DOI: 10.5433/1679-0359.2020v41n5Supl1p2429

Proliferative enteropathy caused by *Lawsonia intracellularis* in rabbits in Southern Brazil

Enteropatia proliferativa por *Lawsonia intracellularis* em coelhos no Sul do Brasil

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Highlights:
The disease was characterized by poorly absorbed diarrhea which culminated in death.
This outbreak presented an acute development of clinical signs.
Important economic losses to the farmer due to mortality and treatment costs.
The necessity of adequate sanitary standards.

Abstract

Proliferative enteropathy (PE), also known as ileitis, is a disease caused by the bacterium *Lawsonia intracellularis*. This disease is characterized by diarrhea and ill-thrift. The aim of this study is to describe a PE outbreak in rabbits that occurred in Southern Brazil. The farm had 700 rabbits at the time the outbreak occurred. The clinical signs were severe diarrhea, dehydration, and apathy. Necropsy was performed in 33 rabbits, and the most evident macroscopic findings were thickening of the intestinal wall, intestinal loops distended by large amounts of gas, and liquid feces. Histopathological examination demonstrated a marked proliferation of enterocytes in intestinal crypts, decrease number of goblet cells, and crypts microabscesses. Silver impregnation technique (Warthin-Starry) demonstrated in intestinal crypts inside of enterocytes cytoplasm, curved vibrioid bacteria compatible with *L. intracellularis*. Immunohistochemical staining with anti-*L. intracellularis* confirmed the agent presence. PCR was performed and *L. intracellularis* was confirmed as the etiological agent.
Key words: Lagomorphs. Bacterial disease. Ileitis. Diarrhea.

Resumo

A enteropatia proliferativa (EP), também conhecida como ileíte, é uma doença causada pela bactéria *Lawsonia intracellularis*, que é caracterizada por diarreia com redução do ganho de peso. O objetivo deste trabalho é descrever um surto de EP em coelhos na região sul do Brasil. A propriedade possuía 700

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Received: Apr. 08, 2019 - Approved: Mar. 24, 2020

coelhos, durante o período do surto. O quadro clínico caracterizava-se por diarreia severa, desidratação e apatia. Realizou-se o exame de necropsia em 33 coelhos, e as alterações macroscópicas mais evidentes eram; alças intestinais acentuadamente distendidas por gás e fezes líquidas, além de serosa rugosa e parede intestinal moderadamente espessada. Na análise histopatológica do intestino delgado visualizou-se marcada hiperplasia de enterócitos de criptas, moderada diminuição do número de células caliciformes e microabscessos de criptas. A etiologia das lesões foi confirmada pela técnica de impregnação pela prata (Warthin-Starry), evidenciando bactérias vibrioides compatíveis com *L. intracellularis* no ápice de enterócitos das criptas intestinais. Ainda, foi obtida imunomarcação positiva de enterócitos de criptas na imuno-histoquímica com anticorpo anti-*L. intracellularis* e a PCR positiva em amostras de intestino.
Palavras-chave: Lagomorfos. Doença bacteriana. Ileíte. Diarreia.

Proliferative enteropathy (PE), also known as ileitis, is a disease caused by *Lawsonia intracellularis* (McOrist & Gebhart, 2012), an obligate intracellular vibrioid, gram-negative bacterium that colonizes immature enterocytes in the intestinal crypts and causes enterocyte hyperplasia and prevents the differentiation of infected enterocytes leading to thickening of the intestinal mucosa, and resulting in malabsorption syndrome (Pusterla, Mapes, & Gebhart, 2012; Vanucci & Gebhart, 2014). The intestinal colonization and clinical disease caused by this microorganism have been reported in several species such as rats, ferrets, hamsters, and guinea pigs (Percy & Barthold, 2007), horses (Vanucci & Gebhart, 2014), rabbits (Peixoto, França, Ribeiro, Bezerra, & Driemeier 2008), and endemic in pig herds (McOrist & Gebhart, 2012).

