





Use of oral fluids to detect anti *Lawsonia intracellularis* antibodies in experimentally infected pigs¹

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ABSTRACT.- Gabardo M.P., Sato J.P.H., Resende T.P., Otoni L.V.A., Rezende L.A., Daniel A.G.S., Pereira C.E.R. & Guedes R.M.C. 2021. **Use of oral fluids to detect anti-*Lawsonia intracellularis* antibodies in experimentally infected pigs.** *Pesquisa Veterinária Brasileira* 40(12):970-976. Departamento de Clínica e Cirurgia, Escola de Medicina Veterinária, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Belo Horizonte, MG 31270-901, Brazil. E-mail: guedesufmg@gmail.com

Several pathogens and antibodies derived from serum or produced in tissues associated with the oral cavity are present in the oral fluid (OF). Considering the applicability of this alternative sample, recent studies in veterinary medicine have tested OF as a replacement for serum in diagnostic assays. The aim of this study was to standardize the immunoperoxidase monolayer assay (IPMA) to detect anti-*Lawsonia intracellularis* immunoglobulin A (IgA) and immunoglobulin G (IgG) in OF samples from experimentally infected pigs. Sixty-two pigs were divided into two groups: control (T1, n=30) and inoculated with *L. intracellularis* (T2, n=32). Blood, OF and fecal samples were collected at 0, 7, 14, 21, 28 and 42 days post-inoculation (dpi). Some adaptations of the standard technique for serum were made to IPMA for the detection of IgA and IgG in OF. The IPMA showed high specificity and sensitivity for serum samples and high specificity and moderate sensitivity for the detection of IgA and IgG in OF. There was high agreement between the results of serum IgG and OF IgA and IgG. Based on our results, oral fluid samples may be used for the evaluation and determination of anti-*L. intracellularis* antibodies in pigs, but not for individual diagnosis of swine proliferative enteropathy.

INDEX TERMS: Oral fluid, *Lawsonia intracellularis*, antibody, pigs, diagnostic, enteric disease, porcine proliferative enteropathy, serology, swine.

RESUMO.- [Utilização de fluidos orais para detecção de anticorpos anti *Lawsonia intracellularis* em suínos experimentalmente infectados.] Vários patógenos e anticorpos derivados do soro ou produzidos em tecidos associados a cavidade oral estão presentes no fluido oral (FO). Considerando a aplicabilidade dessa amostra alternativa, estudos recentes em medicina veterinária têm testado o FO como substituto do soro para testes diagnósticos. O objetivo desse estudo foi padronizar a imunoperoxidase em monocamada

de célula (IPMC) para a detecção de imunoglobulina A e imunoglobulina G anti-*Lawsonia intracellularis* em amostras de FO de suínos experimentalmente infectados. Um total de 62 suínos foram divididos em dois grupos: controle (T1, n=30) e inoculados com *L. intracellularis* (T2, n=32). Sangue, FO e amostras de fezes foram coletados aos 0, 7, 14, 21, 28 e 42 dias após a inoculação (dpi). Algumas adaptações da técnica foram realizadas na técnica padrão da IPMC para a detecção de IgA e IgG. A IPMC demonstrou alta especificidade e sensibilidade para amostras de soro e alta especificidade de moderada sensibilidade para a detecção de IgA e IgG em FO. Houve alta concordância entre resultados de detecção de IgG em soro com a IgA e IgG em amostras de FO. Baseado em nossos resultados, amostras de fluido oral podem ser usadas em avaliações e detecção de anticorpos anti-*L. intracellularis* em suínos, porém não de forma individual.

TERMOS DE INDEXAÇÃO: Fluido oral, anticorpo, *Lawsonia intracellularis*, suínos, diagnóstico, doenças entéricas, enteropatia proliferativa, sorologia.

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INTRODUCTION

Lawsonia intracellularis is an obligate intracellular bacterium that causes porcine proliferative enteropathy (PPE) (McOrist et al. 1995). PPE is an intestinal infectious disease of grow-finish pigs. PPE has three manifestations: acute, characterized by bloody diarrhea and death of the pigs at slaughter age; chronic, characterized by a performance decrease and transitory diarrhea in pigs from 6 to 20 weeks of age; and subclinical, characterized mainly by decreased production performance (Guedes 2007).

