SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2021v42n5p2867

Occurrence of *Lawsonia intracellularis* in horses raised in three regions of the state of Paraná, Brazil

Ocorrência de *Lawsonia intracellularis* em equinos criados em três regiões do estado do Paraná, Brasil

Tatiane Caleffo[1]; Vinicius Dahm[2]; Jéssica Gonçalves dos Santos[3]; Arthur Colombari Cheng[4]; Mayane Faccin[5]; Monica Regina de Matos[6]; João Pedro Cavasin[7]; Michelle de Paula Gabardo[8]; Roberto Mauricio Carvalho Guedes[9]; Aline de Marco Viott[10]*

Highlights

First report of detection of *L. intracellularis* in horses in the state of Paraná.
Low serological occurrence (5.55%) of *L. intracellularis* in the state of Paraná.
Higher occurrence in animals older than one year.

Abstract

Lawsonia intracellularis is a bacterium already described in several species and most prevalent in pigs, in which it causes enteric problems. Horses can also be affected, developing a disease known as equine proliferative enteropathy, which results from the proliferation of intestinal crypt cells in response to infection by the bacterium. Despite the existence of reports of the disease in several countries, including

[1] Prof. Msc., Sindicato da Educação do Sudoeste do Paraná, União de Ensino do Sudoeste do Paraná, UNISEP, Dois Vizinhos, PR, Brazil. E-mail: taticaleffo86@gmail.com
[2] Student of the Undergraduate Course in Veterinary Medicine, Universidade Federal do Paraná, UFPR, Palotina, PR, Brazil. E-mail: vinicius_dahm@live.com
[3] PhD Candidate of the Veterinary Microbiology and Preventive Medicine Program, Iowa State University, ISU, Ames, IA, United States of America. E-mail: jessicasantosufpr@gmail.com
[4] Anatomic Pathology Resident, Veterinary Medicine Residency Program, Universidade Federal do Paraná, UFPR, Palotina, PR, Brazil. E-mail: aarthurcheng@gmail.com
[5] Graduate Assistant, Veterinary Pathobiology, Texas A&M University, TAMU, College Station, TX, United States of America. E-mail: mayanefaccin@hotmail.com
[6] Profa Msc., Pontifícia Universidade Católica do Paraná, PUCPR, Toledo, PR, Brazil. E-mail: monicareginadematos@gmail.com
[7] Anatomic Pathology Resident, Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, CU, Ithaca, NY, United States of America. E-mail: joaopedrocavasin@gmail.com
[8] Profa Dra, Department of Agricultural Sciences, Instituto Federal de Minas Gerais, IFMG, Bambuí, MG, Brazil. E-mail: michelle.gabardo@ifmg.edu.br
[9] Prof. Dr., Department of Veterinary Clinic and Surgery, Universidade Federal de Minas Gerais, UFMG, Belo Horizonte, MG, Brazil. E-mail: guedes@vet.ufmg.br
[10] Profa Dra, Department of Veterinary Sciences, UFPR, Palotina, PR, Brazil. E-mail: alinedemarco@yahoo.com.br
* Author for correspondence

Received: Mar. 05, 2021 - Approved: Apr. 06, 2021

Brazil, there are still no reports of the disease or epidemiological studies of its occurrence in symptomatic or asymptomatic horses in the state of Paraná. Thus, the present study was conducted to examine the occurrence of L. intracellularis in asymptomatic horses raised in the west, northwest and north regions of Paraná by means of serological testing and the real-time polymerase chain reaction (qPCR) technique. In the serological approach, the immunoperoxidase monolayer assay (IPMA) technique was employed. Feces were processed and subjected to qPCR. In total, samples were collected from 162 animals from 20 farms. Of these, 9/162 (5.55%) showed specific antibodies against L. intracellularis. Real-time PCR, on the other hand, identified 7/162 (4.32%) fecal samples positive for the presence of the bacterium. When the techniques were compared, none of the samples was positive by both, demonstrating that, for a better diagnosis, they must be performed together. In contrast to most reports in horses, the present study describes higher serological and molecular occurrence in animals older than two years. These results are of great epidemiological relevance, as they indicate that the bacterium is present in the sampled regions of the state of Paraná. Therefore, the disease must be included in the differential diagnosis of diseases with similar clinical manifestations.
Key words: Equine proliferative enteropathy. Immunoperoxidase monolayer. Polymerase chain reaction.