In the acute presentation, there are reports of high mortality due to diarrhea in neonatal, young, and adult rabbits (Peixoto et al., 2008). Otherwise, rabbits with chronic lesions may present thickening of the small intestine (Percy & Barthold, 2007). Although, *L. intracellularis* infection is considered silent, outbreaks in pig herds can be associated with severe economic losses due to mortality, and reduction of weight gain (McOrist & Gebhart, 2012). The disease is usually transmitted through fecal contamination of food and water; however, reports suggest that there may be vectors and carriers of the bacteria serving as a source of infection (Guedes, 2002; McOrist & Gebhart, 2012). In pigs, the control can be achieved by implementing adequate hygiene management practices, vaccination and treatment of sick animals (Guedes, 2008; McOrist & Gebhart, 2012).

The objective of this study was to characterize an outbreak of proliferative enteropathy caused by *Lawsonia intracellularis* in rabbits in Rio Grande do Sul, Brazil, describing epidemiological, clinical, pathological and molecular aspects, and discuss the main sources of contamination which may have led to the occurrence of the outbreak in rabbits.

Diagnostic investigation of mortality in a rabbit herd in the municipality of Feliz, Rio Grande do Sul, Brazil (29º27'03 "S; 51º18'23" W) was carried out. Clinical and epidemiological data were obtained from the owner and several visits were made to the farm. Twenty-eight weaned rabbits and five adult rabbits were submitted to necropsy. During the post-mortem examination, organ fragments especially the small and large intestines, as well as mesenteric lymph nodes were collected and fixed in a 10% buffered formalin solution. These samples were routinely processed for histopathology, cut at 3µm and stained by Haematoxylin and Eosin (HE). Intestinal sections were submitted to silver-impregnation technique (Warthin-Starry).

Immunohistochemistry was performed on intestinal fragments of all animals examined using the L-SAB streptavidin-biotin-peroxidase (Dako, Corporation, cat. K0690, Inc. North America, Carpinteria, California USA) assay with the anti-*Lawsonia intracellularis* polyclonal antibody at 1:15.000 dilution (Guedes, 2002). Antigen retrieval was conducted in a microwave oven, with citrate

buffer, pH 6.0, in two cycles of five minutes each. Reactions were revealed using 3-amino-9-ethylcarbazole (AEC, Biocare Medical, Pacheco, California, USA). Intestinal section of an affected pig with swine proliferative enteropathy was used as positive control and a swine and a rabbit intestine fragments without lesions as negative control.

To extract DNA, a pool of refrigerated fecal samples were used. A 375-bp segment of a DNA fragment from the IS-intracellularis-specific DNA clone p78 was sequenced using Sequenase and a commercial kit (Sequenase kit; U.S. Biochemical Corp., Cleveland, Ohio). As a positive control a sample previously tested positive through PCR to *L. intracellularis* was used, and for the negative control, a sample prepared with a water aliquot and an aliquot of total DNA negative to *L. intracellularis*.

The outbreak occurred from September 2002 to October of 2003 in a commercial rabbitry. The herd had approximately 700 rabbits of the New Zealand White, Chinchilla and California breeds from age groups that varied from 20 day-old to 6-month-old. The adult rabbits were kept isolated in metal cages, and the weaned rabbits were kept in groups in a semi-open shed. They were fed with commercial pelleted ration and ramie also known as Chinese grass (*Boehmeria nivea*). Pigs were raised on the same farm, as reported by the owner and confirmed during several visits. There was not direct contact between pigs and rabbits; however, the same staff was responsible for both herds, in addition pig waste was used to fertilize the ramie provided to the rabbits.

The rabbits were weaned at 30 day-old and began to receive commercial feed and a small amount of ramie cultivated on the farm. Three to four days after starting to receive ramie, 33 rabbits began to develop clinical signs such as apathy, anorexia, diarrhea (Figure 1.A), and dehydration that lasted 24 to 48 hours.

Post-mortem examinations were performed on 33 rabbits, in which 28 were weaned rabbits and 5 adult rabbits. The gross lesions observed in the weaned rabbits were roughened serosa of intestinal loops (Figure 1.B) and moderate gas distention; in the lumen, there was a large amount of feces characterized as semifluid. In addition, the intestinal mucosa displayed segments with reddish coloration and accentuated thickening of the wall mainly in the ileum segment. The adult rabbits showed no signs of lethargy or dehydration and during necropsy, intestinal mucosa was moderately thick.

