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YUNIBESITHI YA PRETORIA

HAEMORRHAGIC BOWEL SYNDROME IN GROWER PIGS

by

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Presented in partial fulfillment of the requirements of

the degree
MMedVet (Suill)

IN THE DEPARTMENT OF PRODUCTION ANIMAL STUDIES
FACULTY OF VETERINARY SCIENCE
UNIVERSITY OF PRETORIA
PRETORIA
2009

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Abbreviations

ADG	Average daily gain
CDAC	*Clostridium difficile* associated disease
FCR	Feed conversion ratio
HBS	Haemorrhagic bowel syndrome
H&E	Haematoxylin and eosin
IFA	Immunofluorescence assay
MMC	Migrating myo-electric complex
NE	Necrotic enteritis
PCR	Polymerase chain reaction
PHE	Porcine haemorrhagic enteropathy
PIA	Proliferative intestinal adenomatosis
PRRS	Porcine reproductive and respiratory syndrome
RI	Regional ileitis
SPF	Specific pathogen free
TGE	Transmissible gastro-enteritis

Summary

In the past five years generally well managed farms reported an increase in acute deaths in their grower herds to their consulting veterinarian. At the same time reports from across the world indicated that this is not a problem seen only in South Africa. The syndrome is generally referred to as haemorrhagic bowel syndrome (HBS), red gut or balloon pig. Veterinarians generally believed that the cause of these acute deaths were due to the acute form of *Lawsonia intracellularis*, also known as porcine haemorrhagic enteropathy (PHE). Because neither the clinical symptoms present prior to death, nor the post mortem changes were typical for a *L. intracellularis* case it was decided to investigate this syndrome in more depth. Five commercial farms were purposefully selected where growers that died peracutely were necropsied and intestinal samples collected for histological as well as bacteriological examination. A total of 28 pigs were sampled with the histological sections from all samples indicating a *Clostridium* species as the cause and from 11 of samples *Clostridium perfringens* were cultured as the predominant bacterium. Although pigs on the farms were seropositive for *Lawsonia intracellularis*, there was no evidence that this bacterium was the cause of death in the pigs. Rather the aetiology points to *C. perfringens* being the cause, possibly together with other predisposing factors such as rapid growth, high ambient temperatures and interruption in fedding patterns. Based on these results further studies to determine the toxin type as well as predisposing factors should be done.

Opsomming

Gedurende die afgelope vyf jaar het plase met ’n algemene goeie bestuur ’n verhoging in akute vrektes in hulle groeikuddes opgemerk en hulle het hulle kommer oor die vrektes aan hulle konsulterende veeartse oorgedra. Diè verhoging in groeivrektes is nie uniek aan Suid Afrika nie. Dieselfde tendens is regoor die wêreld opgemerk, maar niemand is seker wat presies die oorsaak van die akute vrektes is nie. In die literatuur word daar na “haemorrhagic bowel syndrome (HBS)” oftewel hemoragiese derm sindroom verwys. Boere verwys na die sindroom as rooiderm of “balloon pig”. Tot nou toe het veeartse aanvaar dat die oorsaak moontlik *Lawsonia intracellularis* is. Die organisme is verantwoordelik vir ’n groep sindrome waarvan “porcine haemorrhagic enteropathy” die akute form is. Omrede die kliniese simptome en die nadoodse ondersoek nie tipies vir ’n *L. Intracellularis* geval is nie, is daar besluit om die akute vrektes verder te ondersoek. Vyf plase, waar die sindroom baie voorkom, is geidentifiseer en dermonsters is geneem vir histopatalogiese sowel as mikrobiologiese ondersoeke. In totaal is monsters van 28 varke geneem. Die histologies seksies van al die monsters het gedui op ’n Clostridium spesie as die hoofoorsaak van vrekte en Clostridium perfringens is uit 11 van die monsters geisoleer. Alhoewel al 5 plase serologies positief getoets is vir *Lawsonia intracellularis*, was daar geen bewyse gewees dat die bakterium verantwoordelik vir die vrektes was nie. Die etiologie dui eerder op C. perfringens as die oorsaak. Daarby saam speel ander faktore soos vinnige groei, hoë omgewingstemperature asook onderbrekings in beskikbaarheid

van voer heelwaarskynlkik ’n belangrike rol in die sindroom. Verdere navorsing om die toksien tipe te identifiseer asook die identifikasie van moontlike faktore wat die sindroom aanhelp moet gedoen word.

Acknowledgements

I thank my promoters Dr B.T. Spencer and Dr J. A. Pickard for all their help and constructive criticism. I also want to thank them for the moral support during the period of my studies.

To Prof Mark C. Williams, Section of Pathology, Dept. of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria I want to express my gratitude for performing the histopathology as well as writing parts of the histopathology results.

I want to thank the partners of Charles Street Veterinary Consultancy for their support and permission to further my studies.

To the management and staff of the five piggeries where this study was conducted I want to express my gratitude for informing me of possible cases. Without their help this study would not have been possible.

All laboratory personnel involved in this study are thanked.

I thank my family for their moral support and their motivation.

CHAPTER 1: INTRODUCTION, AIMS AND OBJECTIVES

Introduction

In the last five years generally excellent pig farmers in South Africa with optimal management systems have reported to the consulting veterinarians that their grower pigs die acutely with little warning. Most of these pigs die from what can be described clinically and pathologically as haemorrhagic bowel syndrome (HBS). In South Africa farmers refer to this syndrome as “red gut” due to the intense red discolouration of the entire intestinal tract. Elsewhere in the world farmers refer to it as balloon pig due to the rapid distension of the abdomen during and shortly after death.

Deaths due to this syndrome tend to be in the hot summer months resulting in a post-weaning mortality of 4% (2% above the acceptable norm). Furthermore, affected farms are situated in the northern parts of South Africa where summer temperatures can soar to 38°C. These farms are generally well managed with modern buildings that have more than 1000 sows and don’t castrate their grower boars. All are multi-site farms that make use of the all-in all-out management system. Two of the units that form part of the study are specific pathogen free (SPF) units that are free from contagious respiratory diseases such as *Actinobacillus pleuropneumoniae* and *Mycoplasma hyopneumoniae* as well as the

enteric disease *Brachyspira hyodysenterica*. The remaining three are normal health farms where they vaccinate the weaners against *Mycoplasma hyopneumoniae*. Under South African circumstances it appears as if farms that suffer from endemic respiratory disease in grower pigs, mortalities due to HBS are perceived to be of lesser importance. However, on these farms where respiratory disease is well controlled and the pigs genetically superior (such as the farms that were included in this study), the economic importance of this syndrome was considered important enough for further investigation.

Mortalities tend to occur in the early hours of the morning usually affecting the best growing pig. Once a pig starts showing clinical signs it usually dies within 45 minutes. Initially the pig squeals as if in severe pain with abdominal distension being visible after 15 minutes. It then shows signs of severe respiratory distress with open mouth breathing and after a few minutes it becomes cyanotic. Just prior to death the pig becomes very weak and its movements become jerky and uncoordinated (personal observation).

At necropsy the skin is usually pale without signs of trauma. There is often a bloody discharge from the nostrils and a markedly distended abdomen. The most remarkable finding on incision of the abdomen is the severely distended intestinal tract that is usually dark maroon in colour, but in some cases the intestines only have a slight reddish tinge. In 30% of the cases there is an intestinal torsion at the level of the mesentery implant. Other than the liver which tends to be pale and

pushed cranially the remaining organs appear normal. Indicative of a peracute disease, the stomach is always filled with food. Other than the first 10 centimeters of the duodenum which is normal, the luminal surface of the rest of the intestines is highly congested and the wall appears thinner than normal. The intestinal content is reddish, foul-smelling with a watery consistency. The cardio-respiratory system is normal. The carcasses of these animals show an accelerated rate of decomposition.