Among *ante mortem* diagnostic tests, PCR is highly specific; however, there are inhibitory factors that interfere with sensitivity in fecal samples. Additionally, the results are influenced by the course of the disease and variations in fecal shedding (Vannucci et al. 2012). Serological tests, such as ELISA, indirect immunofluorescence and the immunoperoxidase monolayer assay (IPMA), are also used (Kroll et al. 2005). Seroconversion occurs approximately 14 days after exposure to the agent and lasts for a long period; serology can be used to evaluate the kinetics of infection in the herd and to establish disease control strategies (Walter et al. 2004).

Traditionally, serum is used in diagnostic tests, but there are an increasing number of studies in veterinary medicine that used oral fluid (OF) to detect viruses, bacteria and specific antibodies against these agents (Prickett et al. 2008). OF samples may provide practical and economic advantages, including easier collection and reduced stress (Prickett & Zimmerman 2010).

Additionally, several studies have shown a high correlation between the detection of infectious agents and antibodies from serum and OF (Kittawornrat et al. 2013, Panyasing et al. 2014, 2013). Tests based on OF have demonstrated good sensitivity, specificity and reproducibility (Kittawornrat et al. 2013, Ouyang et al. 2013). To date, there have been no reports of the use of OF to detect anti-*L. intracellularis* antibodies in experimentally infected pigs. This study aimed to detect immunoglobulin A (IgA) and immunoglobulin G (IgG) anti-*L. intracellularis* antibodies in OF and to verify the persistence of the detection of these isotypes in OF in experimentally infected pigs. ^e

MATERIALS AND METHODS

Animals and experimental design. The experiment was approved by the Animal Experimentation Ethics Committee of the "Universidade Federal de Minas Gerais" (#77/2011 - June 8, 2011). Sixty-two 5-week-old pigs (10.0±0.64kg/b.w.) from a commercial swine herd free of clinical signs of PPE and other enteric diseases were used. All pigs were weighed, ear-tagged and randomly divided into the control (T1) and infected (T2) groups (30 and 32 pigs each, respectively). Each group was housed in separated rooms with four suspended pens with plastic floors, meshed-wired separations, deposit feeders and nipple drinkers. Four pigs were grouped per pen; however, there were only three pigs in two pens of the T1.

Management of temperature, and feeding were the same for all pens. All daily management activities were initially performed in control rooms, changing boots and overalls between the control room and infected challenge pigs. No parenteral or feed antimicrobial agents were used throughout the experimental period.

At 42 days post inoculation (dpi), all pigs were individually weighed. Pigs in poor clinical condition were euthanased and

necropsied. The remaining pigs were euthanased and postmortem evaluated at 49 dpi. At necropsy, gross lesions were evaluated and ileum and cecum samples were collected to histopathologic and immunohistochemistry (IHC) to confirm *Lawsonia intracellularis* infection (Guedes et al. 2002a). For the IHC the labeled streptavidin³ method with polyclonal antibodies specific for *L. intracellularis* was used (Guedes & Gebhart 2003a).

Preparation of inoculum and inoculation. The inoculum was prepared from porcine intestines affected by PPE, according to a previously described model (Guedes et al. 2002c). On day 0, pigs in T2 received 1 dose of 60mL of the inoculum via stomach tube. The total dose was approximately 8.8×10^{10} *L. intracellularis*/pig. Pigs in T1 received a sham inoculum. Bacteriologic testing of the inoculum did not detect any other confounding enteropathogenic organisms, such as *Salmonella* sp., *Brachyspira* spp. or enteropathogenic *Escherichia coli*.

Clinical evaluation and sample collection. Clinical observations of diarrhea were made by the same person twice a week. The scoring was based on Guedes et al. (2002d). The OF, blood and fecal samples were collected individually 0, 7, 14, 21, 28, 35 and 42 dpi. The pen OF sample was collected 0, 4, 14, 21, 28, 35 and 42 dpi. To collect OF, 15cm long cotton ropes were individually provided for pig. To prevent other pigs in the pen to have access to the rope of a given individual, the cotton ropes were marked and provided directly into the mouth of the animal. To collect pen samples, rope bundles were tied to the side of each pen for a time period sufficient to acquire an average of 10mL of OF per pen or until the pigs lost interest in the rope, in general for 30min. OF were extracted by twisting the damp part in plastic bags, then transferring to 50mL conical tubes, centrifuged at 1000xg for 10min, aliquoted and stored at -20°C until tested. Blood samples were collected at the same days, processed and serum stored at -20°C until tested.