Microscopically, lesions in weaned rabbits were more evident in the ileum segment. Various degrees of mucosal thickening were noticed associated with hyperplastic epithelial cell proliferation of the Lieberkühn crypts, with multilayer enterocytes, that form transverse branches (Figure 1.C). In addition, there were a decreased number of goblet cells, some crypts were dilated and filled with cellular debris and inflammatory infiltrate composed of heterophils often degenerated (crypt abscesses). Adjacent to proliferated epithelial crypts, there was a marked inflammatory infiltrate of lymphocytes, eosinophils, heterophils and plasma cells. In some sections of ileum, multifocal moderate areas of ulceration were noticed followed by severe hemorrhage. The lesions found in the adult rabbits were similar to those described in weaned rabbits; however, the presentation was mild.

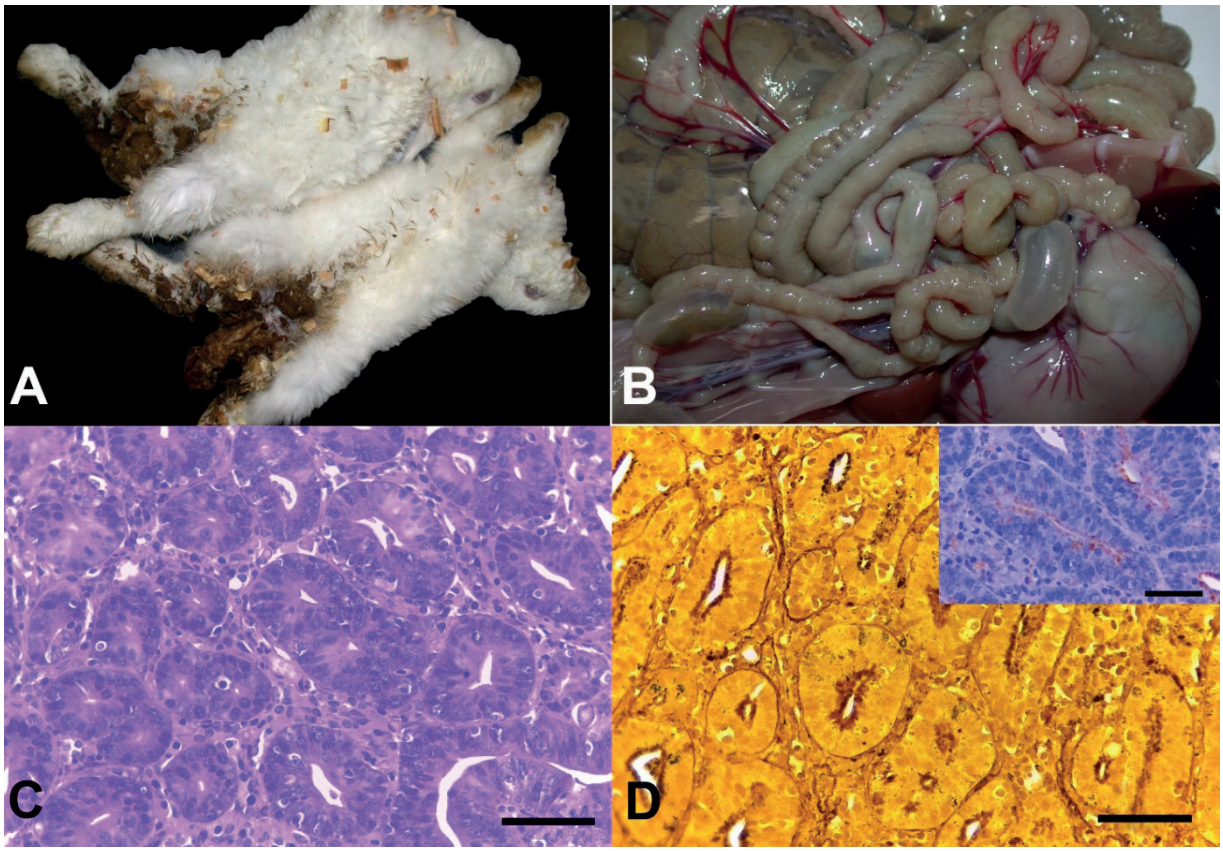

Figure 1. A- Rabbits with large amount of feces in the perineum, compatible with diarrhea. B- Roughened serosa of intestinal loops and moderate gas distention. C- Hyperplastic epithelial cell proliferation of the Lieberkühn crypts, with multilayer enterocytes. HE Bar 50µm. D- Vibrioids with morphology compatible with *L. intracellularis* visualized at the apex of the enterocytes. Warthin-Starry Bar 50 µm. Inset: Strong labeling for anti-*L. intracellularis*. IHC Bar 50 µm.

Through impregnation by silver, vibrioids with morphology compatible with *L. intracellularis* were visualized at the apex of the enterocytes of weaned and adult rabbits' crypts (Figure 1.D). The immunohistochemistry showed intense immunostaining for *L. intracellularis* at the apical cytoplasm of epithelial cells in affected crypts of the small and large intestine. In addition, PCR was positive to *L. intracellularis*.

The diagnosis of proliferative enteritis caused by *L. intracellularis* was based on the clinical, epidemiologic, gross and microscopic lesions, PCR, and immunohistochemistry findings. In this study, the most affected animals were between 40 to 50-day-old. Schoeb and Fox (1990) reported that in a study with 28 rabbits with lesion compatible to *L. intracellularis* most of the animals were weanlings; although, adult rabbits were also showing similar clinical signs.

