Brazilian Journal of Microbiology 44, 1, 145-151 (2013)
ISSN 1678-4405
Copyright © 2013, Sociedade Brasileira de Microbiologia
www.sbmicrobiologia.org.br

Research Paper

The prevalence of swine enteropathogens in Brazilian grower and finish herds

A.M. Viott[1], A.P. Lage[2], E.C.C. Cruz Junior[3], R.M.C. Guedes[3]

[1]Laboratório de Patologia Veterinária, Universidade Federal do Paraná, Palotina, PA, Brazil.
[2]Departamento de Medicina Veterinária Preventiva, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.
[3]Departamento de Clínica e Cirurgia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

Submitted: September 10, 2011; Approved: July 02, 2012.

Abstract

Diarrhoea among growing and finishing pigs is an important problem in many herds. The prevalence of *L. intracellularis*, *B. pilosicoli*, *B. hyodysenteriae*, *Salmonella* spp., enterotoxigenic *E. coli*, *Trichuris suis* and the occurrence of mixed infection were investigated. Fecal samples for forty-six herds with diarrhea or a history of diarrhea were randomly collected in Minas Gerais state, Brazil. The enteric pathogens were detected by culture (*E. coli* and *Salmonella* sp.), PCR (*L. intracellularis* and *Brachyspira* spp.) and eggs counts (*T. suis*). The overall herd prevalence of *L. intracellularis*, *Salmonella enterica* serotype Typhimurium and enterotoxigenic *E. coli* were 19.56%, 6.52%, 10.86% respectively. Mixed infection was diagnosed in 30.43% of herds, and *L. intracellularis* and *Salmonella enterica* serotype Typhimurium are main pathogens association (10.87%). *B. pilosicoli* was diagnosed only in two herds, always associated with mixed infections. *B. hyodysenteriae* and *T. suis* were not demonstrated in any sample. These pathogens have been reported world-wide but studies regarding epidemiology in Brazil are few. This study contributes to establish of prevention programs for the control enteropathogens in grower finish herds in Brazil.

Key words: swine, enteropathogens, grower finish herds, prevalence, Brazil.

Introduction

According to research, Brazil is the fourth largest producer of pigs, behind China, the European Union and United States. The state of Minas Gerais is the fourth largest producer of pork in Brazil, and the growth of pig production in the state has been increasing year by year (Abipesc 2009). Because of this it is necessary to accurately detect and identify porcine pathogens in order to devise proper treatments and prevention programs (Baccaro *et al.*, 2003).

The enteric infections are among the most frequent diseases in pig production, being responsible for significant losses and significant economic impact on industry. The damages are represented primarily by reducing weight gain, mortality and expenses with antibiotics (McOrist 2005). Further, some of these diseases can be transferred to humans (Weneger and Bager, 1997). Several agents have been suggested as possible causes of diarrhea in growing/finishing pigs. Infection by *L. intracellularis* causing porcine proliferative enteritis, swine salmonellosis caused by *Salmonella enterica* serotype Typhimurium, porcine intestinal espirochetosis caused by *B. pilosicoli,* swine dysentery caused by *B. hyodysenteriae,* and *Trichuris suis* causing intestinal trichuriasis are among the most prevalent at this age (Batte *et al.*, 1977; Thomson *et al.*, 1998; Stege *et al.*, 2000; Baccaro *et al.*, 2003). Enterotoxigenic strains of *Escherichia coli* may also be present among growing pigs weighing 30-50 kg with or without symptoms (Stege *et al.*, 2000).

The risk of diarrhea increases when the herd is infected by two or more pathogens simultaneously (Stege *et al.*, 2000; Suh and Song, 2005). Usually the pigs with mixed infection have lesions more pronounced with lower development of the herd. Suh and Song (2005) analyzing 462 swine fecal samples observed the occurrence of mixed infections, represented by 3% of the samples simulta-

Send correspondence to: R.M.C. Guedes. Departamento de Clínica e Cirurgia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
E-mail: guedes@vet.ufmg.br.

neously infected with *Salmonella* sp. and *B. hyodysenteriae*, 2.2% with *L. intracellularis* and *Salmonella enterica* spp. and 1.3% with *L. intracellularis* and *B. hyodysenteriae*.

