herds in the major swine production regions of Brazil, including the states of São Paulo, Santa Catarina, Paraná, Rio Grande do Sul, Minas Gerais, Mato Grosso do Sul, Goiás, Rio de Janeiro, Pernambuco, Ceará and Distrito Federal. A farm was considered positive when at least one sampled pig was PCR positive.

### DNA extraction from feces

Total DNA was extracted by using a modification of the procedure based on the binding of DNA to silicates in the presence of high concentrations of guanidine thiocyanate [GuSCN]- (BOOM *et al.*, 1990).

### DNA amplification

The PCR amplification mixture (25  $\mu$ L) consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.001% (w/v) gelatine, 200  $\mu$ M each of the four deoxynucleoside triphosphates, 100 ng (each) primer, and 0.5 U of Taq DNA polymerase (Life Technologies- Grand Island, NY). The primers used in the process were commercially synthesised (Life Technologies) as follows: primer A - 5'TATGGCTGTCAAACACTCCG 3' and primer B - 5'TGAAGGTATTGGTATTCTCC3' (JONES *et al.*,

1993). Amplification was conducted on a DNA thermocycler (Model Touch down, Hybaid, UK) as described by JONES *et al.* (1993). In all experiments, PCR amplification was carried out in negative control samples, without DNA. A sample of purified *Lawsonia intracellularis* DNA was provided by Dr. Steven McOrist (Veterinary Pathology Services, Glenside, Australia) and used as positive control.

### Detection of PCR products

The 319-bp amplified products were separated by electrophoresis in 1.5% agarose gel and stained with ethidium bromide. An HaeIII digest of  $\phi$ X174 replicative form DNA (Life Technologies) was used as a molecular size marker.

### Statistical analysis

The animals were classified into five age groups, and the relationship between age and *L. intracellularis* detection was analyzed by stratified Chi-square through SPSS 9.0.1 for Windows (SPSS Inc.).

## RESULTS

Amplification of DNA extracted from the pure culture of *Lawsonia intracellularis* and from the 181 fecal samples produced a 319-bp band. Positive animals represent 15.0% of the total examined (181/1,215). Samples from the 207 swine herds showed that 63 of them (30%) had animals infected by *L. intracellularis*.

The pigs under examination were separated in five groups according to their ages: (A) 25-42 days old, (B) 43-70 days old, (C) 71-120 days old, (D) 121-180 days old, and (E) > 180 days old. The distribution of positive cases according to age showed a higher frequency in the group over 180 days old and a lower frequency in the 43 to 70 days old group (Table 1)

Table 1 - Frequency of *Lawsonia intracellularis* detection in faecal samples from pigs from Brazil according to age between January 1997 and December 1999.

Ages	Number of positive cases	Total samples	% positive
(A) 25 - 42 days	16	84	19
(B) 43 - 70 days	18	260	6.9 <sup>a</sup>
(C) 71 - 120 days	95	616	15.4
(D) 121 - 180 days	31	196	15.8
(E) > 180 days	20	44	45.4 <sup>a</sup>

a-values with superscript are significantly different  $P < 0.001$ , IC= 95%.

Table 2 - Frequency of *Lawsonia intracellularis* positive swine herds according to Brazilian states examined between January 1997 and December 1999.

States	Number of positive herds	Total herds	% positive
São Paulo	22	59	37.0
Santa Catarina	28	81	34.5
Paraná	5	30	16.6
Minas Gerais	3	19	16.0
Goiás	2	5	40.0
Mato Grosso do Sul	2	5	40.0
Rio Grande do Sul	1	4	25.0
Distrito Federal	0	1	0
Rio de Janeiro	0	1	0
Pernambuco	0	1	0
Ceará	0	1	0

The frequency of *L. intracellularis* positive herds in the various states was 37% in São Paulo; 35% in Santa Catarina; 20% in Paraná; 16% in Minas Gerais; 40% in Goiás; 40% in Mato Grosso do Sul and 25% in Rio Grande do Sul. In other Brazilian regions, such as Distrito Federal and the states of Pernambuco, Ceará and Rio de Janeiro, no positive results were found in the herds included in the study (Table 2).