Even though the clinical and pathological picture was unusual, it was thought that *Lawsonia intracellularis* infection was at least in part responsible for this disease syndrome. This belief stemmed from the fact that *L. intracellularis* infection is known to be endemic on the affected farms and it is known to cause haemorrhagic enteritis. This fact was borne out by a recent serological study, in South Africa, in association with Boehringher Ingelheim that showed that most pigs seroconvert at 6 weeks of age with a higher prevalence in inland farms as opposed to coastally situated farms. Bigger herds, with fully slatted floors and wet or wet/dry feeding systems tended to be more prone to *L. intracellularis* infections[15].

However, the farms under this study control *Lawsonia intracellularis* infections with either in-feed or in-water tetracyclines. Furthermore, the clinical and pathological picture best fits with another cause of acute gastrointestinal disease such as *Clostridium perfringens* infection. This bacterium is a common cause of mortalities

in neonatal pigs but has not yet been recognized as an important cause of disease in grower pigs[22].

Problem statement

Haemorrhagic bowel syndrome has, on farms in South Africa, especially in Limpopo province, increased the mortalities by 2%. Furthermore by the time a pig reaches the grower phase the farmer has invested a lot of money in it, making it essential to control any disease, like HBS, that would result in its death. It is also known that there are many causes of HBS, making treatment and prevention of the disease syndrome very difficult. Although most veterinarians are of the belief that the main causative agent for HBS is *L. intracellularis*, the clinical presentation on farm does not correlate with that of acute PHE. PHE, caused by *L. intracellularis*, is the acute form of the disease complex. It occurs either sporadically or as an outbreak and is characterized by rapid onset of severe dysentery and a mortality rate of up to 50% of HBS-like cases. The mucosa of the ileum is thickened and the lumen is filled with blood[18]. Nowhere in the literature is it ever described that the animal vocalizes due to severe distress and pain. In contrast to this HBS is a peracute disease with a mortality rate of 100%. Once an animal starts showing clinical signs, it will be dead within the hour. The affected pig is in severe distress and vocalizes a lot. No signs of dysentery are present and often abdominal distension is visible prior to death, which is not the case in PHE. HBS does not

occur as an outbreak but daily mortalities would be recorded in different age groups. *Clostridium* spp. could play a role in the pathogenesis of HBS. Thus the aetiology of HBS in pigs in South Africa should be further investigated.

Hypotheses

Hypothesis

Lawsonia intracellularis in the acute form is the causative agent of HBS.

Alternative Hypothesis

Lawsonia intracellularis is not the only cause of the acute haemorrhagic grower death syndrome. Other pathogens or management aspects may also be involved in the aetiology of this disease.

Study aim and objectives

Aim:

To investigate the cause of deaths of pigs that have died from HBS on farms in Gauteng and the Limpopo Province over period of 3 months in summer.

Objectives:

To:

1. Identify well managed farms that are experiencing problems with acute deaths in their grower pigs.
2. Collect farm data pertaining to locality of the farm, the time of year when the mortalities peak, age of pigs affected and any clinical signs that were noted.
3. Perform necropsies on all available grower pigs that have died from suspected HBS over a period of 3 months.
4. Collect intestinal samples for histological and bacteriological analyses.
5. Place all the data into an Excel spreadsheet.
6. Analyze and interpret the data.

CHAPTER 2: LITERATURE REVIEW

Haemorrhagic bowl syndrome (HBS) is very often a last resort type of diagnosis. This diagnosis is made for a case which presents it as an acute death with features described for HBS, and after thorough investigation, no other cause is determined. The cause of HBS is unknown and the general consensus is that is a multifactorial disease complex[31]. HBS has become more important in the last decade. Initially it was mainly seen in Europe with an occasional outbreak in the USA. Because it affects pigs almost ready to go to market, it is a costly disease.

Haemorrhagic bowel syndrome primarily affects rapidly growing pigs between 4 and 6 months of age (70 – 120kg live mass)[36]. Other distinguishing characteristics of this disease syndrome include:

- A previously healthy pig found dead
- Marked pallor of the skin and abdominal distension
- The small intestine is congested and contains intra-luminal blood or bloody fluid
- No other reason for cause of death or intestinal haemorrhage[31].

Because the cause of HBS is not known and literature is often confusing, it will be useful to look at causes of intestinal haemorrhage and consider their relevance in this disease.

When considering intestinal haemorrhages in the pig there appear to be three distinct pathological entities that need to be differentiated:

- Intestinal haemorrhagic bowel syndrome (entero-haemorrhagic syndrome). This is characterized by congestion and gaseous distension of the entire intestine except the first 10 cm of the duodenum. Death is usually sudden, but when clinical signs are observed, they include abdominal distension and pain.
- Haemorrhagic bowel syndrome. This is characterized by massive haemorrhage from the ileal mucosa into the ileum, caecum and colon. At autopsy there is a distinctive lesion in the ileum.
- Haemorrhage from gastro-oesophageal ulcers[23].

The main differential diagnoses for intestinal haemorrhage in the grower pig are the following:

- Intestinal haemorrhage bowel syndrome
- Intestinal torsion
- Porcine haemorrhagic enteropathy
- Gastric ulcers
- Whey feeding
- Salmonella enterocolitis
- Swine dysentery

- Trichuris suis
- Lawsonia intracellularis

The Intestinal haemorrhage bowel syndrome

This syndrome mainly affects pigs of 35kg and heavier. Affected pigs become pale, develop distended abdomens and they die suddenly. The small intestines are almost always autolytic. There is also an inflammatory change with the small intestine shedding its villous epithelium. There is a loss of villi depth and migration of inflammatory cells into both the *lamina propria* and the lumen, which is filled with cells, blood and bacteria[37].

Currently it is not clear what causes this syndrome but the following has been suggested:

- Whey feeding[24]
- Allergy. Allergy to milk protein or some other compound in the diet. This is based on the large numbers of eosinophils present in lesions[37]
- Manipulation – animals that were turned during anaesthesia developed this syndrome[37]
- Infections. *Lawsonia intracellularis* has been suggested as a contributory factor in cases without volvulus. Enteropathogenic *E. coli, C. perfringes* types A and C and *Salmonella* serotypes may also be involved[37]

Intestinal torsion

Colonic displacement is a condition sometimes affecting grower pigs. The entire intestine twists on the long axis at the mesenteric implant and death follows rapidly. On necropsy the abdomen is extremely distended. The intestinal loops are blackish-red in colour and filled with a bloody fluid. Causes of this condition are not fully understood, but factors such as sudden movement with full intestines are thought be in part responsible.

Porcine haemorrhagic enteropathy (PHE)

Porcine haemorrhagic enteropathy is part of a group of chronic and acute disease syndromes (usually referred to as prolific enteropathies) caused by *Lawsonia intracellularis.* Porcine haemorrhagic enteropathy usually affects the terminal ileum. The affected intestine is often thickened and dilated with some degree of serosal oedema. There is usually one to a few blood clots in the lumen of the intestine with no other food or fluid present. The surface of the affected part shows little damage except for marked epithelial hyperplasia. Histological examination shows extensive degeneration, congestion and haemorrhage within the mucosa with the presence of numerous *L. intracellularis*-like organisms intracellularly within the apical mucosa. There is a marked accumulation of bloody cellular debris containing numerous *L. intracellularis* organisms above the affected mucosa[18].

Gastric ulcers

The most common form of gastric ulcers in pigs is ulceration of the *pars oesophagea*, the non-glandular region of the pig's stomach. The *pars oesophagea* is frequently stained yellow as a result of bile staining. This is especially true if the surface is rough and thickened due to hyperkeratosis and/or parakeratosis. Although ulceration of the glandular area of the stomach does occur it is uncommon. Oesophageal ulceration can lead to sudden death due to acute intragastric haemorrhage or it can be the cause of chronic ill-thrift.

The exact cause of gastric ulceration is not clear although there are many factors that contribute to the condition. Diet components, particle size, mixing of pigs, season and concurrent disease are a few factors that can cause gastric ulceration.