The disease was characterized by profuse, poorly absorbed diarrhea, leading to a significant reduction in the absorption of glucose and electrolytes, which culminated in death. Diarrhea is correlated with the loss of function of the intestinal mucosa due to changes in permeability related to chronic infection stage (Percy & Barthold, 2007). Diarrhea and weight loss were reported by Schoeb and Fox (1990) and Horiuchi, Watarai, Kobayashi, Omata and Furuoka (2008). In our study, diarrhea lasted about 24 to 48 hours and frequently concluded in

death, corroborating with Schoeb and Fox (1990) were the rabbits died few days after diarrhea onset.

The primary lesion in all *L. intracellularis*-affected species can be observed as cell proliferation, described as hyperplasia of the intestinal crypt cells (Schoeb & Fox, 1990). The gross lesions observed were consonant to those described by Percy and Barthold (2007), who demonstrated that animals in the acute phase of the disease have liquid feces, while in chronic presentation proliferative lesions characterized by thickening of the intestinal mucosa and rough appearance of the serosa are observed. The jejunum and proximal ileum are commonly affected in rabbits (Percy & Barthold, 2007), in accordance with the present study, in which most lesions were found in the proximal ileum.

In rabbits three histological lesions pattern of enteritis associated with *L. intracellularis* has been described: proliferative, histiocytic, and necrosuppurative (Schoeb & Fox, 1990). As observed in other species, crypt proliferation is commonly severe and leads to changes in the ratio of villi and crypt cells, shifting the ratio of 3:1 to 1:1 (Peixoto et al., 2008). However, in cases with an acute presentation, such as the ones described in this study, the proliferation may be little or moderate. The acute course that led to death in 24-48 hours was already described in an outbreak of *L. intracellularis* in rabbits, and the animals only showed diarrhea and dehydration, and the ileum displayed thickness and firm consistency (Peixoto et al., 2008). Another author infers that proliferative lesion is the primary feature in rabbits (Sampieri et al., 2013); nevertheless, it can vary from histiocytic enteritis and necrosuppurative (Schoeb & Fox, 1990). Horiuchi et al. (2008) had described lesions similar to the ones found in this study. Combinations of these lesions can occur and vary depending on the stage of the disease (Vanucci & Gebhart, 2014).

