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The discovery of *Lawsonia intracellularis*

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Introduction

The cause of porcine proliferative enteropathy (PE, ileitis) is the obligate intracellular bacterium *Lawsonia intracellularis*, which preferentially grows within the cytoplasm of intestinal epithelial cells. This bacterial growth is invariably accompanied by localised proliferation of infected immature crypt epithelial cells. This bacterium has not as yet been cultivated in cell-free media, probably because it has a metabolic requirement for pre-formed mitochondrial triphosphates or a similar energy source located within the host cell. This “energy parasite” reaction is similar to that for other obligate intracellular bacteria such as Chlamydia and Rickettsia species, but *Lawsonia* is not related to these other bacteria.

History of ileitis research

Harry Biester and others in the 1930’s first described the lesions of PE in pigs at the Veterinary Medical Research Institute in Ames, Iowa. These workers provided a pathologic description of the lesions and suggested that they may have been associated with the high-corn, low-vitamin diets common at that time. Subsequent workers throughout the 1950 have provided a series of case descriptions in Scandinavia and elsewhere, also focusing on pathologic discussions. In the late 1960’s, intensification procedures started to get into swing in the pig industry and in-feed antibiotics, such as tylosin, also started being widely registered and used. It is likely that these changes led to both an upsurge in *Lawsonia* transmission and in susceptible pig populations. This in turn led to further clinical and pathologic descriptions, with the workers at the University of Edinburgh: Alan Rowland, Giles Rowntree and Gordon Lawson investigating major outbreaks occurring in the United Kingdom. As part of this, they developed a productive research program and examined the proliferative intestinal changes in pigs via electron microscopy and special stain techniques.

They discovered that when lesions were examined ultrastructurally or using silver stains, small, curved intracellular bacteria were consistently present within the abnormal proliferating cells (see Rowland and Lawson 1974). These bacteria are curved to straight vibrioid Gram-negative rods with either tapered or rounded ends and measure 1.25 – 1.75 μm in length by 0.25 – 0.43 μm in width. The bacteria typically lie free in the apical cytoplasm of infected epithelial cells and are not membrane-bound during the important stages of infection. An early transmission experiment using homogenised PE-affected mucosa from a natural case, as an oral challenge inoculum was successful (see Roberts et al 1977), suggesting these bacteria were the causative agent of the lesions. However, a number of attempts to repeat this work, even with the same inoculum by the same workers, failed. This problem of failed inocula, later recurring with several other PE studies in the USA, Australia and elsewhere, was concluded to be due to loss of infective titre during older ultra-cold storage facilities. Some of this earlier literature refers to the intracellular bacterium as a Campylobacter-like organism; however, that designation was only based on its morphologic similarity to that genus. Over many years of work, a variety of *Campylobacter* species, particularly *C. mucosalis*, *C. hyointestinalis*, *C. jejuni*, “*C. hyoilei*” (later corrected to being a *C. jejuni* variant) and *C. coli*, were all recovered from the proliferative lesions by various groups chasing this apparent causative agent in pure culture. However, the specific proliferative lesions or intracellular colonisation have never occurred when pigs are inoculated with any of these bacteria. A wide variety of pigs (old ones, young ones, germ-free, SPF etc) and a wide variety of challenge techniques with these bacteria produced no meaningful results for PE. This string of failed experiments expended most of the 1970’s and 1980’s and led to wilder notions of viruses, *Chlamydia* spp or dual infective agents being involved. In fact, these *Campylobacter* species are second-
Secondary agents taking advantage of the altered conditions in the gut for colonisation, which causes their apparent increase in numbers in PE lesions. Similar colonisation by other secondary bacteria is a feature of other enteric diseases in pigs, such as swine dysentery.

The discovery of the intracellular agent in PE in pigs had led researchers to note the presence of similar agents in the similar lesions of proliferative ileitis seen in affected laboratory hamsters in the USA (see Frisk and Wagner 1977). This led to successful challenge studies affecting hamsters with intracellular bacteria derived from PE-affected lesions from pigs or hamsters and the suggestion that they were therefore the same causative agent in both hosts. Unfortunately, culture and other studies in the 1970’s and 1980’s in hamsters aimed at identifying the correct agent (Lawsonia) were as unrewarding as those in pigs.