These pathogens have been reported in world-wide but (Thomson *et al.*, 1998; Stege *et al.*, 2000 Jacobson *et al.*, 2005; Suh and Song, 2005; La *et al.*, 2006) the importance and involvement of some of these organisms in clinical disease still remains to be clarified in the Brazilian swine herds. The purpose of this study was to determine the prevalence of the enteric pathogens *L. intracellularis*, *B. pilosicoli*, *B. hyodysenteriae*, *Salmonella enterica*, enterotoxigenic *E. coli, T. suis* and the occurrence of mixed infections in Brazilian growing/finishing pigs from swine farms with diarrhea or a history of diarrhea in the state of Minas Gerais, Brazil.

Materials and Methods

Herds and animals

Farrow-to-finish herds with diarrhea or a history of diarrhea were randomly selected from four different geographical regions of the state of Minas Gerais, Brazil [South and southwest (SSW), metropolitan area of Belo Horizonte (MBH), Zona da Mata (ZM) and Triângulo Mineiro e Alto Paranaiba (TMHP)]. The sample size was calculated at two levels, property and animals. The feces were collected between January 2008 and February 2009. To determine the number of herds, simple sampling was used with an estimated prevalence of 50% confidence level of 95% and an estimated error of 15% (Noordhuizen *et al.*, 2001).

In each farm, between 10 and 15 fecal samples were randomly collected from 60 to 14 day-old pigs. Feces were collected directly from the rectum and shipped under refrigeration to the Laboratory of Veterinary Pathology in the Federal University of Minas Gerais.

Microbiology investigation

For the isolation of Salmonella sp. 1 g of feces was mixed in 4 mL sterile phosphate buffer saline (PBS). For pre enrichment 1 mL of this solution was added to 9 mL of peptone water (DIFCO) (37 °C/18 h). After incubation aliquots of 0.1 mL were seeded in selective broth Rappaport-Vassiliadis (DIFCO) (42 °C/24 h). Subsequently aliquots of the selective broth were plated on XLT-4 agar (DIFCO) and brilliant green agar (DIFCO). The suspected colonies were identified (Bergey and Holt, 1994). These colonies were grown in Brain Heart Infusion agar (BHI agar) (DIFCO) and submitted to the following biochemical tests: urea broth (DIFCO), lysine broth (DIFCO) and triple sugar iron agar (TSI) (DIFCO). Serology using anti "O" polyvalent antiserum (PROBAC®) was used to confirm the isolation of *Salmonella enterica* sp. Colonies confirmed were selected for serotyping at the Institute Oswaldo Cruz, Rio de Janeiro, Brazil.

Aliquots of the feces mixed in PBS were plated in MacConkey agar (37 °C/24 h) for the isolation of lactose positive colonies (Lac+ colonies). These colonies were frozen in glycerol at -80 °C for later PCR multiplex for enterotoxigenic *E. coli.*

Detection of *L. intracellularis*, *B. hyodysenteriae* and *B. pilosicoli*

The methods use for the diagnostic of *L. intracellularis, B. hyodysenteriae* and *B. pilosicoli* and the sensitivity of detection are a multiplex –PCR for rapid detection (La *et al.*, 2006). The positive controls were kindly provided by department of Veterinary and Biomedical Sciences at University of Minnesota, EUA.

Extraction of DNA and multiplex PCR primers

DNA was extracted from all faeces using the QIAamp DNA Stool Mini Kit (QIAGEN) according to the manufacturer's instructions. The respective primer sequences were: H1 (5'-ACTAAAGATCCTGATGTATTTG-3') and H2 (5'-CTAATAAACGTCTGCTGC-3') targeting a 354 bp region on the nicotinamide adenine dionucleotide hybride (NADH) oxidase (*nox*) gene of *B. hyodysenteriae*; P1 (5'-AGAGGAAAGTTTTTTCGCTTC-3') and P2 (5'-GCACCTATGTTAAACGTCCTTG-3') targeting a 823 bp region of the 16S rRNA gene of *B. pilosicoli*; and Lint-146F (5'-GATAATCTACCTTCGAGACGG-3') and Lint -745R (5'-TGACCTCAGTGTCAGTTATCGT-3') targeting a 655 bp region of 16S rRNA gene of *L. intracellularis.*