Individual fecal samples from all pigs were collected at the same days and stored at -20°C for PCR testing. DNA of fecal was extracted from fecal samples using a commercial kit⁴ according to the manufacturer's protocol, and subsequently tested by PCR (Jones et al. 1993).

Serum and oral fluid analysis. Serum samples were tested by IPMA for anti-*L. intracellularis* IgG (Guedes et al. 2002b, 2002c). For the verification of titers, positive serum samples at the 1:30 cut-off point were serially diluted to 1:120, 1:480 and 1:1920.

The IPMA used for detection of *L. intracellularis*-specific IgA and IgG in OF was a modified version of the IPMA used for serum described by Guedes et al. (2002b). The standardization of the IPMA for both isotypes in OF was initially performed on samples from a farm with PPE cases and pigs with high serum antibody titers. To for standardization, briefly, 96-well plates containing acetone fixed monolayer of McCoy cells highly infected with *L. intracellularis* were rehydrated with distilled water or PBS Tween 0.05% (PBS with 0.05% Tween 20, pH 7.2) for 10 and 45 minutes at 37°C. OF and secondary antibody (anti-pig IgA⁵ and IgG⁶ -peroxidase conjugate) were diluted in phosphate-buffered saline (PBS; pH 7.2), or 0.25% skimmed milk 1x PBS solution or PBS Tween 0.05%. Secondary antibodies were also diluted in IPMA buffer (PBS with 2.5% serum

³ K675 Dako, Corporation, Carpinteria, CA 93013 USA.

⁴ PSP Stool DNA Spin Kit, Molecular STRATEC Campus Berlin-Buch, Germany.

⁵ Bethyl Laboratories, Montgomery, Texas, USA.

⁶ Sigma-Aldrich, St. Louis, MO.

fetal bovine, 1% rabbit serum, and 0.08% Tween 80) (Guedes et al. 2002b). OF were tested undiluted and diluted 1:2 and 1:3, and the secondary antibodies were tested using 1:45, 1:50, 1:100 and 1:200 dilutions. OF samples were gradually thawed in the refrigerator at 4°C for 12 hours to avoid interference of temperature on the integrity of the antibodies (Prickett et al. 2010, Decorte et al. 2013, Jones & Muehlhauser 2014) and to sediment gross material.

Statistical analysis. Comparisons of serum anti-*L. intracellularis* IgG, and OF anti-*L. intracellularis* IgA and IgG between the groups were performed by the Kaplan-Meier test in the statistical program STATA. The level of significance was 5% ($P < 0.05$). The sensitivity and specificity of IPMA were calculated in Microsoft Excel using the control and infected groups, at 28 dpi, for the determination of negative and positive samples, respectively. The Spearman correlation test was used to evaluate the correlation between the detection of serum IgG and IgA and IgG in OF. The Kappa method was used to verify agreement between the tests and the agreement between the detection of specific IgA and IgG in individual and collective samples. The program used was BioEstata 5.0. The pens were considered positive if at least one animal tested positive for the studied immunoglobulin isotype in the individual collection.

RESULTS

Animals and inoculation

All pigs were seronegative and PCR negative for *Lawsonia intracellularis* in fecal samples on day 0.

In the third week post-inoculation an animal in T2 had bloody diarrhea from 19 to 23 dpi, and watery diarrhea

until 34 dpi, when it was euthanased due to its poor clinical condition and persistent diarrhea. At necropsy, there were typical PPE lesions confirmed by IHC. *L. intracellularis* fecal shedding was first detected in T2 pigs 7 dpi and peaked at 14 dpi. Pigs in the T1 remained negative up to 28 dpi (Fig.1). At the end of the experiment, macroscopic lesions were more frequent, extensive and severe, as well as IHC scores were more intense in the T1 group.

Four pigs from each group died or were euthanized in the first and second week of the study due to conditions not related to the challenge.

IPMA in serum and oral fluid

The main modifications of IPMA for OF were the hydration of the plates with PBS Tween 0.05%, no dilution of the samples, and the time of incubation of OF sample was extended to 45 min, as described by Barrera-Zarate (2019). All tested concentrations of anti-pig IgA and anti-pig IgG secondary antibodies were adequate, but the 1:100 dilution in PBS 0.05% Tween had less background; thus, specific immunostaining was easier to visualize.