Resumo

Lawsonia intracellularis é uma bactéria já descrita em várias espécies, sendo mais comum em suínos, ocasionando problemas entéricos nesses animais. Dentre estes, equinos podem ser acometidos, levando à uma doença conhecida como Enteropatia Proliferativa Equina que é decorrente da proliferação das células da cripta intestinal em reação à infecção pela bactéria. Apesar de existirem relatos da doença em diversos países, inclusive no Brasil, no estado do Paraná ainda não se tem relatos da doença e estudos epidemiológicos da ocorrência em equinos sintomáticos ou assintomáticos. O objetivo do presente estudo foi avaliar a ocorrência de *L. intracellularis* em equinos assintomáticos criados nas regiões Oeste, Noroeste e Norte do estado do Paraná através de sorologia e qPCR. Para a sorologia, utilizou-se a técnica da Imunoperoxidase em Monocamadas (IPMA). As fezes foram processadas e submetidas à técnica de Reação em Cadeia pela Polimerase em Tempo Real (qPCR). Ao todo, foram coletadas amostras de 162 animais de 20 propriedades. Destas, 9/162 (5,55%) apresentaram anticorpos específicos contra *L. intracellularis.* Já a qPCR, identificou 7/162 (4,32%) amostras de fezes positivas para a presença da bactéria. Ao se comparar as técnicas, nenhuma amostra foi positiva em ambas, demonstrando que, para um melhor diagnóstico, as mesmas devem ser realizadas em conjunto. Em contraste à grande parte dos relatos em equinos, o presente estudo encontrou uma maior ocorrência sorológica e molecular em animais com mais de dois anos de idade. Esses resultados são de grande relevância epidemiológica, pois indicam que a bactéria está presente nas regiões amostradas do estado do Paraná, levando à necessidade de incluir a doença no diagnóstico diferencial de enfermidades que cursam com manifestações clínicas semelhantes.
Palavras-chave: Enteropatia proliferativa equina. Imunoperoxidase em monocamadas. Reação em Cadeia pela Polimerase.

Introduction

Lawsonia intracellularis is a gram-negative, curved bacterium capable of causing clinical disease in pigs, rabbits, hamsters and horses. It is an obligate intracellular agent that affects mainly cells in the proliferative zone of the intestine (crypt cells) (Gelberg, 2017; Resende et al., 2019).

The bacterium causes a disease known as proliferative enteropathy (Resende et al., 2019). Infected crypt cells have their multiplication rate increased and cell differentiation inhibited, which leads to hyperplasia with simultaneous thickening of the intestinal mucosa the ileum, mainly (Zachary, 2017).

In swine, the disease causes macroscopic lesions in the gastrointestinal tract, mesenteric lymph nodes and abdominal cavity (Guedes et al., 2017). Symptoms include anorexia, vomiting and diarrhea with or without the presence of blood, which can develop into a necrotic and hemorrhagic mucosal condition (Guedes et al., 2017; Gelberg, 2017).

Unlike most infections in swine, the proliferative lesion in horses can occur in the duodenum and in segments of the large intestine (Gabardo, Sato, Resende, & Guedes, 2015). Foals at weaning age are the most affected and may develop lethargy, anorexia, weight loss, colic, diarrhea and hyperthermia and death (Gabardo et al., 2015; Bohlin, Olsen, Laursen, Ohman, & Van Galen, 2019). However, adult animals can also occasionally develop a similar condition (Pusterla & Gebhart, 2013).

Among the existing serological techniques, immunoperoxidase monolayer assay (IPMA) has been used in several studies, constituting a reference method for the detection of specific antibodies in horses (Hassenin, Sena, Gebhart, & Goyal, 2017). The disadvantage of serology is encountered in recently infected animals, whose organism has not produced an efficient humoral response (Pusterla & Gebhart, 2013).

Real-time polymerase chain reaction (qPCR) has been used to detect *L. intracellularis* in animal feces with suspected proliferative enteropathy (Bohlin et al., 2019). However, because some factors can reduce the sensitivity of this technique, the serological and molecular methods must be performed together to increase the chances of diagnosing the infection in animals (Pusterla & Gebhart, 2013).

In Brazil, cases of the clinical disease in horses have already been described in the central-west region of the country and in the state of Rio de Janeiro (Guttmann, Viscardi, Lessa, & Guedes, 2014; Gabardo et al., 2015). However, there are still no reports of EPE or studies demonstrating the occurrence of this pathogen in the state of Paraná. Information about the epidemiology of this disease is of paramount importance so it can be included as a differential diagnosis of enteric diseases in horses, since it can involve clinical manifestations similar to those of other pathologies (Gabardo et al., 2015; Bohlin et al., 2019).