Multiplex PCR

The DNA was amplified in 25 μL total volume. Amplifications mixtures consisted of 1 X PCR buffer (containing 1, 5 mmol L^{-1} of Mg Cl_2), 1.25 U of Taq DNA polymerase (Cenbiot, Porto Alegre, Brazil), 0.1 mmol L^{-1} of each dNTP (Invitrogen, Carlsbad, CA), 0.2 mmol L^{-1} of each primer pair (H1 and H2, P1 and P2, Lint-146F and Lint-745R) (Prodimol, Wisconsin, USA) and 2.5 μL chromosomal template DNA. Cycling conditions involved an initial 5 min Taq DNA polymerase activation step at 95 °C, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 90 s, and a primer extension step at 68 °C for 2 min. A final 10 min extension step at 68 °C. The PCR products were subjected to electrophoresis in 1% (w/v) agarose gels in 1 x TAE buffer, stained with ethidium bromide and viewed over UV light. The sensitivity of detection of the multiplex PCR was estimated (La *et al.*, 2003).

Multiplex PCR for detection of *Escherichia coli* enterotoxigenic virulent genes

For the diagnosis of enterotoxigenic *E. coli* genes a multiplex-PCR was used (Macedo *et al.*, 2007). As positive

controls for the factors of virulence, four reference samples of *E. coli* were kindly provided by the Veterinary Diagnostic Laboratory at the University of Minnesota.

Extraction of DNA

After thawing the Lac + colonies were plated on MacConkey agar. Isolated colonies were resuspended in microtube containing 500 μL sterile PBS. The bacteria were centrifuged at 7000 rpm for 10 min for sedimentation. Sediment was added 270 μL lysis solution (Tris HCl 1.0 M, pH 8.0; EDTA 0,5 M; NaCl 4.5 M; SDS 10%) and 200 μL of High TE (Tris HCl 1.0 M, pH 8.0; EDTA 0.5 M). After mixing and vortex for 20 to 30 seconds, the mixture was incubated at 55 °C for at last 3 h. The lysate containing the bacterial DNA was subjected to DNA extracting by phenol-chloroform (Sambrook *et al.*, 1989).

Multiplex PCR

The PCR technique used four pairs of primers for the toxins Stb, StaP, LT and Stx2e and five pairs for the fimbriae K99, F18, 987P, K88 and F41. Briefly the DNA was amplified in 10 μL total volume containing 10 ng of bacterial DNA. Amplifications mixtures consisted of 0.2 mmol L^{-1} of each dNTP (Prodimol, Wisconsin, USA), 1.5 mmol L^{-1} of $MgCl_2$ (Cenbiot, Porto Alegre, Brazil), 0.5 mmol L^{-1} of each primer (Invitrogen, Carlsbad, CA) and 5 U of TAQ polymerase (Cenbiot, Porto Alegre, Brazil). Cycling conditions involved an initial 1 min Taq DNA polymerase activation step at 94 °C, followed by 25 cycles of denaturation at 94 °C for 1 min, at 55 °C for 1 min, and a primer extension step at 70 °C for 2 min. A final 10 min extension step at 72 °C. The PCR products were subjected to electrophoresis in 6% (w/v) polyacrylamide gels in 1 x TBE buffer, stained with silver.

Parasitological exam

For detection of *T. suis* eggs in swine feces, fecal samples were individually examined by flotation technique (Willis-Mollay) with a hyper saturate solution of NaCl (Hoffmann 1987). The infection intensity was scored according the number of eggs per gram of feces: 0, none; 1-5 mild; 5-10, moderate; and more than 10 severe.

Statistics

For statistical analyses Fischer's exact test (SAS Institute, Cary, North Caroline, USA 1999) was used to determine the association between herd size and pathogen prevalence.

Results

The study comprised 46 farrow-to-finish pig herds. Forty six farms were analyzed, 31 with 101-500 sows and 15 with > 500 sows. The number of herds collected from each region and is in Table 1. A total of 512 faecal samples were examined. The analysis by the Fisher exact test showed no significant association between the pathogen and the size of the farm.

Salmonella spp.

The only subspecies of *Salmonella* spp. found, potentially pathogenic for pigs, was *Salmonella enterica* subspecies Typhimurium. The overall herd prevalence of *Salmonella enterica* subspecies Typhimurium was 6.52%. *Salmonella enterica* subspecies Typhimurium was diagnosed in 3 herds and was detected in 31 (6.05%) samples. One herd with 101-500 sows and two herds with more than 500 sows were positive for *Salmonella enterica* subspecies Typhimurium (Table 2).