One animal in T2 seroconverted 14 dpi, and 100% of the pigs were seropositive at 42 dpi. T1 pigs seroconverted at 35 dpi, and an increasing number of seropositive pigs were detected at 42 dpi, but the proportion of seropositive pigs was significantly lower ($P < 0.05$) than T2 from 14-42 dpi (Fig.1).

The first detection of specific *L. intracellularis* IgA and IgG, respectively, in OF occurred at 14 dpi and 21 dpi in T2,

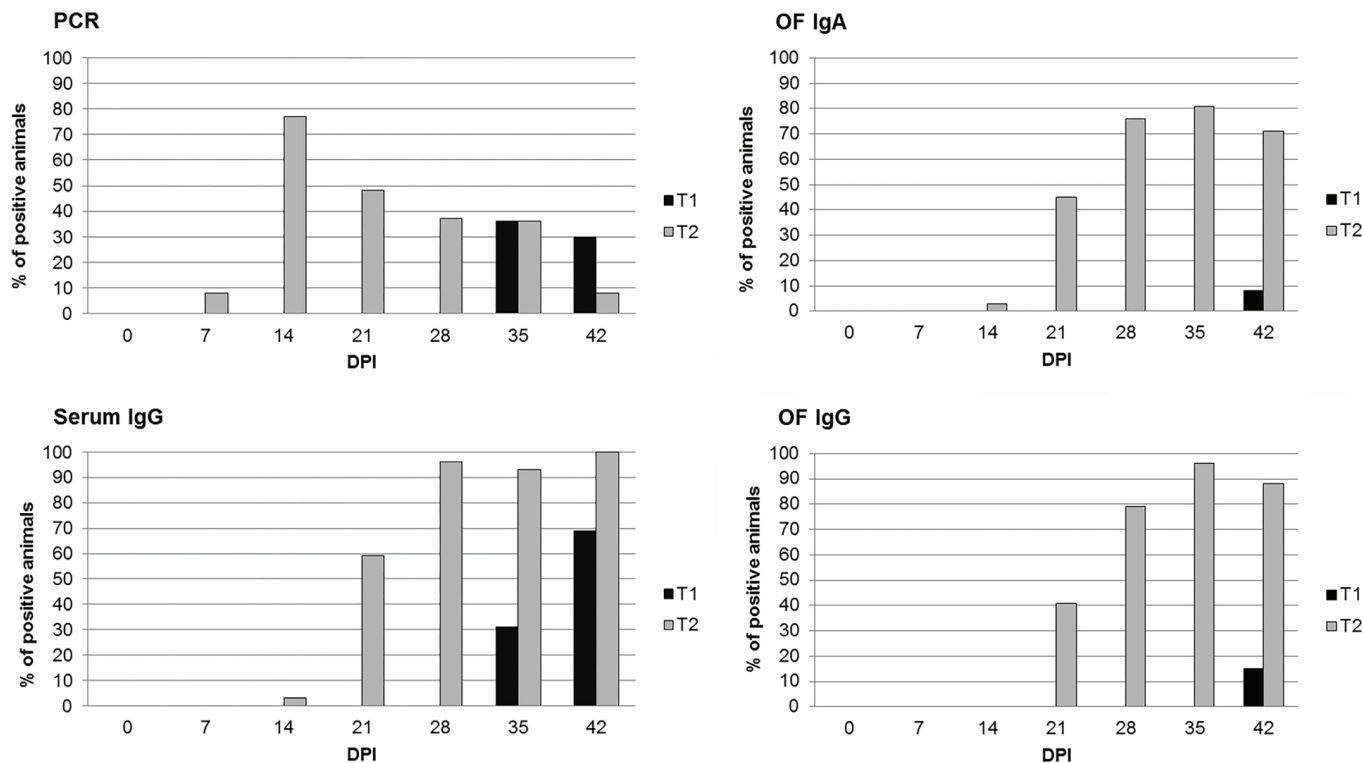


Fig.1. Percentage of positive pigs by PCR for *Lawsonia intracellularis* in fecal samples, percentage of positive pigs using immunoperoxidase monolayer assay (IPMA) to detect IgG in serum samples and Percentage of positive pigs using IPMA to detect IgA and IgG in oral fluids (OF) samples. All pigs used in the experiment were considered in these analyses. There was a difference ($P < 0.05$) in positive animals for *L. intracellularis* between groups on 7 to 28 dpi. There was a difference ($P < 0.05$) in the detection of OF serum IgG between the groups on 21 to 42 dpi.