In view of the above-described situation, this study proposes was to examine the occurrence of *L. intracellularis* in asymptomatic horses raised in the west, northwest and north regions of the state of Paraná, Brazil, by serology and detection of the pathogen using a qPCR-based method.

Materials and Methods

Ethics committee

The present study was approved by the Ethics Committee on Animal Use (CEUA) of the Federal University of Paraná - Palotina (approval no. 32/2016).

Study site

The study was conducted on 20 farms, in the municipalities of Entre Rios do Oeste, Iporã, Londrina, Marechal Cândido Rondon, Maringá, Palotina, Santa Helena, Toledo, Umuarama and Vera Cruz do Oeste, located in the west, northwest and north regions of the state of Paraná, Brazil (Figure 1).

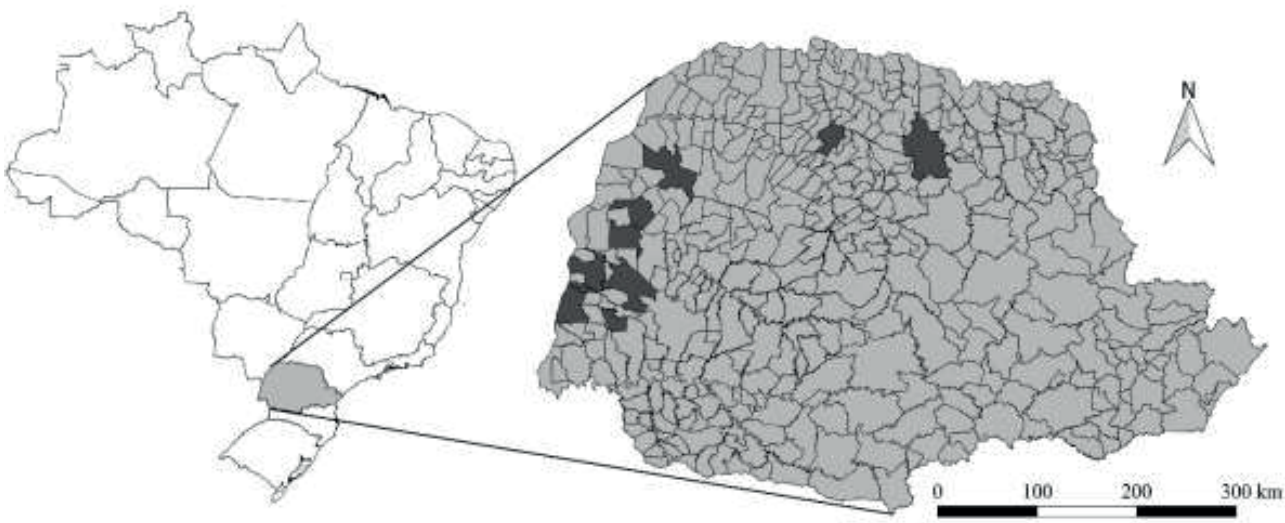

Figure 1. Location of the state of Paraná (light gray) in Brazil and municipalities studied within the state (dark gray). Geoprocessing by QGIS 2.14.20 software.

Sample collection

Samples of feces and blood were collected from healthy horses between November 2016 and May 2017. The animals were used in sports competitions and, therefore, had a history of travel within the state of Paraná. In addition, they were raised on pasture and in individual stalls. The younger animals, which did not leave the property, had contact with the older ones.

Feces were collected directly from the rectal ampulla of the horses and stored in sterile plastic bottles with lids at -20 ºC until analysis.

Approximately 5 mL of blood were collected directly from the jugular vein of the animals, using Vacutainer collection tubes (BD, Franklin Lakes, United States) without anticoagulant. The blood samples were centrifuged to obtain the blood serum, which was frozen at -20 ºC until processing.

Serological testing

The serum samples were sent to the Laboratory of Veterinary Pathology at the Federal University of Minas Gerais. The applied technique was immunoperoxidase monolayer assay (IPMA), which was validated by Guedes, Gebhart, Deen and Winkelman (2002).

Extraction of genetic material and fecal qPCR

Fecal DNA was extracted using the QIAamp DNA Stool Mini commercial kit (Qiagen, Hilden, Germany), following the manufacturer's instructions.