Others subspecies of *Salmonella enterica*, not pathogenic for pigs were found in seven herds (15.2%). *Salmonella enterica* subspecies Agona was observed in one herd (2.17%) and two samples (0.4%) in a MBH herd with 101-500 sows. The *Salmonella enterica* subspecies Panama was diagnosed in three herds (6.52%) and 13 samples (2.54%). All herds were localized in ZM, two with 101-500 sows and one with > 500 sows. The *Salmonella enterica* subspecies Schwarzenground was observed in one herd (2.17%) and two samples (0.4%) in a MBH herd with > 500 sows. *Salmonella enterica* untypable was observed in two herds (4.34%) and two samples (0.4%). The herds were located in MBH one with 101-500 sows and one with > 500 sows.

L. intracellularis, B. pilosicoli and *B. hyodysenteriae*

The limit of detection obtained in PCR was 103 cells per gram of faeces for *B. pilosicoli* and 10^4 for *L. intracellularis* and *B. hyodysenteriae*. The 10^4 cells per gram of feces were obtained when the three species were applied together in equal number in the fecal samples.

B. hyodysenteriae was not isolated from any of the samples from the 46 piglet-producing herds. The overall herd prevalence of *B. pilosicoli* was 4.36%. This pathogen was demonstrated in two herds and in nine (1.75%) samples, always in mixed infections (Table 2). *L. intracellularis* was diagnosed in 9 herds (19.56%) and was detected in 59 samples (11.52%). This bacteria was more prevalent in ZM (8.69%) and TMHP (6.52%) regions (Table 2).

Table 1 - Number of properties samples and herd size in each region of Minas Gerais.

Regions	ZM[a]	TMHP[b]	MBH[c]	SSW[d]	Total
Herd size					
101-500	11	5	8	7	31
> 500	7	5	2	1	15
Total	18	10	10	8	46

[a]Zona da Mata" (ZM), [b]Triângulo Mineiro e Alto Paranaíba (TMHP), [c]Metropolitan area of Belo Horizonte (MBH), [d]South and southwest (SSW).

Table 2 - Distribution of prevalence according to herd size, region and pathogen; *Lawsonia intracellularis*, enterotoxigenic *E. coli*, *Salmonella* Typhimurium and mixed infection.

Pathogen / Region	Herds 101-500 nº	101-500 %	> 500 nº	> 500 %	Total	Prevalence (%)	IC (95%)[e] IL (%)	IC (95%)[e] SL (%)
L. intracellularis	5	10.87	4	8.69	9	19.56	8.10	31.02
TMHP[a]	1	2.18	2	4.34	3	6.52		
MBH[b]	1	2.18	0	0	1	2.18		
ZM[c]	2	4.34	2	4.34	4	8.69		
SSW[d]	1	2.18	0	0	1	2.18		
E. coli enterotoxigenic	2	4.36	3	6.52	5	10.87	1.87	19.87
TMHP[a]	0	0	1	2.18	1	2.18		
MBH[b]	0	0	0	0	0	0		
ZM[c]	2	4.34	1	2.18	3	6.52		
SSW[d]	0	0	1	2.18	1	2.18		
S. Typhimurium	1	2.18	2	4.34	3	6.52	0	13.65
MBH[b]	1	2.18	1	2.18	2	4.34		
ZM[c]	0	0	1	2.18	1	2.18		
*S.*Typhimurium/*L.intracellularis*	4	8.69	1	2.18	5	10.87	1.87	19.87
TMHP[a]	2	4.34	0	0	2	4.34		
MBH[b]	1	2.18	1	2.18	2	4.34		
ZM[c]	1	2.18	0	0	1	2.18		
L.intracellularis/E. coli	1	2.18	2	4.34	3	6.52	0	13.65
TMHP[a]	0	0	1	2.18	1	2.18		
ZM[c]	0	0	1	2.18	1	2.18		
SSW[d]	1	2.18	0	0	1	2.18		
*L.intracellularis/E.coli/ S.*Typhimurium	4	8.69	0	0	4	8.69	0.55	16.83
TMHP[a]	1	2.18	0	0	1	2.18		
MBH[b]	1	2.18	0	0	1	2.18		
ZM[c]	2	4.34	0	0	2	4.34		
B.pilosicoli/L.intracellularis	1	2.18	0	0	1	2.18	0	6.4
MBH[b]	1	2.18	0	0	1	2.18		
B.pilosicoli/L.intracellularis/S Typhimurium	0	0	1	2.18	1	2.18	0	6.4
MBH[b]	0	0	1	2.18	1	2.18		

Fischer's exact test (p = 0.0075, Prob = 0.9020). [a]Triângulo Mineiro and Alto Paranaiba (TMHP), [b]metropolitan area of Belo Horizonte (MBH), [c]Zona da Mata (ZM),[d]South and southwest (SSW). [e]IL (%) lower 95% confidence interval, SL (%) upper 95% confidence interval.