with higher percentages of positive pigs at 35 dpi for both isotypes. In T1, both isotypes were detected in OF at 42 dpi. The detection of both isotypes in this group remained different from T2 from 14 and 21 dpi until 42 dpi ($P < 0.05$) for IgA and IgG (Fig.2), respectively. Figure 2 shows the curve of serum IgG titers and the percentage of IgA and IgG positive pigs in OF.

The IPMA sensitivity and specificity for the detection of serum IgG and OF IgA and IgG were determined using 52 samples (26 pigs from each group) at 28 dpi. The IPMA sensitivity for detecting IgG in serum was 100% and 84.62% and 88.46% for OF IgA and IgG, respectively. The specificity was 100% for both serum and OF.

There was an increase in the number of IgA- and IgG-positive pigs based on the OF analysis concomitant with the increase in IgG serum titers. There was a high correlation between the detection of serum IgG and the detection of IgA and IgG in the OF ($r_s = 0.82$ and $r_s = 0.89$, respectively).

There was high agreement between the IPMA results for specific *L. intracellularis* IgG in serum and OF IgA and IgG at 28 dpi in individual samples (Table 1). In the OF pen samples, the IPMA detected one positive pig housed together with three negative animals (Table 2 and 3), and there was high agreement between the pen samples IPMA results and the individual animal samples.

DISCUSSION

The naive condition of the pigs used in this study was confirmed by serology and PCR of samples collected on

the day of inoculation. The success of the challenge was confirmed by the high number of pigs that shed *Lawsonia intracellularis* in their feces, the seroconversion rate and the detections of lesions at the end of the experiment. Although the T1 was housed in separate room with strict management, due to the long period of the experiment, there was indirect contamination confirmed by *L. intracellularis* fecal shedding and seroconversion at 35 dpi. This period of T1 contaminations was confirmed with detection of more evident macroscopic lesions and immunolabelling compared to T2. The period between the inoculation of T2 pigs and euthanasia (49 dpi) justify the regression of gross lesions by the end of the trial, as previous demonstrated by Guedes & Gebhart (2004).

Both clinical manifestations and the detection of *L. intracellularis* in feces of T2 were consistent with other experimental challenge studies using mucosal homogenate as the inoculum (Guedes et al. 2002b, 2002d). Fecal and serum samples from pigs of both groups were negative by PCR and serology during the first two weeks and remained PCR negative until 35 dpi in T1.

Table 1. Percentage of agreement between immunoperoxidase monolayer assay (IPMA) results for specific *Lawsonia intracellularis* IgG in serum and IgA and IgG in oral fluid (OF) at 21 and 28 dpi

	21 dpi	28 dpi
Serum IgG/IgA OF	79.00 ^a (0.46) ^b	90.38 (0.81)
Serum IgG/IgG OF	80.77 (0.50)	94.23 (0.88)
IgA OF/IgG OF	86.54 (0.58)	92.31 (0.84)
Individual pig IgA/pen IgA	93.75 (0.86)	100.00
Individual pig IgG /pen IgG	100.00	100.00

Twenty-six animals were used in each group; ^a percentage of agreement, ^b Kappa-index.

Table 2. Number of positive animals per pen and pan positive for IgA in individual and collective samples, respectively, of pig oral fluid on different days post-inoculation with *Lawsonia intracellularis*