Clinical signs tend to reflect the degree of blood loss. Very often an apparently healthy pig is found dead and its carcass is extremely pale. If the blood loss occurs more slowly, pigs will show signs associated with anaemia.

Histologically the lesion is the result of hyperplasia and parakeratosis, with nucleated cells present on the mucosal surface. Neutrophils and eosinophils are often present at the tips of the proprial papillae. Epithelial separation and erosion usually occur beneath a band of cells with cytoplasmic pallor and nuclear

degeneration. Usually lesions only involve the epithelium and submucosa, but occasionally the *muscularis externa* and the serosa can also be involved[38].

Whey feeding

Whey has been suggested as a cause of haemorrhagic enteropathy in swine. The clinical signs are sudden death, carcass pallor, marked abdominal distension and a haemorrhagic effusion into the lumen of the entire small intestine except the duodenum. Whey is a highly-fermentable substrate. It is thought that there is an inadequate availability of lactase in the intestine for lactose digestion. The lactose is therefore not broken down to glucose and galactose and is then available for fermentation by intestinal microbes into lactic acid, with the release of methane and hydrogen gasses[40]. This gas formation can lead to displacement of the caecum which could result in mesenteric volvulus. The haemorrhagic enteropathy is a consequence of the torsion and occlusion of the mesenteric veins. Since the duodenum is separately drained, it is not affected[39]. The intestinal content is blood-stained, resembling port wine. The colon contains similar fluid mixed with normal intestinal content.

Salmonella enterocolitis

This disease syndrome is most common in pigs from weaning up to about 4 months of age. The main serotypes responsible for this disease are *S*. Choleraesuis, *S*. Typhimurium and sometimes *S*. Heidelberg. Salmonellosis has a world wide distribution and the major source of infection is affected pigs and environments contaminated by infected pigs. Salmonellae are very hardy bacteria and it is known that these organisms can survive for years in suitable organic materials[7]. The initial clinical sign is watery yellow diarrhoea, usually without blood and mucous. It spreads quickly, often involving all the pigs in the pen. The disease has a waxing and waning disease pattern because of the occurrence of carrier pigs. Pigs are initially febrile, they have decreased feed intake, are dehydrated and have diarrhoea. Blood can occur in the faeces, although not in the same quantity as with swine dysentery or PHE. Mortality is low, but if pigs die, the gross lesion is a focal or diffuse necrotic enteritis, colitis or typhlitis. Mesenteric lymph nodes are generally enlarged with a moist appearance. The gross lesions may extend to involve the descending colon and rectum and it is often seen as button ulcers when the lesions resolve. The typical enteric lesion is necrosis of the surface and crypt enterocytes. This varies from focal to diffuse. The necrosis often extends into the muscularis mucosa, submucosa, and lymphoid follicles. Diagnosis is confirmed by bacteriology and histopathology[7].

Swine dysentery

Swine dysentery is caused by *Brachyspira hyodysenteriae.* It is a Gram-negative, oxygen-tolerant anaerobic spirochaete. Swine dysentery has a world-wide distribution although there are differences between countries spatially and temporally. The disease is not common on well managed South African farms (personal observation 2008). The disease is most common among grower and finisher pigs, with a peak a few weeks after the pigs have been moved out of the weaner facilities. The primary mode of infection is oral/faecal. This is particularly evident in farrow-finish units with a continuous flow pattern. The incubation period can vary from 2 days to 3 months although the disease usually occurs within 10 – 14 days in naturally exposed animals. Diarrhoea is the most prominent clinical sign. It usually starts with the presence of soft yellow to grey faeces. The animals are also anorexic at this stage. As the disease progresses, large amounts of mucous, blood and shreds of white mucofibrinous exudate are seen in the faeces. The perineum is also stained at this stage. Macroscopic lesions are present in the large intestine but absent in the small intestine. In the acute stages the wall of the large intestine is hyperaemic and oedematous. The mesenteric lymph nodes may be swollen. The serosa is covered by mucus and fibrin with flecks of blood. As the disease progresses oedema in the walls of the large intestines decreases and mucosal lesions become more severe with an increase in fibrin exudation. At this stage the serosa is covered by a thick, mucofibrinous pseudomembrane containing

blood. In chronic cases the serosa is covered by thin, dense fibrinous exudates. The only significant microscopic lesions are found in the caecum, colon and rectum. Acute lesions include thickening of the mucosa and submucosa due to vascular congestion and extravascularzation of fluids and leukocytes. There is hyperplasia of goblet cells. Spirochaetes may enter the goblet cells in the colonic crypts and enter the intercellular gaps in the epithelium. There is a loss of cohesion between colonic enterocytes, with subsequent necrosis and shedding of the epithelium. Bleeding occurs from small vessels located under areas of eroded epithelium. Blood becomes trapped in the overlying mucus. Later changes include the accumulation of large amounts of fibrin, mucous and cellular debris in mucosal crypts and on the luminal surface of the large intestine. Deep ulceration is not typical, but superficial erosion of the mucosa may be extensive. Chronic changes are non-specific, although superficial necrosis of the mucosa can be more advanced. Healing does occur as the pigs get older and therefore lesions are seldom seen in pigs of baconer mass[8].

Trichuris suis

Trichuris suis occurs naturally in pigs and wild boars. Adult female nematodes measure 6 – 8 cm long and males are half the size. Eggs are passed in the faeces and require 3 – 4 weeks to reach infectivity. Infective eggs hatch in the small intestine and caecum and adults are found in the rectum. The released L1

penetrates the crypt cells, followed by a histotropic phase persisting for 2 weeks. The larva migrates gradually from the deeper *lamina propria* to the submucosa. Luminal development begins in the third week after infection. *Trichuris suis* causes enterocyte destruction, ulceration of the mucosal lining, loss of capillary blood and secondary bacterial infection[35].

Considering the initial hypothesis, local vets as well as the researcher considered *Lawsonia intracellularis* as a major cause of HBS. Therefore this disease syndrome is dealt with in greater detail.

Lawsonia intracellularis

Characteristics of *Lawsonia intracellularis*

Lawsonia intracellularis (formerly ileal symbiont intracellulare) is an important pathogen in animals, especially in pigs. It has been established as the aetiological agent of proliferative enteropathy. It is an obligate intracellular, Gram-negative bacterium belonging to the delta division of Proteobacteria[32]. Its closest relatives are *Bilophila wadsworthia* and *Desulfovibrio* species. However, *L. intracellularis* differs significantly from them in that it is entero-invasive (with tropism for immature epithelial cells and no extra-intestinal location) and cannot grow in cell-free media[33].

Clinical signs

Lawsonia intracellularis can manifest itself as four different disease syndromes. It varies from sub-acute enteritis (porcine intestinal adenomatosis - PIA) to fatal ilelitis (haemorrhagic enteropathy – PHE). The former is more common in pigs aged 6 weeks to 4 months of age. The predominant clinical signs are anorexia, diarrhoea and poor growth. The diarrhoea is usually moderate, with loose pasty stools (resembling cow pats) of normal colour. There may be some undigested food particles in the faeces. Another typical sign is variation in body condition within the group and growth in the grower herd[2].

The acute form of the disease is a much more dramatic and severe type of disease. Haemorrhagic enteropathy has an acute onset with intestinal blood loss. Affected animals are usually older than 5 months. During an acute outbreak, pigs are often found dead[16]. Mortalities can reach anything from 12% to 50%. The main clinical feature is the acute onset of red-black tarry faeces[2]. Due to the blood loss in the faeces, some pigs appear very pale. The diarrhoea is profuse, watery and foul smelling, very often staining all the pigs as well as the walls in the affected pen. Many pigs in a group can be affected at the same time. Mortalities start occurring 8 – 24 hours after the onset of the diarrhoea[18].