Enterotoxigenic *E. coli*

The overall prevalence of enterotoxigenic *E. coli* was 10.87%. The number of positive herds and samples was 5 and 26 (5.49%) respectively. The 512 stool collected only 473 samples showed growth of positive lactose colonies in MacConkey's agar. The enterotoxigenic *E. coli* bacteria was more prevalent in ZM (6.52%) (Table 2).

The enterotoxigenic genotype more observed was the profile StaP-Stb-F18 with 8/26 (30.76%) positive samples, followed by genotype StaP-Stb-987P with 6/26 (23.07%) positive samples. Other genetic profiles found were: LT-K88, StaP-Stb-K99, Stb-987p-K99, StaP-987p all with two samples each and K99-LT, STx2E-987p, LT-K88-STx2E and 987p-LT all with one positive sample detected.

Trichuris suis

T. suis eggs were not diagnosed in any of the samples from the 46 piglet-producing herds.

Mixed Infection

The overall herd prevalence of mixed infection was high 30.43%. Mixed infections were diagnosed in 14 herds

and 67 samples (13.08%). Nine herds were positive for two agents (19.56%), and five herds were positive for three agents (10.87%). There was a higher frequency of mix infection in herds with 101-500 sows, 10 in a total, compared with herds with more than 500 sows four (Table 2). The MBH region had the highest occurrence of mix infection, five herds. The most common mixed infection in herds was *Salmonella enterica* subspecies Typhimurium associated with *L. intracellularis* present in 5 herds (10.87%) and 28 fecal samples (5.47%), following by *L. intracellularis* associated with enterotoxigenic *E. coli* and *Salmonella enterica* subspecies Typhimurium present in 4 properties (8.69%) and 20 samples (3.9%). The concomitant detection of enterotoxigenic *E. coli* and *L. intracellularis* was diagnosed in 3 herds (6.52%) and 12 samples (2.34%). *B. pilosicoli* was detected concomitantly with *L. intracellularis* in 1 herd (2.18%) corresponding a four samples (0.78%), and with both *L. intracellularis* and *Salmonella enterica* subspecies Typhimurium, one herd (2.18%) and three samples (0.58%) (Table 2).

Discussion

Generally, studies on enteric disease have focused on a specific pathogen (Fellstrom *et al.*, 1996), decreasing the chances of a correct diagnosis of the sanitary problems that affect the herd. The prevalence of six enteropathogens with pathogenic potential to grower/finisher pigs was studied and this prevalence was associated with de herd size. It was been reported that outbreaks of *L. intracellularis* and *Brachyspira* spp. occur more often in the large production units (Holyoake *et al.*, 1996), indicating an association between herd size and disease outbreak. However, the results in this study did not show this.

The prevalence of hers infected with *L. intracellularis* was 19.56%. This result is in agree with previously reported in other countries, where the prevalence of *L. intracellularis* varies between 15% and 93.7% (Thompson *et al.*, 1998; Stege *et al.*, 2000; Jensen *et al.*, 2006; Biksi *et al.*, 2007) and in Brazil. In Brazil works reported a prevalence of 15% of positive faecal samples (146/971), and 30% of 203 herds from seven different regions (Moreno *et al.*, 2002; Baccaro *et al.*, 2003). The differences among the prevalence numbers observed in this and in others works may be due to regional variations, different swine breeding systems, use of antibiotics and sampled population (sick or healthy animals) and detection assay used.