	Pen	0 dpi	7 dpi	14 dpi	21 dpi	28 dpi	35 dpi	42 dpi
Control pen	1A	0/4	0/4	0/4	0/4	0/4	1/4	2/4
	1B	0/4	0/4	0/4	0/4	0/4	2/4	3/4 ^a
	1C	0/4	0/4	0/4	0/4	0/4	1/4	0/4
	1D	0/4	0/4	0/4	0/4	0/4	1/4	1/4
	2A	0/3	0/2	0/2	0/2	0/2	1/2	0/2
Inoculated pen	2B	0/3	0/2	0/2	0/2	0/2	2/2	1/2 ^a
	2C	0/4	0/4	0/4	0/4	0/4	1/4	0/4 ^a
	2D	0/4	0/2	0/2	0/2	0/2	0/2	1/2 ^a
	3A	0/4	0/4	1/3	3/3 ^a	2/3 ^a	2/2 ^a	2/3 ^a
	3B	0/4	0/4	1/4	2/4 ^a	3/4 ^a	4/4 ^a	4/4 ^a
	3C	0/4	0/4	0/4	1/4 ^a	2/4 ^a	1/4 ^a	1/4 ^a
	3D	0/4	0/4	0/4	0/4	4/4 ^a	4/4 ^a	2/4 ^a
	4A	0/4	0/3	0/3	0/3	1/3 ^a	0/2 ^a	0/2 ^a
	4B	0/4	0/4	0/4	3/3 ^a	3/3 ^a	3/3 ^a	3/3 ^a
	4C	0/4	0/4	0/4	3/4 ^a	4/4 ^a	3/3 ^a	3/3 ^a
	4D	0/4	0/4	0/4	1/4	3/4 ^a	3/4 ^a	2/4 ^a

^a Positive pens in collective oral fluid, ^b cells surround indicates pens with negative pens in collective samples, but positive results in individual samples; * Positive in collective sample, but negative in individual samples.

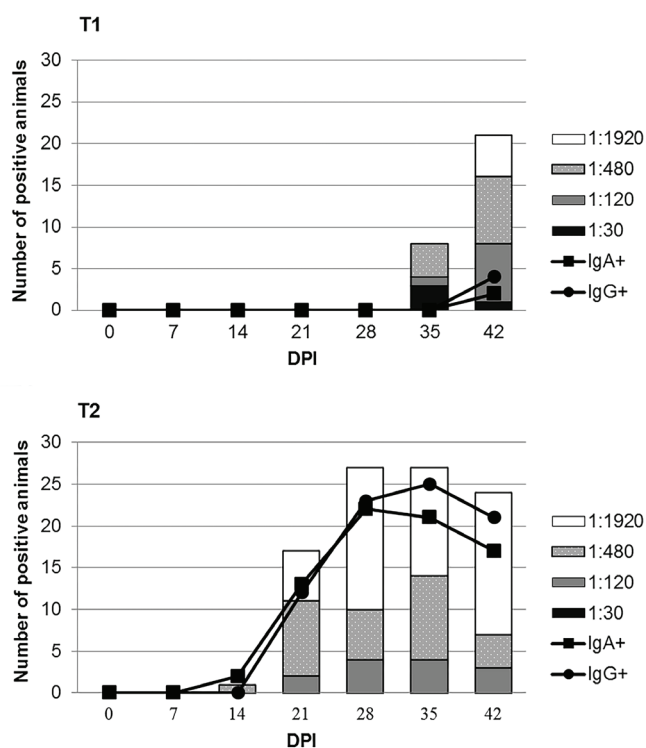


Fig.2. Positivity of pigs detected in serum and oral fluids (OF). Numbers and titers of *Lawsonia intracellularis* IgG seropositive pigs (bars) and numbers of positive pigs and specific *L. intracellularis* IgA and IgG titers (lines) in OF samples. T1 = control group and T2 = inoculated group.

OF contains pathogens and antibodies derived from serum or produced in tissues associated with the oral cavity. A number of studies in animals have demonstrated the usefulness of OF compared to serum (Pacheco et al. 2010, Prickett et al. 2011, Giménez-Lirola et al. 2013, Vosloo et al. 2015). These studies used ELISA for the detection of serum and OF antibodies. IPMA was chosen in the present study because this methodology was already established in our laboratory and had demonstrated high sensitivity and specificity in previous studies (Guedes et al. 2002b, 2002c).

Similar to serum samples, the present study also demonstrated high specificity of IPMA in OF, as no false positive animal was detected (at 21 and 28 dpi). Despite the low concentrations of antibodies detectable in OF (Escribano et al. 2012), the IPMA showed moderate sensitivity for detecting IgA (84.62%) and IgG (88.46%) in OF. Kittawornrat et al. (2012) also obtained good sensitivity and specificity for the detection of anti-PRRSV IgG in field samples after standardization of ELISA in OF. Control pigs showed positive OF after indirect infection, demonstrated by positive PCR and increase of seropositive pigs. This result demonstrated that even naturally infected pigs have detectable antibodies specific for *L. intracellularis* in the OF. Although not planned, this result demonstrated that this type of sample might also be applicable in field conditions.

