Enterisol® Ileitis, what we have learned in twenty years controlling *Lawsonia intracellularis*

Fernando Leite, DVM, MS, PhD[1]; Greg Cline, DVM[1]; John Waddell, DVM[1], MBA; Jeremy Kroll, PhD[1]; Jeff Husa, DVM[1]; Edgar Diaz, DVM[1]; Oliver Gomez-Duran, DVM, PhD[1]
[1]Boehringer Ingelheim Animal Health USA Inc.

Introduction

The goal of every swine veterinarian is to solve health challenges with effective tools to promote wellbeing and production performance. Porcine proliferative enteropathy (PPE) or "ileitis", is one such health challenge. Thankfully, the swine industry has had an effective tool to help in controlling this disease for twenty years.

PPE was first described by Biester and Schwarte in the 1930s who reported on field cases and experimental reproduction of disease (Biester and Schwarte 1930). They described epithelial proliferative lesions in the intestine of swine that were reproducible by feeding intestinal contents and mucosal scrapings to susceptible pigs. The etiology could not be determined at the time and they commented that animals in field cases were infected with *Salmonella*, which they thought could potentially contribute to the disease. In the 1970s the research group of Dr. Gordon Lawson began investigating this disease making crucial findings (Gebhart and Guedes 2010). Gebhart et al. (1993) found that the bacterium responsible for proliferative enteropathy was a new species that was named in 1995, *Lawsonia intracellularis*. This was after much effort to achieve pure cultures of the bacterium and to fulfill Koch's postulates (Lawson and Gebhart 2000; McOrist et al. 1995; Gebhart et al. 1993). Six years later, in 2001, the first vaccine against the disease, Enterisol® Ileitis, was launched in the United States.

Enterisol® Ileitis use in controlling PPE

Enterisol® Ileitis is an oral live attenuated vaccine, containing a non-virulent strain of *L. intracellularis* to mimic natural infection and induce mucosal immunity with one dose. The efficacy of Enterisol® Ileitis against PPE was first demonstrated by Kroll et al. at the 31st AASV Annual Meeting in 2001. The study showed that providing vaccine in the drinking water to pigs conferred immunity and significantly reduced the colonization of *L. intracellularis* as well as gross and microscopic lesions (Kroll et al., 2001). These findings were reproduced and peer-reviewed in 2004, when Kroll et al. (2004a) proved the vaccine could be provided both by oral drench and in the drinking water of pigs to confer significant protection against PPE.

Field studies followed; at the 33rd AASV Annual Meeting, Kolb J. & Sick F. (2003) presented a summary of 5 field trials evaluating the efficacy of Enterisol® Ileitis in the United States. This evaluation included a total of 55 grow/finish groups, with over 46,900 vaccinated pigs. Kolb found that average daily gain (ADG) was significantly improved in all 5 trials ($p<0.05$), and mortality and cull rates were also improved in the herds that were experiencing clinical signs due to PPE. McOrist & Smits (2007) conducted a randomized, blinded, negative-controlled clinical field efficacy study to evaluate the effect of Enterisol® Ileitis in three herds in Australia. The authors found that vaccination improved ADG and concluded it was efficacious in conditions of both clinical and subclinical presentations of PPE. Since then, several studies have evaluated Enterisol® Ileitis under different field conditions and in different countries including Mexico, Germany, Philippines, Japan, Netherlands, Finland, Denmark and South Korea. All of these studies demonstrated improved performance metrics with the use of Enterisol® Ileitis (Diaz & Chevez, 2006; Viekilde et al., 2006; Bulay et al., 2006; Yamaguchi et al., 2006; Voets & Hardge 2006; Klien et al., 2010; Park et al., 2013; Schuttert & Seenaert, 2014; Peiponen et al., 2018; Ochoa et al., 2020).