The DNA samples were sent to a private diagnostic laboratory. The genetic material was amplified by the real-time PCR (qPCR) technique. The reaction was performed using SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, United States). The oligonucleotides used were those proposed by Lindecrona, Jensen, Andersen and Moller (2002), and the amplification protocol was as proposed by Stahl, Kokotovic, Hjulsager, Breum and Angen (2011).

Results and Discussion

In total, 162 blood and feces samples were collected from 20 farms, consisting of 23 from animals under one year of age, and 139 from animals between one and five years old (Table 1). None of the animals had a history of diarrhea or other clinical changes at the time of collection.

Of the samples analyzed by the serological technique (IPMA), 9/162 (5.55%) had specific antibodies against *L. intracellularis* (Table 2). These animals were distributed across seven farms, which corresponded to 35% (7/20) of the collected units. This overall number of positive animals is relatively low when compared with values described in other studies, e.g., Steinman et al. (2014), who found an overall seroprevalence of 30.5% in horses raised on 14 farms in Israel. In addition, the farm with the highest serological prevalence in this study showed only 25% of occurrence (farm E), which is still low in relation to most of the data found on each farm by Steinman et al. (2014). The difference is probably related to the different locations where the studies were developed, since although Steinman et al. (2014) used the ELISA technique, unlike the present study, it had previously been adapted and validated for the detection of antibodies in horse (Page, Stills, Chander, Gebhart, & Horohov, 2011), and both (IPMA and ELISA) have good accuracy (Pusterla & Gebhart, 2013). Resende et al. (2015), obtained a seroprevalence of 34.7% in pigs in the state of Minas Gerais, demonstrating that, even in swine, serological prevalence in Brazil is lower than in horses from other countries.

Table 1
Location of farms and number of animals sampled on each, by age group

	FARM	MUNICIPALITY	0-11 MONTHS	1-5 YEARS	TOTAL
	A	Maringá	1	1	2
	B	Palotina	2	26	28
	C	Iporã	1	7	8
	D	Santa Helena	3	2	5
	E	Iporã	0	8	8
	F	Iporã	1	4	5
	G	Umuarama	2	0	2
	H	Palotina	3	0	3
	I	Marechal Cândido Rondon	1	11	12
	J	Iporã	1	4	5
	K	Vera Cruz do Oeste	2	7	9
	L	Iporã	4	10	14
	M	Iporã	0	1	1
	N	Iporã	0	6	6
	O	Marechal Cândido Rondon	1	2	3
	P	Iporã	1	27	28
	Q	Iporã	0	5	5
	R	Toledo	0	1	1
	S	Londrina	0	7	7
	T	Entre Rios do Oeste	0	10	10
TOTAL	20		23	139	162

Table 2
Farms with samples positive in serological testing and by real-time PCR, with the respective animal ages

FARM	AGE	IPMA	FARM	AGE	qPCR
B	3 YEARS	+	B	2.5 YEARS	+
E	5 YEARS	+	B	4 YEARS	+
E	2 YEARS	+	K	2.3 YEARS	+
I	2.2 YEARS	+	N	2.6 YEARS	+
K	6 MONTHS	+	P	2.6 YEARS	+
N	5 YEARS	+	P	2 YEARS	+
P	2.3 YEARS	+	P	2 YEARS	+
T	3.6 YEARS	+			
T	2.6 YEARS	+			

In terms of fecal samples, 7/162 (4.32%) were positive by qPCR, from 4/20 (20%) of the evaluated farms (Table 2). Zmudzki et al. (2012) investigated the efficiency of qPCR, using the same primer as the present study, and found good sensitivity and specificity values in the detection of *L. intracellularis* in swine feces, demonstrating the possibility of using the technique in the diagnosis of proliferative enteropathy. These animals, which were positive in molecular biology, originated from four out of the seven (4/7) farms with positive serology, indicating the circulation of the agent on them.

No sample was positive by both techniques (serological testing and qPCR). This divergence is probably correlated to the different stages of infection in which the animals were. These data suggest that horses that tested positive for anti-*L.intracellularis* IgG did not eliminate the bacteria in the feces, or did, but in a lesser amount than the technique is capable of detecting, possibly because they are at an advanced stage of infection (Pusterla & Gebhart, 2013). Moreover, animals with positive fecal qPCR may be in the early stage of infection and have been sampled before seroconverting (Pusterla & Gebhart, 2013).