Due to IgA antibodies representing mucosal immunity, we expected the detection of IgA prior to IgG, however, as observed that there was no time difference in the detection of both, only two pigs were positive one week before IgG detection in T2. In T2, the percentage of IgG detection in OF was higher than IgA from 21 to 42 dpi. This difference may be explained by the influence of the rope material used (cotton). In two studies using synthetic (i.e., polyamide) and natural fiber (i.e., cotton) rope material, higher detection of IgA was observed using the synthetic materials. However, there was no difference

between the materials for the detection of IgG (Olsen et al. 2013, Decorte et al. 2014). Another explanation is that sick pigs have higher concentrations of total immunoglobulin in their OF compared to healthy pigs; this is especially true for IgG, which significantly increases among immunoglobulins (Escribano et al. 2012) and remains useful for a longer time in standard concentrations for the diagnosis in both experimental and field samples (Kittawornrat et al. 2012, 2014). Therefore, it can be inferred that the use of cotton rope for the detection IgG anti-*L. intracellularis* in OF may be used for the monitoring the disease.

The first detection and the peak of antibodies occurred at the same time in the OF and serum in T2 group. Our findings support those Nogueira et al. (2013), who demonstrated that the peak of detection for serum and mucosal IgG against *L. intracellularis* were correlated. A similar correlation between serum and OF IgG was observed in other studies (Prickett et al. 2011, Kittawornrat et al. 2013, Mur et al. 2013). In general, the kinetics of antibodies response in the OF is dependent on the kinetics of antibodies in the serum and mucosa (Prickett & Zimmerman 2010). In the present study, there was a significant correlation between the serum IgG and the two isotypes in the OF. The percentage of agreement between the IPMA results in serum and OF was high at 28 dpi, with 90% for IgA and 94% for IgG. In agreement with our result, Kittawornrat et al. (2013) observed a high correlation between serum and OF and high agreement of positive results using ELISA to detect anti-PRRSV-specific antibodies.

In the present study, the majority of the pigs with IgG titers lower than <1:480 in serum were negative for IgA and IgG in the OF. Similar results were observed in the study of Mur et al. (2013) for African swine fever. The low initial antibody serum titers in control pigs could be explained by the low number of pigs with positive OF one week after IgG seroconversion

Table 3. Number of positive animals per pen and pan positive for IgG in individual and collective samples, respectively, of pig oral fluid on different days post-inoculation with *Lawsonia intracellularis*

	Pen	0 dpi	7 dpi	14 dpi	21 dpi	28 dpi	35 dpi	42 dpi	
Control pen	1A	0/4	0/4	0/4	0/4	0/4	0/4	1/4 ^b	
	1B	0/4	0/4	0/4	0/4	0/4	0/4	1/4 ^b	
	1C	0/4	0/4	0/4	0/4	0/4	0/4	0/4	
	1D	0/4	0/4	0/4	0/4	0/4	0/4	0/4	
	2A	0/4	0/3	0/3	0/3	0/3	0/3	0/3	
	2B	0/3	0/2	0/2	0/2	0/2	0/2	0/2	
	2C	0/4	0/4	0/4	0/4	0/4	0/4	1/4 ^a	
	2D	0/2	0/2	0/2	0/2	0/2	0/2	1/2 ^a	
	Inoculated pen	3A	0/4	0/4	0/3	2/3 ^a	2/2 ^a	2/2 ^a	2/2 ^a
		3B	0/4	0/4	0/4	1/4 ^a	2/3 ^a	4/4 ^a	4/4 ^b
3C		0/4	0/4	0/4	1/4 ^a	4/4 ^a	4/4 ^a	3/4 ^a	
3D		0/4	0/4	0/4	0/4	4/4 ^a	4/4 ^a	2/2 ^a	
4A		0/4	0/3	0/3	3/3 ^a	1/3 ^a	1/2 ^a	2/2 ^a	
4B		0/4	0/4	0/4	3/3 ^a	3/3 ^a	3/3 ^a	3/3 ^a	
4C		0/4	0/4	0/4	1/4 ^a	4/4 ^a	3/3 ^a	3/3 ^a	
4D		0/4	0/4	0/4	0/4	2/4 ^a	4/4 ^a	3/4 ^a	

^a Gray marking indicates the positive pens in collective oral fluid, ^b cells surround indicates pens with negative results in collective samples, but positive results in individual samples.