There are two other syndromes which are of lesser importance. In necrotic enteritis (NE) the lining of the intestine is covered in yellow or greyish masses of necrotic material. Regional ileitis (RI), also known as “hosepipe gut”, presents itself as a smooth, rigid lower small intestine. The terminal region of the ileum in particular is affected. These two syndromes are however not differential diagnoses for haemorrhagic bowel syndrome[37].

Pathogenesis and infection

Like most other important enteric bacteria, *L. intracellularis* relies on faecal-oral transmission for their propagation[25]. The pathogenesis for PIA and PHE appears to be similar. The major pathogenic mechanism of this bacterium is infection and hyperplasia of enterocytes.

Little is known of the events that take place in the first 5 days after infection that leads to crypt cell infection. It has been shown that intestinal microflora influence the development of disease[16]. Experiments were done where gnotobiotic piglets or piglets contaminated by simple non-enteric bacteria were given pure cultures of *L. intracellularis*. These piglets did not develop disease and immunological assay of the ileum did not show any evidence of intra- or extra-cellular colonization. In contrast to this, gnotobiotic piglets that were co-inoculated with *L. intracellularis* and an undefined population of intestinal bacteria developed disease. These

findings suggest that intestinal microflora modifies or supports the ability of *L. intracellularis* to colonize the intestinal tract and without the presence of these bacteria infection would fail[32]. It has been suggested that the presence of an intestinal microflora modifies pathogenicity by altering the bowel oxidation-reduction potential or by affecting the rate of enterocyte division[16].

During infection, *L. intracellularis* localizes on the ileal epithelial surface where it adheres and invades the epithelial cells. Once it has entered the cells, it must escape from the vacuole and initiate intracytoplasmic growth. Finally it is released for onward transmission[33].

Currently the processes involved in host cell attachment are not clearly understood. Specific adhesions or receptors for *L. intracellularis* have not been characterized yet[2]. Bacteria appear to enter the cells individually, after which there is a loss of microvilli and a thickening of the cell border adjacent to the bacteria. A depression forms beneath the adhering bacteria in the cell membrane. The cell membrane appears thickened but it stays intact as the bacteria enter into the cell. The vacuole surrounding the bacterium therefore forms progressively as the bacteria enter the cytoplasm. Breakdown of the vacuole occurs shortly after entry and within 3 hours the majority of the bacteria are located in the cytoplasm[32]. Studies have indicated that *L. intracellularis* produce a member of a novel family of haemolysin. This potential virulence factor plays an important role in the release of the bacteria from the vacuole[33].

Roughly 10 days after hamsters were infected, the number of cellular bacteria had increased and cellular hyperplasia was evident. It is assumed that lesions develop at the same rate in pigs as in hamsters[16]. Cellular hyperplasia is the most obvious alteration induced by *L. intracellularis*. This host cell proliferation is a prerequisite for *Lawsonia* replication[33]. To understand this bacterial replication one must consider the underlying processes of enterocyte multiplication. At the base of the epithelium are tubular crypts. These crypts contain mitotically active cells. Dividing cells migrate from the crypts to form villi that extend into the luminal surface of the intestines. Mature differentiated cells have a well-developed brush border. This is in contrast with the poorly differentiated crypt cells, which have limited brush border development. The research done by Smith *et al*[33] indicates that the crypt cells are targeted for attachment and bacterial entry. This tropism for crypt cells indicates physiological needs of the bacterium. These cells divide and migrate to populate the top of the villi. The continued growth and migration of infected cells is the mechanism by which *L. intracellularis* infects the epithelium. It is suggested that dividing cells promote the growth of the bacterium better than non-dividing cells[32]. By day 15 – 20 the affected villi were 3 times longer than normal and entirely composed of hyperplastic cells[16]. Although the bacteria initially stimulate the crypt cells to divide at an increased rate, this stimulatory effect does not persist once the lesion becomes fully developed[32].

Cells infected by bacteria show a poor major histocompatibility complex class 2 expression. This probably leads to immunosuppression[16]. Uncomplicated cases of *L. intracellulare* infection are generally considered as non-inflammatory and it is accompanied by poor specific immune responses. Pigs affected by PHE show a more vigorous response as well as the presence of IgM secreting B-cells. A constant feature in all forms of the disease is large amounts of IgA within cells containing bacteria. At this stage it is not certain whether this is due to failure to excrete this immunoglobulin or whether it is a specific IgA stimulated by the organism directly. Many have credited the differences between PIA and PHE to differences in immune response. The different age groups affected support this clinically. Another important difference between the two syndromes is that bacteria are more widely distributed in PHE (it appears free, in macrophages within the lamina propria, submucosa and tonsil as well as in the capillaries and lymphatics of the intestine) [32].

The dilation of blood vessels causes a reduction of blood flow rate through the entire area. Vascular endothelium becomes damaged to such an extent that further exudation and even escape of cells from blood vessels occur. This compromised blood flow is critical to the tips of the villi, explaining their extensive involvement in the lesion seen. This compromised blood flow results in fibrin clots, which reduce blood flow even more. The end result is extensive blood loss and necrosis of the intestinal mucosa[18].

When the intestinal epithelium starts to return to normal, cells containing bacteria are found free in the lumen. Cells containing apoptic bodies which appear shrunken are found throughout the mucosa. The appearance of free cells in the lumen provides a mechanism by which *L. intracellularis* can infect intestinal tissue distal to the original lesion. It also makes faecal shedding possible[16].

Pathology

The lesions of PHE resemble lesions consistent with an acute bacterial infection. Pigs usually die within 24 hours after the onset of clinical disease. The mucosa of the terminal ileum is much thickened and the lumen of the ileal tract is filled with blood. Other lesions include oedema of the mesenteric attachment of the affected part of the ileum and an abundance of clear ascitic fluid[18].

Histology typically appears as follows: Lesions are usually restricted to the mucosa of the affected parts of the ileum. There is often an extensive accumulation of proteinaceous fluid in the interstitium and lacteals of the lamina propria at the tips of the affected villi. With Warthin-Starry stain, curved organisms are seen in the apical cytoplasm of epithelial cells, within cells in vessels and free in tissue spaces of both the lamina propria and in the submucosa. Cells containing organisms in vesicles are also abundant. Mast cells and thrombosed blood vessels are also present[18].

Epidemiology

Infection is wide-spread in herds with all types of production systems in all parts of the world. It appears that white breed hybrid stock develop disease more easily than Duroc and Duroc-cross pigs[16].

There are two patterns of *L. intracellularis* infection. This is based on whether age-separation of groups occurs on infected farms and whether infection occurs in breeding stock. The first pattern is where early infection occurs at 4 – 7 weeks of age. This is usually seen on farrow-to-finish units. If animals were to become ill they usually have the more chronic form of the disease i.e. PIA. The second pattern is characterized by a delayed infection (14 – 18 weeks). It is typically seen in farms with separation of ages at weaning in an all-in all-out manner on multiple sites. The disease usually manifests itself as PHE in these cases[5, 16].

Herd size is also considered a risk factor with bigger herds having an increased incidence of *L. intracellularis* infection. The use of partially slatted floors for finishers is also associated with an increased risk of infection. Reason for this could be that these floors are often inadequately cleaned. This will lead to the spread of disease between different batches as well as within batches[3, 5].

Diagnosis

It is important to obtain the correct diagnosis of diarrhoea, sudden death and poor performance in grower pigs. There are various methods of diagnosing *L. intracellularis.*

Serology

1. A competitive blocking ELISA is now available commercially as a semi-quantitive test to test herds routinely[2].
2. Immunofluorescence assay (IFA) is available for routine testing of swine serum antibodies against *L. intracellularis*[2].

Molecular methods

1. Detection of *L. intracellularis* DNA. A 5' nuclease assay was developed to detect *L. intracellularis* in porcine faecal samples. The specific probe and primers were chosen by using the 16S ribosomal DNA gene as a target. A two step polymerase chain reaction (PCR) is used to detect genomic DNA from *L. intracellularis*. The test can be used on necropsy samples such as intestinal mucosa as well as on faeces. *Lawsonia intracellularis* is not consistently excreted in the faeces. It is usually only detected early after

infection and prior to seroconversion. This method is highly sensitive and specific[17].