Gabardo et al. (2015) described similar results, in which none of the three animals that were positive in serological testing showed positivity by the PCR technique. Vannucci, Pusterla, Mapes and Gebhart (2012) observed that foals inoculated with horse isolates eliminate *L. intracellularis* 12 to 38 days after infection and that serological detection occurrs only from 14 days after contact with the bacterium. On this basis, animals that eliminate the agent in their feces do not necessarily have positive serology. The results found by both authors, as well as those of the present study, demonstrate that for the best detection of positive animals in the herd, the serological and molecular techniques should be performed together so the advantages of both diagnostic methods will be combined, allowing the identification of positive animals at all stages of infection (Pusterla & Gebhart, 2013).

Among the positive animals (16), only 1/16 (6.25%) were less than one-year old at the time of collection, which was detected by serological testing (Table 2). Most literature reports of equine proliferative enteropathy (EPE) are of growing foals aged up to 12 months (Bohlin et al., 2019; Pusterla & Gebhart, 2013). However, of the 23 animals under one year of age, only one (4.35%) was positive (Table 2). This discrepancy with the literature may be due to a lower pressure of environmental infection or the shorter time exposure of the foals to the environment and, consequently, to the bacterium.

When we compare the farms with positive serology with the samples positive by qPCR, 3/7 (farms E, I and T) showed serologically positive animals, but there were no positive animals by the molecular technique. This may be due to the animals having a history of travel for equestrian sports, and it is possible that they were contaminated in other locations, since there is seemingly no active circulation of the bacteria on the farm.

To date, there have been no reported cases of EPE in the state of Paraná. However, the detection of positive animals by both qPCR and serological testing demonstrates that the bacterium is circulating on the farms of the west and northwest regions of the state, even if not apparently, without the development of the clinical disease itself. These data are of great relevance, because as EPE has clinical

signs similar to those of other diseases, it must be considered as a differential diagnosis for these diseases (Gabardo et al., 2015; Bohlin et al., 2019), especially in areas with known circulation of the agent. Additionally, it should be emphasized that the state of Paraná has a great contribution to Brazilian pig farming, accounting for around 19.85% of pigs slaughtered in the country in 2019. The western region of the state had the greatest share in this percentage, having achieved great leaps in productivity due to investments in the sector (Associação Brasileira de Proteína Animal [ABPA], 2020; Associação Brasileira dos Criadores de Suínos [ABCS], 2016). For these reasons, the knowledge of probable sources of disease infection for pigs makes this epidemiological study even more relevant.

Conclusions

Lawsonia intracellularis affects horses in the west and northwest regions of the state of Paraná. Thus, EPE should be included in the differential diagnosis of diseases that cause diarrhea in horses. Proliferative enteropathy is also a condition that affects pigs, and the establishment of new and probable sources of infection or transmission makes this study even more important. Therefore, serological and molecular surveillance measures must be implemented on the farms so that the occurrence can be identified and EPE diagnosed early.

Acknowledgments

To Prof. Dr. Roberto Mauricio Carvalho Guedes for his assistance in carrying out the serological tests.

References

Associação Brasileira de Proteína Animal (2020). *Relatório anual 2020.* São Paulo, SP: ABPA.

Associação Brasileira dos Criadores de Suínos (2016). *Mapeamento da suinocultura brasileira.* Brasília, DF: ABCS.

Bohlin, A. M., Olsen, S. N., Laursen, S. H., Ohman, A., & Van Galen, G. (2019). Lawsonia intracellularis associated equine proliferative enteropathy in Danish weanling foals. *Acta Veterinaria Scandinavica, 61*(12), 1-8. doi: 10.1186/s13028-019-0447-3

Gabardo, M. P., Sato, J. P. H., Resende, T. P., & Guedes, R. M. C. (2015). Equine proliferative enteropathy on a Brazilian farm. *Pesquisa Veterinária Brasileira, 35*(5), 443-447. doi: 10.1590/S0100-736X2015000500009

Gelberg, H. B. (2017). Alimentary system and the peritoneum, omentum, mesentery, and peritoneal cavity. In J. F. Zachary (Ed.), *Pathologic basis of veterinary disease* (pp. 324-411). San Luis, MO: Elsevier.