Table 4. Number of PCR positive animals per pen in individual fecal collect and qPCR positive pen in collective oral fluids (OF) on different days post-inoculation with *Lawsonia intracellularis*

	Pen	14 dpi	21 dpi	28 dpi	35 dpi	42 dpi	
Control pen	1A	0/4 ^a	0/4 ^a	0/4 ^a	1/4 ^a	2/4 ^a	
	1B	0/4 ^a	0/4 ^a	0/4 ^a	2/4 ^a	3/4 ^a	
	1C	0/4 ^a	0/4 ^a	0/4 ^a	1/4 ^a	0/4 [*]	
	1D	0/4 ^a	0/4 ^a	0/4 ^a	1/4 ^a	1/4 ^a	
	2A	0/2 ^a	0/2 ^a	0/2 ^a	1/2 ^a	0/2 [*]	
	2B	0/2 ^a	0/2 ^a	0/2 ^a	2/2 ^a	1/2 ^a	
	2C	0/4 ^a	0/4 ^a	0/4 ^a	1/4 ^a	0/4 [*]	
	2D	0/2 ^a	0/2 ^a	0/2 ^a	0/2 ^b	0/2 [*]	
	Inoculated pen	3A	2/3 ^a	1/3 ^a	0/3 ^a	2/3 ^a	0/3
		3B	2/4 ^a	2/4 ^a	2/4 ^a	2/4 ^a	0/4 ^a
		3C	4/4 ^a	1/4 ^a	1/4 ^a	1/4 ^a	0/4 ^a
		3D	4/4 ^a	2/4 ^a	1/4 ^a	2/4 ^b	0/4 ^a
4A		2/3 ^a	1/3 ^a	1/3 ^a	0/2 ^a	0/2 ^a	
4B		3/4 ^a	2/3 ^a	1/3 ^a	1/3 ^a	1/3 ^b	
4C		3/4 ^a	3/4 ^a	3/4 ^a	1/4 ^a	0/3 [*]	
4D		1/4 ^a	2/4 ^a	1/4 ^a	1/4 ^a	0/4 [*]	

^a Gray marking indicates the positive pens in collective oral fluid, ^b cells surround indicates pens with negative pens in collective samples, but positive results in individual samples; ^{*} Positive in collective sample, but negative in individual samples.

compared to the inoculated pigs. The difference in the infective initial dose of *L. intracellularis* (which was presumably lower in the control group due to natural exposure) could explain the difference in the initial immune response of the control pigs, as humoral and mucosal responses are dose dependent (Nogueira et al. 2013).

The application of OF-based testing facilitates the monitoring, surveillance and detection of diseases in animal populations. The samples can be collected from various pigs in pens at the same time, thereby facilitating collection and increasing the sample size (Prickett & Zimmerman 2010). In a study of the detection of anti-PRRSV-specific antibodies in OF collected in pens, it was observed that IgM, IgG and IgA were readily detected in populations in which the infection was controlled by experimental challenge. However, this was not observed in field samples (Kittawornrat et al. 2012). Natural exposure in the T1 group may have resulted in pigs at different stages of the humoral response. Thus, in the present study, we compared individuals with pen OF to verify whether collective samples could be representative of the group. The results demonstrated high agreement between individual OF and pen OF samples, and IPMA could detect one positive housed together with three negative pigs (Table 2-4).

Similar results were found by Barrera-Zarate et al. (2019) that detected IgG positive oral fluid collected in the pen that not all pigs tested were positive in serum samples. However, just the correlation of positivity of FO and serum was possible in farms with clinical signs, in the growth and finishing pigs.

CONCLUSIONS

The immunoperoxidase monolayer assay (IPMA) technique has demonstrated moderate sensitivity and high specificity for the detection of anti-*Lawsonia intracellularis* IgA and IgG in oral fluids (OF) in experimentally inoculated pigs and has the ability to detect positive pigs in OF pen samples.

The simultaneous detection of antibodies in both serum and OF samples and the high agreement between the IPMA in serum and OF demonstrate that OF may be used for serologic profile analysis, but not for individual diagnosis of EPS.

In addition, IgG antibodies remain in the oral fluid for a minimum of three weeks.

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