Histopathology and immunohistopathology (IHC)

The cytological examination of formalin-fixed ileum or large intestine provides a definite diagnosis of the proliferative intestinal enteropathies. In the small intestines the villi are shortened and the surface epithelium is cuboidal or flattened. The mucosa is thickened by the proliferating crypts. The mitotic rate in the crypts is increased. There is a mild to moderate inflammatory response in the lamina propria which can extend to the deeper layers. *Lawsonia intracellularis* cannot be detected by haematoxylin and eosin (H&E) stain but will stain black when a silver stain (Warthin/Starry) is used. More specifically IHC can also be used to demonstrate *L. intracellularis*. Monoclonal antibodies are labeled for a direct *in situ* immunoperoxidase staining or immunofluorescense staining. The reaction is detectable at the luminal aspect of the infected crypt cells and sometimes in macrophages of the lamina propria[2].

Treatment

In acute PHE, where both morbidity and mortality can be high, the use of injectable antibacterials is recommended for the “most at risk” pigs. This is usually followed by in-water or in-feed medication. In-water medication is preferable because pigs generally consume water when they are ill, even when they are anorexic[2].

The minimum inhibitory concentrations of various antibacterials against this agent have been examined[2]. Tiamulin, tylosin, lincomycin and chlortetracycline have shown to be effective *in vivo* against *L. intracellularis*. A possible explanation could be that these drugs concentrate in the enterocytes infected with *L. intracellularis*. The performance enhancer antimicrobials: bacitracin, virginiamycin and salinomycin as well as the antimicrobial classes consisting of penicillins, aminoglycosides and fluoroquinolones are ineffective for treating this disease. Heavy metals (copper or zinc), probiotics, acids and enzymes have up to date not shown any real effect on ileitis or its causative agent (personal observation 2008).

Most farms treat pigs as they enter the grower lines prophylactically i.e. from 10 weeks of age usually until 13 weeks of age[2]. On some farms pigs are treated in a metaphylactic manner which is in line with prudent antimicrobial use. This means that they are not continuously on antimicrobial treatment, but that they are rather treated at the time when infection is most likely. This treatment regime is implemented when clinical disease is noted. Tetracyclines are the treatment of choice on most farms. Other farms treat pigs in a pulse manner. When the pigs enter grower accommodation they are placed on antibacterials for a week, the treatment is stopped for a week and thereafter repeated 2 – 3 times. On farms where pigs are on continuous antibiotic treatment the situation can actually worsen because this strategy lowers exposure of pigs to the endemic disease and hence their immunity is lowered.

Pigs can be vaccinated as a prophylactic measure. A vaccine has been available in South Africa for almost 2 years. To determine timing of vaccination, the herd must be bled to establish at what age the pigs seroconvert. Vaccination must be done at least 3 weeks prior to the first seroconversion which, under South African conditions seroconversion appears to be 3 weeks of age[15]. It is a live vaccine which can be given *per os* or in the drinking water. Because it is a rather expensive option, most farms are still using antibacterials to control *L. intracellularis* infections.

Clostridium species

This genus is known to cause peracute haemorrhagic enteritis and toxaemia in neonatal pigs as well as neonates and rapidly growing animals of other animal species that are intensively reared. Therefore, even if not well described in the literature in the age group in question, it should always be included as a possible cause of peracute death.

The peracute nature of the syndrome suggests that a toxin could be involved in the pathogenesis. Various toxin-types of *C. perfringens* cause food poisoning in humans as well as several enterotoxaemic diseases in production as well as other animal species. The organism is characterised by its ability to produce several

extracellular toxins[28]. The *C. perfringens* species is a very heterogenous group of organisms with respect to their metabolic by-products, toxins and pathogenic potential. The species is divided into 5 toxin types, namely A – E, based on their ability to produce any of the four major toxins. These toxins together with enterotoxin and β2 toxin are thought to play an important role in the pathogenesis of several serious enteric and septicaemic diseases in domestic animals[11].

Table 1: Toxins produced by different *Clostridium* spp.

	Toxins			
Type	Alpha	Beta	Epsilon	Iota
A	++	-	-	-
B	+	++	+	-
C	+	++	-	-
D	+	-	++	-
E	+	-	-	++

Key
++ Produced as a predominant toxic fraction
+ Produced in smaller quantities
- Not produced.

Previously *C. perfringens* was mainly associated with disease and mortalities in piglets. The ubiquitous *C. perfringens* type A which are found in high numbers, and *C. perfringens* type C which is found in small numbers in the colonic microflora of healthy pigs are associated with disease in pigs. *Clostridium perfringens* type C

causes haemorrhagic, often fatal, necrotic enteritis in primarily 3-day-old piglets, although clinical signs can be seen as early as 12 hours after birth[34]. Characteristic lesions include deep mucosal necrosis and emphysema in the small intestine. Microscopically, the pathognomonic lesion is haemorrhagic necrosis of the intestinal wall, which starts in the mucosa but usually progresses to affect all layers of the intestine[34]. These lesions sometimes extend into the caecum and proximal portion of the colon.

Clostridium perfringens type C is considered a primary pathogen, even though disease is more severe following other infections such as coccidiosis. The organism is transmitted via direct contact between piglets and spores can survive in the environment for quite some time due to their resistance to heat, disinfectants and ultraviolet light. The ultimate source of the organism is the sow, although isolating it from normal sow faeces is difficult[34]. This disease is mainly controlled by vaccination of the sow herd.

Infections with strains of *C. perfringens* type A tend to be opportunistic and like *C. perfringens* type C affects mainly the neonatal piglet and occasionally the weaner resulting in non-haemorrhagic, mucoid diarrhoea. Histopathology shows a mucosal necrosis, villous atrophy and serositis. Lesions are usually most severe in the jejunum and ileum[34]. The diagnosis of disease due to this bacterium is difficult as it is a common inhabitant of the intestinal tract and a common post-mortem invader.

However, clostridial overgrowth and toxin production by this bacterium is indicative that it was involved in the disease process[34].

Clostridium difficile-associated disease (CDAD) is a cause of enteritis in neonatal pigs. Affected piglets are usually 1 – 7 days old with a history of scours and sudden death. There is usually oedema of the mesocolon and the contents range from pasty to watery with a yellow colour. Affected litters experience lost productivity due to decreased weaning weights. Microscopic lesions include suppuration of the *lamina propria*, infiltration of mononuclear inflammatory cells and colonic and serosal oedema. Segmental erosion of colonic mucosal epithelium is common and volcano lesions may be evident[34]

The literature is scanty regarding the involvement of *C. perfringens* in HBS in grower pigs. There are, however, several studies implicating *C. perfringens* type A, B and C as the causative agents of similar syndromes in other production animals such as cattle and poultry.

In dairy cattle the syndrome is described as a newly emerging, highly fatal intestinal disease. The disease is most commonly seen in adult dairy cows in early lactation. On most farms it occurs as a sporadic disease. Affected cows are simply found dead or dying, mainly due to the sudden and massive haemorrhage into the small intestine. Tachycardia and tachypnoea is common with the extremities often cool and rectal temperature subnormal. When viewed from

behind the abdominal contour is pear-shaped in the standing animal. Rectal examination does not reveal distended intestinal loops because the blood-filled loops sink to the ventral abdomen. Although the cause is currently unknown, there are several reports indicating an association between *C. perfringens* type A and HBS. This association is based upon the following observations[41]:

- Affected cows have positive faecal cultures for this organism,
- *Clostridium perfringens* is readily isolated from intraluminal blood clots in the jejunum of affected cows,
- There is histological evidence of intestinal necrosis associated heavy intra-luminal growth of *C. perfringens* type A, and
- Other organisms usually associated with haemorrhagic enteritis are rarely isolated or identified from tissues from affected animals.