The identification of the source of infection in this outbreak is challenging, once many routes of transmission has been described in the literature, including oral-fecal contamination through direct contact, contaminated food, and water. Carriers have been identified as potential transmitters to domestic animals regarding *L. intracellularis* (Hwang, Seo, & Yeh, 2017). Firstly, we have considered the possibility of food contamination through the ramie, due to the use of swine waste as fertilizer. Swine waste is widely used as a fertilizer to fodder and some procedures must be carried out to reduce and inactivate pathogenic microorganisms and toxic agents. The swine waste used as ramie fertilizer could be the source of contamination to the rabbit herd, since the infection of susceptible animals can occur by the fecal-oral route (McOrist & Gebhart, 2012). As reported by the owner and confirmed during visits, the animals were housed separately avoiding contamination through direct contact.

Moreover, a stressful environment and bad hygiene practices favor the occurrence of this disease in susceptible animals; in addition to the fact that a small number of organisms are required to induce the infection contributing to the high prevalence in global herds of swine (Boesen et al. 2004). *L. intracellularis* transmission can occur interspecies, and rabbits are susceptible to equine-derived isolate that can induce typical lesions in the rabbits. However, pig-derived isolated has not been correlated with proliferative enteropathy in rabbits yet, and those challenged with pig isolate did not develop typical intestinal lesions (Sampieri et al., 2013). Nonetheless, no molecular assay was performed to confirm the presence of the agent in the ramie or in the pig population.

Secondly, the diseases could be present in the herd subclinically. Klein, Gebhart and Duhamel (1999) have demonstrated the disease could occur due to its enzootic presence in a colony of rhesus monkey. Rabbits experimentally infected with *L. intracelullaris* has shown to act as a host and amplifier of the bacteria and shed it through feces and transmit to foals. This might add to the fact that a possible positive rabbit was present or introduced

to the herd started to shed the bacteria that leads to the outbreak (Pusterla et al., 2013). The exposition of immune naïve animals to positive ones may lead to clinical signs of the diseases, this mechanism is well described in pigs (Jordan, Knitted, Schwartz, Roof, & Hoffman, 2004). The owner did not elucidate any other episode of diarrhea or mortality that could indicate the presence of the agent in the herd before.

Finally, we hypothesize carriers as a feasible route of infection. A research conducted by Hwang et al. (2017) pointed out an increasing evidence demonstrating the potential of *L. intracelullaris* infection in feral cats and rodents and possible interspecies transmission route. Shedding on feces of five different wild animals was reported. For the maintenance of hygiene measures, it is necessary the control of animals determined as pests, which includes the rodents (Boesen et al., 2004). Rodents are a common plague in any animal production, which includes pig and rabbit herds, and can be involved in the transmission of pathogens, such as *Brachyspira* spp. (Backhans, Jansson, Aspán, & Fellström, 2011), and recently the DNA of *L. intracellularis* has been detected in rat feces (Pusterla et al., 2012). Despite carrying small amounts of the agent, around 10^{10} *L. intracellularis*/g feces, this amount is enough to infect susceptible animals, like pigs and rabbits (Percy & Barthold, 2007).

The diagnosis of *L. intracellularis* causing proliferative enteropathy in rabbits was performed through the observation of clinical, macroscopic, and microscopic findings and confirmed by silver impregnation, immunohistochemistry, and PCR. This outbreak presented an acute development of clinical signs culminating in economic losses to the farmer due to mortality and treatment costs. It should be highlighted that adequate sanitary standards, that should include feed source, facility, and pest control, are needed to prevent *L. intracellularis* transmission.

Acknowledgment

The authors thanks CNPq and CAPES for the financial support that made this research possible. DD and RMCG have research fellowship from CNPq.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

Backhans, A., Jansson, D. S., Aspán, A., & Fellström, C. (2011). Typing of *Brachyspira* spp. from rodents, pigs and chickens on Swedish farms. *Veterinary Microbiology*, *153*(1-2), 156-162. doi*:* 10.1016/j.vetmic.2011.03.023

Boesen, H. T., Jensen, T. K., Schmidt, A. S., Jensen, B. B., Jensen, S. M. & Moller, K. (2004). The influence of diet on *Lawsonia intracellularis* colonization in pigs upon experimental challenge. *Veterinary Microbiology*, *103*(1-2), 35-45. doi: 10.1016/j.vetmic.2004.06.008

Guedes, R. M. C. (2002). *Porcine proliferative enteropathy: diagnosis, immune response and pathogenesis*. Doctoral thesis, University of Minnesota, Minneapolis, MN, USA.