Guedes, R. M. C., Gebhart, C. J., Deen, J., & Winkelman, N. L. (2002). Validation of an immunoperoxidase monolayer assay as a serologic test for porcine proliferative enteropathy. *Journal of Veterinary Diagnostic Investigation, 14*(6), 528-530. doi: 10.1177/104063870201400618

Guedes, R. M. C., Machuca, M. A., Quiroga, M. A., Pereira, C. E. R., Resende, T. P., & Gebhart, C. J. (2017). *Lawsonia intracellularis* in pigs: progression of lesions and involvement of apoptosis. *Veterinary Pathology, 54*(4), 620-628. doi: 10.1177/0300985817698206

Guttmann, P. de M., Viscardi, V., Lessa, D. A. B., & Guedes, R. M. C. (2014). Equine proliferative enteropathy caused by *Lawsonia intracellularis* in a foal in Brazil. *Journal of Equine Veterinary Science, 34*(5), 701-703. doi: 10.1016/j.jevs.2013.11.010

Hassenin, A. S. H., Sena, S., Gebhart, C. J., & Goyal, S. M. (2017). Comparison of ELISA and IPMA tests for the detection of Anti-*Lawsonia* antibodies in horses. *International Journal of Advancements in Research & Technology, 6*(3), 17-24.

Lindecrona, R. H., Jensen, T. K., Andersen, P. H., & Moller, K. (2002). Application of a 5′ nuclease assay for detection of *Lawsonia intracellularis* in fecal samples from pigs. *Journal of Clinical Microbiology, 40*(3), 984-987. doi: 10.1128/JCM.40.3.984-987.2002

Page, A. E., Stills, H. F., Chander, Y., Gebhart, C. J., & Horohov, D.W. (2011). Adaptation and validation of a bacteria-specific enzyme-linked immunosorbent assay for determination of farm-specific *Lawsonia intracellularis* seroprevalence in central Kentucky Thoroughbreds. *Equine Veterinary Journal, 43*(40), 25-31. doi: 10.1111/j.2042-3306.2011.00487.x

Pusterla, N., & Gebhart, C. (2013). *Lawsonia intracellularis* infection and proliferative enteropathy in foals. *Veterinary Microbiology, 167*(1-2), 34-41. doi: 10.1016/j.vetmic.2013.06.017

Resende, T. P., Medida, R. L., Guo, Y., Vannucci, F. A., Saqui-Salces, M., & Gebhart, C. (2019). Evaluation of mouse enteroids as a model for *Lawsonia intracellularis* infection. *Veterinary Research, 50*(57), 1-11. doi: 10.1186/s13567-019-0672-9

Resende, T. P., Pereira, C. E. R., Gabardo, M. de P., Haddad, J. P. A., Lobato, Z. I. P., & Guedes, R. M. C. (2015). Serological profile, seroprevalence and risk factors related to *Lawsonia intracellularis* infection in swine herds from Minas Gerais State, *Brazil. BMC Veterinary Research, 11*(306), 1-6. doi: 10.1186/s12917-015-0618-z

Stahl, M., Kokotovic, B., Hjulsager, C. K., Breum, S. O., & Angen, O. (2011). The use of quantitative PCR for identification and quantification of Brachyspira pilosicoli, *Lawsonia intracellularis* and *Escherichia coli* fimbrial types F4 and F18 in pig feces. *Veterinary Microbiology, 151*(3-4), 307-314. doi: 10.1016/j.vetmic.2011.03.013

Steinman, A., Aharonson-Raz, K., Blum, S. E., Shnaiderman, A., Klement, E., Lensky, I. M.,... Page, A. E. (2014). Demographic and environmental risk factors for exposure to *Lawsonia intracellularis* in horses in Israel. *Journal of Equine Veterinary Science, 34*(5), 641-646. doi: 10.1016/j.jevs.2013.12.003

Vannucci, F. A., Pusterla, N., Mapes, S. M., & Gebhart, C. (2012). Evidence of host adaptation in *Lawsonia intracellularis* infections. *Veterinary Research, 43*(53), 1-9. doi: 10.1186/1297-9716-43-53

Zachary, J. F. (2017). *Pathologic basis of veterinary disease*. San Luis, MO: Elsevier.

Zmudzki, J., Szczotka, A., Podgórska, K., Nowak, A., Grzesiak, A., Dors, A., & Pejsak, Z. (2012). Application of real-time PCR for detection of *Lawsonia intracellularis* and *Brachyspira hyodysenteriae* in fecal samples from pigs. *Polish Journal of Veterinary Sciences, 15*(2), 267-273. doi: 10.2478/v10181-011-0144-2