### Discussion

Detection of *L. intracellularis* in animals with PPE is extremely important for the diagnosis of the disease. Traditionally, the disease has been poorly diagnosed. The application of PCR techniques for the detection of *L. intracellularis* offers the opportunity for a rapid detection in a large number of specimens.

The results of this study indicate that the PPE agent was present in about 15% of the pigs involved in the survey (181/1,215). TAKAHASHI et al. (1998) describe a similar frequency of affected pigs in Japan, 14.9% (33/221), but SOCCI et al. (1998) found a higher incidence of the disease in Mexico, 23% (113/484). On the other hand, lower frequencies are described by JORDAN et al. (1996) who report 5% of positive cases (26/621) in studies done in the United States; CHANG et al. (1997) found 5.5% in Taiwan (31/560); KIM et al. (1998) showed 3.3% positive animals in Korea (16/490); and CHIRIBOGA et al. (1999) described 7.2% positive cases in Brazil (46/636).

The number of positive herds, 30% (63/207), was of considerable significance. This number was compatible with those observed by SOCCI et al. (1998) in Mexico (35% - 52/148) and by CHANG et al. (1997) in Taiwan (30% - 12/40). TAKAHASHI et al. (1998) showed a higher incidence (55.2% - 16/29) in Japan. KIM et al.

(1998) reported 20% of positive farms in Korea (7/35) and CHIRIBOGA et al. (1999) described 25% in Brazil (15/60).

The differences among the number of positive animals and positive farms observed by these authors may be due to regional variations, different swine breeding systems, use of antibiotics or size of the samples and sampled population (sick or healthy animals). In addition, variations related to PCR technique could be observed. The study conducted by CHIRIBOGA et al. (1999) in Brazil had some variations in the PCR technique, as the use of pooled samples and DNA extraction protocol from feces based in enzymatic digestion and phenol-chloroform purification. TAKAHASHI et al. (1998) used nested PCR for detection of the agent in the study in Japan. These variations may interfere negatively with the sensitivity of the test or, in some cases, they may improve the DNA detection. The present study was conducted with the PCR protocol described by JONES et al. (1993) and validated by MCORIST et al. (1994) and MCCORMICK et al. (1995), with expected detection limit of  $10^3$  bacteria/g feces.

In 5 farms, MØLLER et al. (1998) reported 22.9% positive results in weaned pigs and 12.9% in growing and finishing pigs. The distribution of positive cases according to the age groups showed a similar frequency of positive animals among pigs 25 to 42 days old (19%), 70 to 120 days old (15.4%), and 121 to 180 days old (15.8%). A significantly higher percentage of positive animals was found in the group older than 180 days (45.4%) and a lower incidence in the 43 to 70 days old group (6.9%) ( $P < 0.001$ ).

The lower occurrence of *L. intracellularis* detection between 43 and 70 days of age can be explained by the wide use of antibiotics and growth promoters in this stage of pig production (LANZA et al., 1996). On the other hand, the high number of positive samples in the group older than 180 days can be justified by the low use of antibiotics and growth promoters, as well as by the large number of risk factors associated with this age group such as transportation, commingling of pigs and repopulation (BANE et al., 1997).

Porcine proliferative enteritis was detected in several Brazilian states with no difference in the manifestations of the disease in the various regions and with a similar incidence. PPE can affect pigs at any stage of production; however, the major impact occurs during the nursery and growing-finishing stages, suggested by the large number of samples received from animals with ages from 25 to 180 days.

The use of PCR as an antemortem test on fecal samples to monitor and diagnose PPE has been a tremendous asset in the prevention and control of the disease. The results presented in this study stress the importance of *L. intracellularis* to Brazilian swine producers in the various states.

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