It is important to consider as well that Enterisol® Ileitis has not only been shown to be an important tool in grow/finish herds but also in protecting gilts and sows against PPE and the hemorrhagic form of PPE, porcine hemorrhagic enteropathy (PHE). Soon after the launch of the vaccine, Waddell et al. 2003 described a vaccination protocol in a newly constructed 1250-sow breeding farm (gilt multiplier) and its downstream gilt offspring wean-finish site, in which immunization against *L. intracellularis* rather than routine feed or water antibiotic medication was used to prevent and control PPE. They observed no outbreaks of any form of PPE at either site in spite of the complete absence of any feed or water antibiotic use when immunizing animals. They also noted that non-

vaccinated gilts from this same original gilt source that were shipped to at least three different non-vaccinated herds during this same stocking timeframe did experience PHE outbreaks. Candor et al., 2008 found similar results in which administering one dose of Enterisol® Ileitis to gilts prior to their selection and shipment led to the cessation of deaths and clinical signs from PHE. Sanford (2006) performed an evaluation of clinical cases of PPE in gilts after vaccination and found field observations that would suggest the duration of immunity for Enterisol® Ileitis vaccinated gilts is at least 2 years and probably greater than 3½ years. A reduction in cases of PHE has also been observed in grow-finish pigs when implementing vaccination in piglets (Seo et al., 2016).

These studies and results demonstrate that one dose of Enterisol® Ileitis, given by oral drench prior to weaning or through the water in later ages is effective in promoting production performance and controlling disease under various field conditions. Maternal immunity interference has not been found to be an issue with this vaccine when used properly (Kroll et al., 2004b).

Vaccination as a tool to reduce antimicrobial use

Several of the studies that implemented Enterisol® Ileitis vaccination noted a beneficial reduction in the use of antimicrobials on the farm. Voets & Hardge (2007) evaluated this specific effect on three different systems. They found it was possible to remove a substantial amount of antimicrobials with the use of vaccination and maintain, if not in some cases improve performance. Nerem (2009) investigated performance differences between continuous tylosin in feed during finishing versus a protocol of receiving one dose of Enterisol® Ileitis prior to entering the finisher. This study also concluded that it is possible to change from routine use of antimicrobials in the finisher to prevention of PE by vaccination without sacrificing performance. It was also noted that vaccination was the most economical strategy.

As it pertains to feed additives, it is interesting to consider that some feed additives have been found to promote or act in synergy with Enterisol® Ileitis. Bourgot et al. (2017) investigated feeding sows a short-chain fructo-oligosaccharide (scFOS) prebiotic during the last third of gestation and throughout lactation. They found that maternal supplementation of scFOS led to an increase of *L. intracellularis* specific IgA levels in piglets that received Enterisol® Ileitis. Muller et. al, (2018) investigated the supplementation of pigs with a zinc amino acid complex while receiving Enterisol® Ileitis and subsequent *L. intracellularis* challenge. They found that in comparison to vaccination alone, the combined use of Enterisol® Ileitis and zinc amino acid complex led to a greater reduction in mortality than vaccination alone. The effects of zinc on the immune system have been known for some time and have also been found to help the pig respond to *L. intracellularis* challenge (Leite et al., 2018).

While a medication free window of three days prior, and three days following vaccination are needed when implementing Enterisol® Ileitis vaccination, not all antimicrobials affect the vaccine. Husa et al. (2010) showed that administration of ceftiofur or tulathromycin did not alter vaccine efficacy. High levels of zinc oxide as well as acidic conditions have also been previously found to not affect Enterisol® Ileitis (McOrist & Smits, 2007).

The cost of PPE and return on investment of vaccination

Reduction in the use of antimicrobials is also a factor that contributes to the return on investment of Enterisol® Ileitis. Voets & Hardge (2007) reported a 53% reduction of antibiotic usage and an $8.60 higher economic benefit per pig in one system that began the use of Enterisol® Ileitis. The cost of PPE has recently been updated by Dr. Holtkamp to range from $5.98 to $17.34 per marketed pig (Holtkamp, 2019). According to Holtkamp, the major source of economic losses associated with ileitis arises from productivity losses caused by the disease. The disease may also result in an increase in the percentage of culled pigs, and in some cases it may cause mortality. Kolb J. & Sick F. (2003) found a benefit cost ratio greater than 7:1 in a summary of 5 field trials as compared to maintaining a conventional continuous feed medication program for ileitis. More recent trials have found increased revenue of $4.80, $4.10 and $5.93 per marketed pig based on production performance with the implementation of Enterisol® Ileitis vaccination (Ochoa et al., 2020; Peiponen et al., 2018; Park et al., 2013).