Histological findings include early haemorrhage into the submucosal layers of the affected segments of the jejunum. There is also dense colonisation of the lumen with large Gram positive rod-shaped bacteria. Trans-mural necrosis of the jejunum with severe intra-luminal haemorrhage is present.

It is unclear whether proliferation of *C. perfringens* type A occurs as part of the primary disease or secondary to another disease or triggering factor. The alpha toxin does cause marked endothelial cell necrosis which could cause intra-luminal haemorrhage and clot formation. However, intra-luminal haemorrhage from

another primary cause could initiate secondary *C. perfringens* proliferation as the organism is likely to multiply in the presence of soluble protein or carbohydrates[41].

Clostridium perfringens type A is also implicated as the causative agent in bovine enterotoxaemia. This syndrome occurs most frequently in beef cattle and it is referred to as "Belgian Blue calf enterotoxaemia" in lay terms. Other breeds can also be affected. Clinical signs include a high mortality rate, sudden deaths, lesions of necrotic and haemorrhagic enteritis of the small intestine and absence of any other clinical signs[19]. In feedlot cattle it is thought to be the causative agent in the so called "sudden death syndrome". This syndrome tends to occur in feedlots with self-feeders where the environmental temperatures are very hot. The primary trigger factor for this syndrome is considered to be variable feed intakes caused by high environmental temperatures. Calves in the finisher stage are worst affected. Control depends largely on proper feedbunk control to ensure regular feed intake[9].

In poultry *C. perfringens* type A is thought to be the causative agent of necrotic enteritis (NE). *Clostridium perfringens* type A is a normal inhabitant of the intestinal flora of birds, but studies have indicated that the normal flora strains are not responsible for the typical lesions associated with the disease after inoculation. As mentioned earlier, *C. perfringens* is genetically diverse, but isolates after an outbreak of NE are genetically identical. One can therefore conclude that NE strains displace non-NE strains and research has been done to examine the population dynamics which cause one strain to displace several other strains. It

was determined that some strains are more virulent for chicks and that NE strains had a selective advantage over non-NE strains and even degrees of selective advantage over each other. *In vitro* studies indicated that NE strains produced substances that strongly inhibited non-NE strains. The reverse did not occur and NE strains did not inhibit each other[1]. The role of alpha toxin in NE has been questioned recently. One viewpoint is that because all *C. perfringens* produce alpha-toxin and only some type A and C cause NE it is reasoned that alpha toxin by itself cannot be sufficient to cause disease. It is therefore reasoned that there must be other virulent factors present to cause disease[12]. Recently a pore-forming toxin called NetB toxin has been identified and is considered to be critical in the development of NE. This novel toxin is the first definitive virulence factor to be identified in avian *C. perfringens* strains capable of causing NE. The NetB toxin has a limited similarity to *C. perfringens* beta-toxin[13, 20].

Other studies have indicated that the alpha-toxin offered significant protection when used to immunise birds. The primary vaccination was given as an alpha toxoid, which was followed by an active toxin as booster. This regime gave much better results than using the toxoid and active toxin separately. The same study has also indicated that proteins other than the alpha toxin that play an important role in the immunity could also be important in the pathogenesis of the disease[14].

A new toxin, β2-toxin, has been identified recently and it is described as the causative agent of enteric diseases in animals. It is produced by *C. perfringens*

that has the *cpb-2* gene. Its activity is similar to β1-toxin; it is lethal to mice and it causes haemorrhagic necrosis of the intestinal wall. In neonatal and weaner pigs it causes diarrhoea or necrotic enteritis very similar to *C. perfringens* type C. It has also been implicated as a cause of disease in foals and lambs as well as bovine enterotoxaemia. When the toxin is divided into two polypeptides by trypsin, it completely loses its cytotoxic activity. The fact that *cpb-2* positive *C. perfringens* has been isolated from healthy animals as well as ill animals (it is isolated more often from ill animals) indicate the disease is multifactorial. Other factors such as low trypsin activity in the intestinal tract, antimicrobial treatment or changes in diet can result in the proliferation of β2-toxigenic *C. perfringens* which could lead to enteritis or enterotoxaemia[30].

The role of management factors in HBS

Some research has been done in an attempt to identify possible factors that could predispose pigs to HBS. It is commonly accepted that HBS targets pigs that have an above average growth rate and excellent health status. This belief was tested in a study where 1900 pigs were individually tagged and weighed daily with automatic sorting scales. Pigs were trained to enter the automatic sorting scales to acquire feed. Water was available in all areas. Analysis of scale visits could therefore correlate with number of feedings. Affected pigs were compared to pigs of similar sex, weight and pen location. There was no statistical difference in

average daily gain (ADG) between affected vs. unaffected pigs. ADG is therefore not a good predictor of HBS. Affected pigs did however visit the feeding station less often than unaffected pigs[29].

Straw *et al* investigated in a recent study several so-called predisposing factors to HBS on two large American farms[36]. It was found that HBS was responsible for a fifth to a third of all deaths. They concluded that HBS probably has a non-infectious aetiology due of the absence of age patterns for mortality typical of primary infectious diseases. It is possible in the case of most primary infectious agents to control them by stocking policies, monitoring, effective treatment protocols, vaccinations and a variety of managerial interventions. Well managed farms are able to control most diseases caused by primary infectious agents. What was noticed in the American study is that HBS is generally a problem on well managed farms, supporting the theory of a non-infectious disease. However, Straw *et al* found that HBS was lower in pigs fed a diet that included one of the following antibiotics: chlortetracycline, bacitracin or bacitracin combined with an arsenical compound. It was reasoned that antimicrobials might inhibit the proliferation of normal intestinal flora that could occur under certain circumstances such as a sudden change in diet or raw materials and out-of-feed events. The fact that the incidence of HBS is higher in summer could be related to a change in feed intake. As pigs get older the number of feedings decrease while the amount ingested at any time increases. Lean pigs also spend less time at feeders and have a faster rate of feeding than obese animals. The larger volumes of feed could

predispose to volvulus/HBS through a mechanical mechanism; the weight of the feed could be enough to displace the intestines[36].

It has been suggested that out-of-feed events could also predispose pigs to HBS. When pigs, usually fed *ad lib*, are restricted, there is a higher incidence of redirected behaviour such as pen mate manipulation and fighting. When the feed supply is re-instated there is an increase in feeding rate (g/min) as well as an increase in fighting and aggression. It has been suggested that out-of-feed events leading to engorgement of the intestinal tract can trigger HBS[4]. Feeding behaviour could trigger HBS in a combination of mechanical and physiological ways. The volume and frequency of feeding causes alterations in the basic migrating myo-electric complex (MMC) pattern. The migrating myo-electric complex can be described as an electro-myography signal that migrates along the small intestine in a strictly organized pattern during fasting. It can be divided in four phases, but in most species, except the dog, the fourth phase is transient and usually skipped. Feeding, and to a certain degree the sight and smell of the food, disrupts the periodic pattern and enhances the secretions from the gall bladder and stomach. In carnivores the MMC is considered a “house keeper” that cleans the gut out of the digesta remnants, bacteria and desquamated epithelia and prepares it for the next prandial pattern. In the herbivorous and omnivorous animals, however, the MMC is an important means of digesta transport. In these animals there are no contractions and no flow of the intestinal content during phase 1 of MMC. During phase II, the backward and forward contractions increase in amplitude to mix the

intestinal content, and the number of propulsive contractions increases during late phase II. During phase III the intestinal contractions are maximal and propulsive thereby allowing transport of all the content along the small intestine caudally[42]. When pigs are fed once a day, their MMC resembles that of carnivores, but when they are fed *ad lib* their intestinal motility pattern resembles that of ruminants, where the MMC pattern persists, regardless of feeding. Out-of-feed events would cause intestinal stasis, therefore preventing food moving along the intestinal tract. Intestinal stasis causes an abnormal proliferation in enteric microflora, thereby predisposing the pig to HBS[36]. Therefore out-of-feed events could be regarded as a definitive risk factor in the HBS disease complex.