Guedes, R. M. C. (2008). Infecção por *Lawsonia intracellularis*: um problema recorrente na suinocultura do Brasil. *Acta Scientiae Veterinariae*, *36*(1), 77-80.

Horiuchi, N., Watarai, M., Kobayashi, Y., Omata, Y., & Furuoka, H. (2008). Proliferative enteropathy involving *Lawsonia intracellularis* infection in rabbits (*Oryctlagus cuniculus*). *The Journal of Veterinary Medical Science*, *70*(4), 389-392. doi: 10.1292/jvms.70.389

Hwang, J. M., Seo, M. J., & Yeh, J. Y. (2017). *Lawsonia intracellularis* in the feces of wild rodents and stray cats capture around equine farms. *BMC Veterinary Research*, *13*, 233. doi*:* 10.1186/s12917-017-1155-8

Jordan, D. M., Knitted, J. P., Schwartz, K. J., Roof, M. B., & Hoffman, L. J. (2004). A *Lawsonia intracellularis* transmission study using a pure culture inoculated seeder-pig sentinel model. *Veterinary Microbiology*, *104*(1-2), 83-90. doi: 10.1016/j.vetmic.2004.09.004

Klein, E. C., Gebhart, C. J., & Duhamel, G. E. (1999). Fatal outbreaks of proliferative enteritis caused by *Lawsonia intracellularis* in young colony-raised rhesus macaques. *Journal of Medical Primatology*, *28*(1), 11-18. doi: 10.1111/j.1600-0684.1999.tb00084.x

Mcorist, S., & Gebhart, C. J. (2012). Proliferative enteropathy. In J. J. Zimmerman, L. A. Karriker, & A. Ramirez (Eds.), *Diseases of swine* (pp. 811-820). Hoboken, NJ: Wiley-Blackwell.

Peixoto, P. V., França, T. N., Ribeiro, C. T., Bezerra, P. S., Jr., & Driemeier, D. (2008). Proliferative enteropathy (*Lawsonia intracellularis*) outbreak in rabbits in Brazil. *Pesquisa Veterinária Brasileira*, *28*(10), 447-451. doi: 10.1590/S0100-736X2008001000001

Percy, D. H., & Barthold, S. W. (2007). Rabbit. In S. Barthold, S. M. Griffey, & D. H. Percy (Eds.), *Pathology of laboratory rodents and rabbits* (pp. 375-376). Oxford, UK: Blackwell Publishing.

Pusterla, N., Mapes, S., & Gebhart, C. (2012). Further investigation of exposure to *Lawsonia intracellularis* in wild and feral animals captured on horse properties with equine proliferative enteropathy. *Veterinary Journal*, *194*(2), 253-255. doi: 10.1016/j.tvjl.2012.04.012

Pusterla, N., Sanchez-Migallon Guzman, D., Vanucci, F. A., Mapes, S., White, A., Difrancesco, M., & Gebhart, C. J. (2013). Transmission of *Lawsonia intracellularis* to weanling foals using feces from experimentally infected rabbits. *Veterinary Journal*, *195*(2), 241-243. doi: 10.1016/j.tvjl.2012.05.028

Sampieri, F., Allen, A. L., Pusterla, N., Vanucci, F. A., Antonopoulos, A. J., Ball, K. R. & Gebhart, C. J. (2013). The rabbit as an infection model for equine proliferative enteropathy. *Canadian Journal of Veterinary Research*, *77*(2), 110-119.

Schoeb, T. R., & Fox, J. G. (1990). Enterocecocolitis associated with intraepithelial Campylobacter-like bacteria in rabbits (*Oryctolagus cuniculus*). *Veterinary Pathology*, *27*(2), 73-80. doi: 10.1177/03009858 9002700201

Vanucci, F. A., & Gebhart, C. J. (2014). Recent advances in understanding the pathogenesis of *Lawsonia intracellularis* infections. *Veterinary Pathology*, *51*(2), 465-477. doi: 10.1177/0300985813520249