**Discovery of Lawsonia intracellularis**

Antigenic studies and the failed challenge studies at the end of the 1980’s both indicated that the intracellular bacterium agent in PE was not a free-living Campylobacter. Its clear intracellular life led to the likelihood that the agent was one of the obligate intracellular bacteria – its life cycle seemed to resemble some Rickettsia species. This led to attempts at co-culture of intracellular bacteria from inocula derived from PE lesions onto cell culture lines. The first report of co-culture of Lawsonia in a cell culture line in fact goes back to 1978, by the group of Robert Jacoby investigating PE in hamsters. Following the failed Campylobacter work in the 1980’s, this initial cell culture work was revisited and taken up in the early 1990’s by Hal Stills and Connie Gebhart in the USA and Gordon Lawson and Steven McOrist in Scotland. Stills published the first account of culture of Lawsonia bacteria from cases of PE in hamsters in 1991, but unfortunately, an unrelated non-pathogenic chlamydial agent contaminated his laboratory co-cultures. This led to an inability to retain the correct Lawsonia agent for animal inoculations and taxonomic work. The identity of these bacteria and their etiologic role in PE in pigs were finally resolved in 1993 with successful co-culture of the intracellular organism and the reproduction of the disease in pigs using a pure culture of this agent (see Lawson et al. 1993 and McOrist et al. 1993). Also in 1993, its taxonomic position was clarified (see Gebhart et al. 1993); its definitive name is Lawsonia intracellularis, within the family Desulfovibrionaceae (see McOrist et al. 1995). The name Lawsonia was chosen to reflect the key role and persistence of the Scottish veterinarian, Gordon Lawson in its discovery. Development of successful co-culture of Lawsonia required attention to the source of PE material, extraction techniques, cell culture atmosphere and maintenance techniques, passage techniques, reduction of various contaminants and many other details. Development of successful challenge exposure studies then required attention to the source of pigs, the bacterial flora of those pigs etc. Challenge studies with PE also need to be carefully monitored for spontaneous cases among negative controls, due to the endemic nature of the agent among herds that might be used to source study pigs.
Features of *Lawsonia intracellularis*

*L. intracellularis* form curved to straight vibrioid-shaped rods with either tapered or rounded ends and measure 1.25 – 1.75 µm in length by 0.25 – 0.43 µm in width. It has a typical Gram-negative trilaminar outer envelope. No fimbriae or spores have been detected. A long, single, polar flagellum and darting motility has been observed in some isolates co-cultured on cell lines, but only when the bacteria are located extracellularly. It has a small, single circular genome and 3 mega-plasmids, totalling only 1.72 million DNA base pairs and 1,324 open reading frames (functional genes). It possesses the small genome size, cell-dependent respiration ("energy parasite"), low G+C ratio % in its DNA, and also the significant expression of the gro EL heat shock proteins, which are all features commonly seen in other obligate and symbiont intracellular bacteria. Genome analysis has also pinpointed several flagellar assembly genes, folate biosynthesis genes and other standard bacterial biochemical genes. Large mega-plasmids have been reported in other intracellular bacteria, such as *Brucella* species. Despite being placed in the Desulfovibrionaceae family, as based on its DNA sequence analysis, it does not appear to have sulfate reduction capacity. Importantly, unlike Desulfovibrio species, it appears to have acquired an active mechanism for cell entry and a mechanism for affecting the target host cells in vivo. Isolates of *L. intracellularis* from pigs and those from a variety of origins and other host species (hamster, horse, deer) show a very high degree (>98%) of similarity among DNA sequences in key taxonomic sites and in their outer membrane proteins, indicating a single homogeneous "strain" of *L. intracellularis* may occur. These features indicate that the considerable genetic shifts from the Desulfovibrio or other ancestor bacteria occurred recently. This is because the development of distinct "strains" would take some time to develop and be detected.

**Explosion of PE research**

Once the causative agent of PE became clear in the mid-1990’s, the groups mentioned and many new groups in Europe and North America were then able to start working quickly on aspects of the disease that really mattered to their pig industries. So the susceptibility of *Lawsonia* to various antibiotics, its epidemiology within and between pig farms and methods of diagnosis in live pigs and many related topics were all taken up for research (see Knittel et al. 1998). In 1994, work commenced on a vaccine for ileitis that led to the first registered vaccine (Enterisol® Ileitis, Boehringer Ingelheim) in 2001. This successful vaccine is now widely registered and used around the world (see Guedes and Gebhart 2003 and Kroll et al. 2004). This rapid ileitis vaccine development is in contrast to the slow pace of development for vaccines for other key enteric diseases of pigs, such as swine dysentery and colibacillosis.
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