Irregular feeding patterns can occur when there is a change in the pig's environment or social status. This could happen when pigs are moved to other pens, general social stress, sorting and adding to pens during the marketing period and when a pig jumps a pen[36]. Pigs maintain a strict social hierarchy and when a "strange" pig is introduced into a group, it upsets this hierarchy. A new hierarchy must be established and this is usually done by fighting. Although feed is available in the bins, the pigs are so busy fighting that they do not feed. This could predispose to HBS either by causing intestinal stasis, or, when the pigs finally eat, they over-engorge, causing large volumes of indigested feed to be present in the large intestine. This excessive feed can also cause proliferation of certain enteric microflora.

Other factors that could cause an abnormal proliferation of enteric microflora include[31]:

- high iron in or poor quality of drinking water,
- high copper or high fat content in the rations
- high levels of protein,
- high protein quality,
- abrupt change in or proportion of feed ingredients
- pelleted rations.

CHAPTER 3: MATERIALS AND METHODS

Introduction

Farmers affected by HBS in their piggeries approached the Pig Veterinary Society, South Africa, to conduct a study as to identify possible causes of the syndrome. The study was conducted during summer, from January to March 2007 on commercial pig farms in the northern parts of South Africa. Farmers informed the author of deaths and she or other qualified persons performed necropsies on the farms. Samples were collected from suitable cases and sent for bacteriology and histopathology.

Model system

Five herds in the northern parts of South Africa with known increased post-weaning mortalities due to HBS were chosen to conduct the study on. Two of the herds are SPF herds and 3 of the herds are of normal health where weaners are vaccinated against *Mycoplasma hyopneumoniae.* Mycoplasmal pneumonia is considered to be well controlled on all three farms. South African piggeries are free of TGE, PRRS and swine influenza. Circovirus 2 is suspected to be present due to the high seropositivity of the national herd.

The weaner accommodation is environmentally controlled and the weaners are either housed in groups of more than 300 or in pens with around 15 weaners per pen. The 70 day weight on all the farms is more than 26kg. The weaner accommodations are all fully-slatted and most of the farms only make use of in-water medication. The earliest mortality can occur at 9 weeks of age.

Four of the farms buy in weaner pellets while the 5th farm feeds the weaners a specialized home mixed meal. All five farms mix their own grower rations and the rations are a maize-soya based ration. Ration formulations are done by nutritionists and veterinarians.

The pigs move to the grower facilities when they are between 10 and 12 weeks old. At this stage they receive a grower ration with an energy and protein content suitable for this period of their life. The grower buildings are naturally ventilated and curtains are controlled with a computer. Four of the farms have fully slatted floors and one farm has mostly half-slatted floors with the newer buildings being fully-slatted. All of the farms have single pig feeders. Boars and gilts are either separately housed or they are kept in separate groups. Boars and gilts do not receive different diets. All the farms feed a grower ration containing Paylean (Elanco) additive 4 weeks prior to marketing. Pigs are marketed from 19 – 22 weeks of age with the boars generally being marketed first and the gilts last. Average carcass mass range from 75kg – 82kg. The average daily gain on all

these farms is more than 880g, with three farms reporting an ADG in excess of 1kg for the grower period.

Necropsies were done on commercially reared, grower pigs (aged from 9 weeks upwards) that have died acutely on farms under study. Necropsies were not carried out on pigs that were dead for more than 8 hours. The reason for this is that the rate of decomposition is accelerated in cases of acute haemorrhagic bowel syndrome and this would have had a detrimental effect on bacteriological as well as histopathological results.

Experimental design

Necropsies

Pig farmers were asked to contact the researcher if grower pigs died acutely and the following criteria were met:

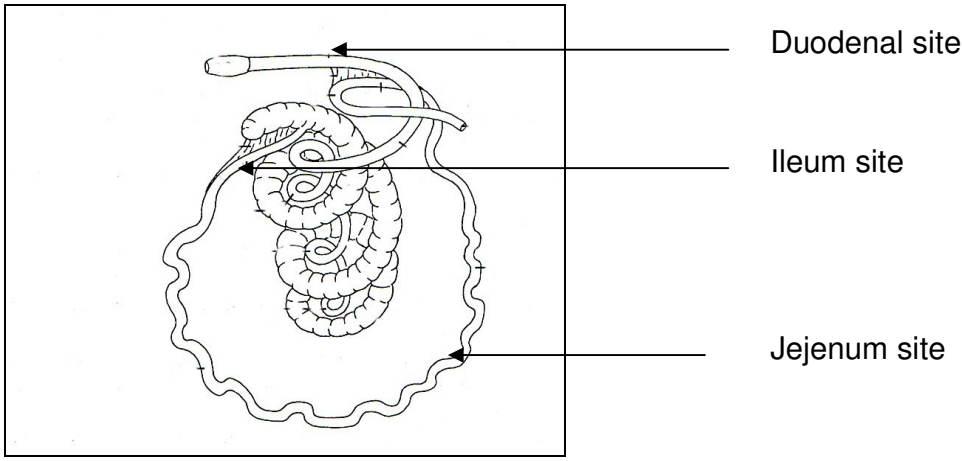
- Pigs weighed between 50 – 110kg,
- Severe abdominal distension was present shortly after death.

The researcher did most of the necropsies herself. When this was not possible she arranged with a colleague to collect the samples. Necropsies were carried out in the following way:

- Age of the animal was recorded.
- The colour of the skin and mucous membranes were examined first. Two of the differential diagnoses – gastric ulcers and acute haemorrhagic bowel syndrome due to *L. intracellularis* – cause severe blood loss into the intestines. Therefore the carcass is very pale.
- The pig carcass was placed on its back and a midline incision was made. The incision started at the cranial part of the sternum and ended as far caudal in the abdominal area as possible.
- If the carcass were severely distended due to gas accumulation in the intestines, special care was taken when making the midline incision as the intestines could have been easily punctured. If the intestines are punctured, the entire abdominal cavity will be filled with a bloody fluid, making proper examination of the abdominal cavity very difficult.
- Once the incision was made the abdominal cavity and its organs were examined for any abnormalities. Particular care was taken to determine whether the organs were not abnormally displaced. This was to rule out intestinal torsion and mesenteric volvulus.
- The degree of gas accumulation in the intestines and the colour of the entire intestinal tract were assessed.

- Thereafter the intestinal tract was removed. The stomach was opened and the type of contents were examined and recorded to determine whether the pig had been eating and for the presence of digested/fresh blood. The stomach wall was also examined for ulcers, especially at the oesophageal entrance.
- A 1cm thick slice of intestine was collected from the following areas for histopathology (see figure 1):
  - Ileum: collected 30cm proximal to the ileo-caecal valve
  - A mid-section of the jejunum
  - Duodenum: 10 cm distal from the stomach

Figure 1: Schematic illustration of collection sites



Each sample was put individually into a 30ml sample bottle filled with 10% buffered formalin in order to fix the tissue.

For bacteriology a 6cm piece of ileum taken just cranial to the histopathology sample was removed and tied off at both ends. It was then sealed in a plastic specimen jar and stored at 4°C in a polystyrene holder. These specimens reached the laboratory within 24 hours of collection.

- Samples were identified as follows: Each farm was given a letter of the alphabet (first letter of the farm's name). Each necropsy done on a farm was given a number, starting at one. Furthermore the ileal, jejunal and the duodenal sample were numbered 1, 2 and 3 respectively i.e. G1.1. Samples for bacteriology were identified by the first letter of the farm's name and the post mortem number i.e. G1.