Using detection techniques, the prevalence of *B. pilosicoli* and *B. hyodysenteriae* in different geographic regions has been estimated. In a study in Danish *B. pilosicoli* was isolated from 19% from 79 herds and *B. hyodysenteriae* in 2.5% (Stege *et al.*, 2000). In a survey conducted in U. K. between 1992 and 1996 that involved 85 pig farms *B. pilosicoli* was identified on 21 (25%) units, and *B. hyodysenteriae* on 6 (7%) units (Thomson *et al.*, 1998). A recent study from Sweden revealed that the presence of *B. pilosicoli* in 34 herds of 105 studied and the absence of *B. hyodysenteriae*, the presence o *B. pilosicoli* was significantly associated with herds having poor performance in growing pigs and with mix infection (Thomson *et al.*, 1998; Jacobson *et al.*, 2005). In our survey the presence of *B. pilosicoli* was associated with mix infection and only in two herds. None positive samples for *B. hyodysenteriae* were detected. This lower prevalence may be explained by the use of additives in the feed, in many countries, including Brazil, the prevalence of *Brachyspira* sp. might be concealed by the use of antimicrobial feed additives (Hampson 2000). In a study conducted in Brazil 38 farms were randomly selected and bacteriologically examined (22 from farms using medicated feed and 16 with non-medicated feed), *B. hyodysenteriae* and *B. pilosicoli* were isolated respectively from 0% and 6.25% in medicated herds and from 31.8% and 45.5% from non-medicated farms (Barcellos *et al.*, 2003)

In addition, variations related to PCR techniques could be observed. There is showed that variations like the use of pooled samples and the DNA extraction protocol may interfere negatively with the sensitivity of the test (Chiriboga *et al.*, 1999) resulting in false negative results. The practical limit of detection of the multiplex PCR when the three species were applied together in the faecal samples was 10^4 cells per gram of feces. These levels of detection are consistent with than those previously reported (Jones *et al.*, 1993; MΦller *et al.*, 1998; La *et al.*, 2003). PCR inhibition is also a problem in the diagnosis of faecal samples prepared directly for PCR. Hence, inhibition might be a problem in the detection of clinical and subclinical carriers (Jacobson *et al.*, 2004). It is known that bacterial isolation for *Brachyspira* sp. is more sensitive than the PCR technique (Komarek *et al.*, 2009), and this fact may have contributed to the low numbers observed.

The overall prevalence for *Salmonella enterica* subspecies Typhimurium (6.52%) and others subspecies of *Salmonella enterica* (15.2%) was high when compared with results of *Salmonella enterica* in others countries like Danish (10.1%) and U.K (13%) (Thomson *et al.*, 1998; Stege *et al.*, 2000). This variation in prevalence can be caused by differences in pig housing (asymptomatic carriers) or feeding regimen (meat meal) which might affect the degree of transmission (Hampson 2000). *Salmonella* spp. were diagnosed as the primary agent in both ZM and MBH regions, it is believed that the highest density of pigs in such areas associated with older facilities have contributed to this finding. *Salmonella enterica* subspecies Typhimurium can cause diarrhea in pigs, but even more important is that *Salmonella* spp. is a zoonotic agent (Weneger and Bager, 1997; Stege *et al.*, 2000). *Salmonella* spp. is a key player in the contamination of pig meat and its derivatives (Weiss *et al.*, 1999).

Pathogenic *E. coli* was previously identified in 49 of 54 pigs from poor performance herds (Jacobson *et al.*,

2003). A survey observed 76.8% of 72 herds infected by hemolytic *E. coli* and other work demonstrated 24.1% of the herds with pathogenic *E. coli* (MΦller *et al.*, 1998; Stege *et al.*, 2000). Despite all these results the significance of the presence of *E. coli* in grower/finish herds has not been well clarified. Enterotoxigenic *E. coli* has been associated with damage to small intestinal villi, loss of villi epithelium and diarrhea in weaned pigs (Macedo *et al.*, 2007). However, this enteric pathogen may alter the ecological balance of the gut flora or change the environment conditions in the gut, which would favors some bacteria (Jacobson *et al.*, 2003). Normally the affected herds had case history of previous problems with post-weaning diarrhea, this may indicate that these herds suffer from a high pathogen load, or that the post-weaning diarrhea might predispose to outbreaks of others enteric diseases (MΦller *et al.*, 1998; Thompson *et al.*, 1998; Stege *et al.*, 2000; Jacobson *et al.*, 2005). An uncharacteristic presentation of F18-positive *E coli* enteritis in 11-weekold pigs, and according to this author, strains of *E coli* positive for the F18 pilus, enterotoxins, and Stx2e endotoxin should be added to the list of differential diseases that cause severe diarrhea and vomiting in grower-finisher pigs (Pittman 2010). Of the 512 samples tested 39 no showed growth for Lac + colonies, it is believed that the overuse of antibiotics in the growing and finishing phase may have inhibited the growth of these bacteria on MacConkey agar.