Immunity to *L. intracellularis* infection and vaccination

Recently it has been shown that *L. intracellularis* can replicate inside of macrophages (Pereira et al., 2020). Macrophages are a significant antigen presenting cell and can be recruited to many sites of the body, including the intestine (Tizard, 2020). It has also been recently shown that when given time to replicate, within three days, *L. intracellularis* is capable of inducing the expression of inflammatory cytokines such as TNF and IL-8 by activation of the innate immune system (Leite et al., 2019). This combination of replication not only in enterocytes but also in macrophages, and the ability to induce inflammatory cytokines is likely significant for the induction of a robust

immune response. This is especially true for the induction of a cellular mediated immune response and activation of CD8 cytotoxic T cells. The latter recognize intracellular antigens and have been suggested to be the main drivers of protection against PPE (Cordes et al., 2012; Tizard, 2020).

Studies have also measured the humoral immune response to *L. intracellularis* and vaccination. While Enterisol® Ileitis does not generally lead to seroconversion in animals, a commercial killed bacterin vaccine does (Kroll et al., 2004a; Roerink et al., 2018). Serum antibody levels are not correlated with protection to PPE (Kroll et al., 2004a; Roerink et al., 2018). Interestingly, it has been found that oral vaccination with Enterisol® Ileitis not only leads to the generation of *L. intracellularis* specific IgA in the ileum of animals, but also *L. intracellularis* specific IgG can be found in intestinal mucosal tissue. Another interesting observation is that not only oral administration but also intramuscular administration of Enterisol® Ileitis can lead to *L. intracellularis* specific IgG levels in the gut (Nogueira et al., 2015).

The Future

As the field of veterinary medicine progresses, so does our understanding of what leads to health, disease and production performance. The gut microbiome, the community of all microorganisms found in the intestine, has been a recent research focus since a strong association has been found among gut microbiome composition and overall health and production performance (Kim & Isaacson, 2012; Neiderwerder, 2017). We have found that *L. intracellularis* not only can lead to lesions, diarrhea and decreased performance but also to a significant change of the gut microbiome community (Bocherwitz et al., 2015; Leite et al., 2020a). Interestingly we have also found that Enterisol® Ileitis in addition to the afore mentioned effects in reducing lesions, clinical signs, improving performance and inducing an immune response, also leads to changes in the composition of the gut microbiome. One of the immediate consequences found of this effect in gut microbiome composition was reduced *Salmonella* shedding in co-infected animals (Leite et al., 2018; Visscher et al., 2018). This is significant as it could have consequences for the transmission of *Salmonella* as well as the promotion of food safety. A change in microbiome composition induced by Enterisol® Ileitis has also been associated with a significant reduction in *L. intracellularis* shedding in vaccinated animals (Leite et al., 2020b). These results suggest that Enterisol® Ileitis prevents not only lesions and traditional measures of disease, but also prevents negative impacts that *L. intracellularis* infection can have on the gut microbiome, which are likely to cause increased susceptibility to other pathogens and decreased intestinal function. The other impacts this could have, as we better understand the relationship of gut health and the gut microbiome on overall health remain to be seen.

While the afore mentioned studies have set the standard for vaccination protocols for PPE, there is still room for more investigation and questions to be answered. Interestingly, successful but temporary eradication of PPE has been achieved when focusing on achieving a negative sow and gilt population (Pia, 2000). Recently, Patton et al., 2021 have described that peri-parturient sows do shed *L. intracellularis* and in fact, the pathogen can be detected in the farrowing crate environment. This raises many questions as to the significance of dams as a source for *L. intracellularis* infection in later stages of production and consequently if there is any value in changing current vaccination protocols. It must also be considered that one of the hypotheses for the occurrence of PHE versus the chronic and non-hemorrhagic form of PPE, is exposure of older naïve animals to *L. intracellularis*. Thus eradication attempts may also benefit from the immunization of animals to prevent this costly form of the disease (Collins, 2013).

As it pertains to vaccination protocols, recent research has also found that Enterisol® Ileitis can confer immunity when administered intramuscularly along with 3FLEX® vaccine (Beckler, 2020). Although this vaccine combination and route of administration remain off label, and cannot be recommended, it may also provide direction for future development efforts.

Conclusion

Few swine vaccines have had the long track record, efficacy, and supporting data that Enterisol® Ileitis has. The future is bright as we better understand all of the benefits of Enterisol® Ileitis and refine ways to use this significant tool to promote swine health and production.