Interactions between pathogens may occur and it is often observed in herds that have or had history of severe diarrhea with dead loss of pigs, usually in this cases there is an increase of lesions in the intestine (Jacobson *et al.*, 2005; Suh and Song, 2005). Mixed infection was present in 30.43% of herds, and the main combination observed was *Salmonella* Typhimurium associated with *L. intracellularis*. This association was already reported (Suh and Song, 2005) 2.2% of the 462 samples positive for *Salmonella* spp and *L. intracellularis*. Other associations have already been reported in Sweden 58% of 320 fecal samples were concomitant infected by *B. pilosicoli* and *L. intracellularis* (Jacobson *et al.*, 2005).

T. suis once relatively common in swine industry (Batte *et al.*, 1977), was not detected in any of the samples. The control in recent years, associated with technological improvements in the swine industry, have resulted in the reduced incidence of helminth parasites.

In conclusion, *L. intracellularis* is the main swine enteropathogen observed in herds in the state of Minas Gerais following by enterotoxigenic *E. coli* and *Salmonella enterica* subspecies Typhimurium. There was a high occurrence of mixed infections in herds with a close relationship of *L. intracellularis* combined with other agents. The occurrence of a large number of herds positive for *E. coli*, suggest that this bacteria may be the cause of diarrhea in growing and finishing pigs.

Acknowledgments

The authors thank the swine producers and veterinarians who participated in this project Julio Maria R. Pupa, Alvimar Lana S. Jalles, Bruno Zinato Carraro, Antonio Rodrigues de Oliveira, Flavia Leite, Israel de Souza. For statistical analysis our special thanks to Msc. Cristiano Nunes Nesi. The authors gratefully acknowledge the financial support of Fundação de Pesquisa de Minas Gerais (FAPEMIG) and from INCT-Pecuária. A.P. Lage and R.M.C. Guedes have a research fellowship from CNPq.

References

ABIPECS Relatório 2008. Available in:http://www.abipecs.org.br/relatorios/rela2008_P.pdf. Setember, 2009.

Baccaro MR, Moreno AM, Shinka LT, Dotto DS (2003) Identification of bacterial agents of enteric disease by multiplex PCR in growing-finishing pigs. Braz J Microbiol 34:225-229.

Barcellos DE, Razia LE, Borowski SM (2003) Ocorrência e identificação de espiroquetas intestinais em suínos em granjas de porte industrial de duas regiões criatórias do estado do Rio Grande do Sul, em relação a medicação da ração. Ciência Rural 33:725-729.

Batte EG, McLamb RD, Muse KE, Tally SD, Vestal TJ (1977) Pathophysiology of Swine Trichuriasis. Am J Vet Res 38:1075-1079.

Bergey DH, Holt JG (1994) Facultative anaerobic Gram-negative rods. In: Bergey's Manual of Determinative Bacteriology. 9th edition. Williams & Wilkins, Baltimore, pp 175-189.

Biksi I, Lorincz M, Molnár B, Kecskés T, Takács N, Mirt D, Cizek A, Pejsak Z, Martineau GP, Sevin JL, Szenci O (2007) Prevalence of selected enteropathogenic bacteria in Hungarian finishing pigs. Acta Vet Hungary 55:219-127.

Chiriboga AE, Guiraes WV, Vanetti MC, Araujo EF (1999) Detection of *L. intracelularis* in feces of swine from the main producing regions in Brazil. Can J Microbiol 45:230-234.

Fellström C, Pettersson B, Johansson KE, Lundeheim N, Gunnarsson A (1996) Prevalence of *Serpulina* species in relation to diarrhea and feed medication in pig-rearing herds in Sweden. Am J Vet Res 57:807-811.

Hampson DJ (2000) The Serpulina Story. In: Cargill C, McOrist S (eds). Proceedings of the 16th International Pig Veterinary Society Congress, Australia, pp 1-5.

Hoffmann RP (1987) *Diagnóstico de parasitismo veterinário*. Porto Alegre: Editora SULINA; 156p.

Holyoake PK, Jones GF, Davies PR, Foss DL, Murtaugh MP (1996) Application of a polymerase chain reaction assay for detection of proliferative enteritis-affected swine herds. J Vet Diagn Invest 8:181-185.

Jacobson M, Aspan A, HeldtanderKonigsson M, Hard CS, Wallgren P, Fellström C, Jensen WM, Gunnarsson A (2004) Routine Diagnostics of *L. intracellularis* preformed by PCR, serological and *post morten* examination, with special emphasis on sample preparation methods for PCR. Vet Microbiol 102:189-201.

Jacobson M, Hard CS, Gunnarsson A, Fellström C, Klimgenberg KV, Wallgren P, Jensen WM (2003) Diarrhoea in the growing pig - Comparison of clinical, morphological and micro-

bial findings between animals from good and poor performance herds. Res Vet Scie 74:163-169.

Jacobson M, Löfstedt MG, Holmgren A, Lundeheim N, Fellström C (2005) The prevalences of *B.* spp. and *L. intracellularis* in Swedish Piglet Production Herds and Wild Boar Population. J Vet Med 52:386-391.

Jensen TK, Christensen BB, Boye M (2006) *L. intracellularis* infection in the large intestines of pigs. Acta Pathol. Microbiol Immunol Scand 114:255-164.

Jones GF, Ward GE, Murtaugh MP, Lin GF, Gebhart CJ (1993) Enhanced detection of intracellular organism of swine proliferation enteritis, ileal symbiont intracelullaris in feces by polymerase chain reaction. J Clinic Microbiol 31:2611-2615.

Komarek V, Maderner A, Spergser J, Weissenböck H (2009) Infections with weakly haemolitic Brachyspira species in pig with miscellaneous chronic diseases. Vet Microbiol 134:311-317.

La T, Collins AM, Phillips ND, Oksa A, Hampson DJ (2006) Development of a multiplex-PCR for rapid detection of the enteric pathogens *L. intracellularis*, *B. hyodysenteriae*, and *B. pilosicoli* in porcine faeces. Lett ApplMicrobiol 42:284-288.

La T, Phillips ND, Hampson DJ (2003) Development of a duplex PCR assay for detection of *B. hyodysenteriae* and *B. pilosicoli* in pig faeces. J Clinical Microbiol 41:3372-3375.

Macêdo NR, Menezes CPL, Lage AP, Ristow LE, Reis A, Guedes RMC (2007) Detecção de cepas patogênicas pela PCR multiplex e avaliação da sensibilidade a antimicrobianos de *Escherichia coli* isoladas em leitões diarréicos. Arq Bras Med Vet Zootec 59:1117-1123.

McOrist S (2005) Defining the full costs of endemic porcine proliferative enterophaty. Vet J 170:8-9.

Moreno AM, Baccaro MR, Coutinho LL (2002) *L. intracellularis* detection in swine feces from important production regions in Brazil. Arq Inst Biol 69:5-8.

MΦller K, Jensen TK, Jorsal SE, Leser TD, Carstensen B (1998) Detection of *L. intracellularis*, weakly beta-haemolytic intestinal spirochaetes, *Salmonella enterica*, and haemolitic *Escherichia coli* from swine herds with and without diarrhea among growing pigs. Vet Microbiol 62:59-72.

Noordhuizen JPTM, Frankena K, Thrusfield MV, Graat EAM (2001) Application of Quantitative Methods in Veterinary Epidemiology, 2nd edition. Wageningen, Paperback, 429 pp.

Pittman JS (2010) Enteritis in grower-finisher pigs caused by F18-positive *Escherichia coli.* J S H P 18:81-86.

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. 2nd edition. Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, 309 pp.

Stege H, Jensen TK, MΦller K, Bækbo P, Jorsal SE (2000) Prevalence of Intestinal Pathogens in Danish Finishing pig Herds. Prev Vet Med 46:279-292.

Suh DK, Song JC (2005) Prevalence of *L. intracellularis*, *B. hyo dysenteriae*, and *Salmonella* in swine Herds. J Vet Scie 6:289-193.

Thomson JR, Smith WJ, Murray BP (1998) Investigations into field cases of porcine colitis with particular reference to infection with *Serpulina pilosicoli*. Vet Rec 142:235-239.

Weiss LHN, Nonnig R, Cardoso MRI (1999) Occurrence of *Salmonella* in finishing pigs in south Brazil. In: International symposium on Epidemiology and ontrol of Salmonella in Pork, Washington, pp 184.

Weneger HC, Bager F (1997) Pork as a source of human Salmonellosis. In: Procedings of the Second International Symposium on Epidemiology and Control of Salmonella in Pork, Copenhagen, Denmark, August, pp. 3-8.

All the content of the journal, except where otherwise noted, is licensed under a Creative Commons License CC BY-NC.