Bacteriology

The ileum sample was used for the following tests:

Smear:

Once the contents had been removed, a mucosal scraping was made with a glass microscope slide and smeared over a clean identified slide. The slide was then stained using the Gram's staining method described in Quinn and Carter (2002)[27]. In brief, the smear was left to dry and heat-fixed. Once cool, a drop of Gram's crystal violet solution was placed on the smear and left for a minute. The slide was

flooded with Gram's iodine for 1 minute and then acetone was flooded on the smear for 20 seconds. Lastly Gram's safranin was placed on the slide for a minute. The smears were rinsed with tap water between each step of the staining procedure. The smear was allowed to dry before it was examined with a microscope using the 100x oil immersion lens. Bacteria were classified as Gram-positive, clostridial like organisms, Gram-positive *Lactobacillus*-like, Gram-positive cocci, Gram-negative *Campylobacter*-like organisms, Gram-negative rods and Gram-negative cocci. The presence of fungi was also noted. A scoring system shown in Table 3 was used to estimate the number of bacteria present.

Aerobic culture:

A mucosal scraping was smeared onto the upper third of a Columbia agar plate (Oxoid, Pty Ltd, UK CM 331) enriched with 5% citrated horse blood (BCA) and MacConkey agar without crystal violet (MAC) (Oxoid Pty Ltd, UK CM 7B). A streaking procedure to spread the bacteria was used by making use of cooled, heat sterilized platinum loops. The agar plates were incubated for 24 hours at 37℃ and examined daily for the presence of bacterial colonies. All β-haemolytic or smooth lactose fermenting and non-lactose fermenting colonies were purified by streaking them onto BCA and MAC and incubating them for 24 hours at 37℃. All Gram-negative, oxidase negative, catalase positive, fermentative, nitrate reducing bacteria were identified using the API10S test (BioMerieux, France 10100).

Salmonella enrichment and selection

Since salmonellae can be present in low numbers a serial pre-enrichment and selection in broth method was used to isolate them[27]. One gram of ileal contents was placed in 9 ml of buffered peptone water (Oxoid Pty Ltd, UK CM509) and incubated in air at 37℃, after 24 hours 1 ml of the broth culture was transferred to 9 ml of Rappaport-Vassiliadis Soya Peptone Broth (RVS) (Oxoid Pty Ltd, UK CM866) and incubated under the same conditions for a further 24 hours. A loopful of this culture was then plated onto Xylose Lysine Deoxycholate agar (XLD) (Oxoid Pty Ltd, UK CM 469) and incubated as for the previous incubations. Any black colonies surrounded by pink media were sub-cultured onto BCA and was these had been incubated overnight at 37℃, non-swarming colonies were identified using the API10S (BioMerieux, France 10100).

Anaerobic culture:

The same specimen that had been used for aerobic culturing plated onto pre-reduced BCA in the anaerobic cabinet using the same method and incubated at 37℃. After an incubation period of 24 hours colonies showing a zone of double β-haemolysis were streaked onto pre-reduced BCA to purify. Furthermore the selected colonies were also inoculated onto BCA and cultured aerobically to check for aerobic growth. Any colonies that consisted of Gram-positive large rods, that were oxidase- and catalase-negative, non-motile and were lecthinase positive, lactose fermenting but were non-proteolytic and lipase negative were considered to be *Clostridium perfringens.* Fresh cultures of each *C. perfringens* strain isolated

were emulsified in a 2.2 mℓ NUNC cryotubes® containing 1.5 mL of brain heart infusion broth (Oxoid) with 0,2% cysteine and 10% glycerol (Merck) and stored at -86℃ (Forma Scientific). Colonies that stained as Gram-negative fusiforms or spirochaetes and did not grow aerobically were identified using the mastring antimicrobial sensitivity test (Difco Laboratories) as well as API 32A (Bio Meneux, France).

Histopathology

Three samples were used for histopathology: a 1cm section of the duodenum, a 1cm section of the jejunum and a 1cm section of the ileum. The intestinal samples were fixed for at least 48 hours. The samples were processed in a Shandon Excelsior automatic tissue processor. The processed samples were embedded in paraffin wax in Tissue Tek moulds, using a Thermolyne Histo-Center 2-N paraffin wax embedding centre. Sections 4µm thick were cut form the wax blocks using a Reichert-Jung Biocut rotary microtome, floated onto warm distilled water in an electrothermal water-bath and picked up on glass slides. The sections were stained by hand with H&E. Cover slips were placed over the stained tissue and the slides were labeled. Sections were examined under a compound light microscope for the presence of microorganisms such as *L. intracellularis*, cell necrosis, haemorrhage, congestion, crypt elongation and immature endothelial cells along the entire surface of the villi.

CHAPTER 4: RESULTS

Introduction

For various reasons to be discussed later the total number of samples collected was less than the initial protocol stated. Although the results did not support the primary hypothesis of this research that *L. intracellularis* is the cause of HBS, the results are of such a nature that it could assist farmers in future to control this syndrome.

Sample collection

Thirty eight samples were collected from January 2007 until March 2007 (see Table 1). Although the initial protocol stated that fifty samples would be collected, it was not possible. Farmers did not always inform the investigator of potential necropsies. The travel distance to some of the farms had a negative influence on sample collection. Three of the units are situated in the Limpopo Province while the investigator is situated in Gauteng. By the time she would arrive at these units, the time of death would be more than eight hours ago. Because of the high ambient temperature autolysis was advanced and no samples were collected of such pigs.

Table 2: Farm profiles and number of samples collected per farm

Farm	Province	Health Status	Number of bacteriology samples	Number of histopathology samples
Farm B	Gauteng	SPF	4	4
Farm G	Gauteng	Normal	1	2
Farm H	Limpopo Province	Normal	11	11
Farm I	Limpopo Province	Normal	3	8
Farm W	Limpopo Province	SPF	10	11

Bacteriology was not done on one sample from Farm G (G2), five samples from Farm I (I4 – I8) and one sample from farm W (W8).

Age distribution of sampled pigs:

The ages of the pigs were recorded when the necropsies were done. Four of the pigs were between 9 and 10 weeks of age and weighed less than 50kg. This is different from the initial sampling plan, but the pigs had a typical HBS appearance. Five pigs were between 10 and 15 weeks of age. Nineteen pigs were between 15 and 20 weeks of age and 2 pigs were older than 20 weeks. The average age of the sampled pigs was 16 weeks and the range is from 9 – 22 weeks of age.

Bacteriology

Samples that were collected for bacteriology were cultured aerobically and anaerobically and smears were made from scrapings of the intestinal wall.

Microscopic evaluation of the ileal wall

Bacterial groups observed from Gram-stained smears of the intestinal mucosa are shown in Table 3.

Table 3: Microscopic morphology of bacteria observed in smears of ileal scrapings.

Organism morphology	Number of samples	% of samples
Gram positive squat rods (*Clostridium*-like*)	25/29	86.2%
Gram positive fine rods (*Lactobacillus*-like‡)	23/29	79.3%
Gram positive cocci§	26/29	89.6%
Gram negative coccobacilli♠	29/29	100%
Gram negative *Campylobacter*-like#	18/29	62%

Key:

* These Gram-positive bacteria were seen to be large Gram-positive or Gram-variable squat rods with rounded edges.
‡ These Gram-positive rods were long and thin
§ Although all Gram-positive cocci were recorded, most took on the shape of short chains with some pleumorphism, indicating that they mainly belonged to the Genus *Enterococcus.*
♠These bacteria are morphologically typical of coliforms which in healthy pigs tend to dominate the micriobial population of the distal intestinal tract.
These bacteria were minute curved, wing to spiral-shaped bacteria that would be primarily represented in the genera: *Campylobacter*, *Lawsonia*, *Arcobacter* and *Helicobacter.*

Aerobic cultures

Results for the aerobic culture are shown in Table 4.

Table 4: Aerobic culture results

Organism	Number of samples	% of samples
Non-pathogenic bacteria	22	75.9%
Smooth *E.coli*	7	24.1%

The non pathogenic bacteria predominantly consisted of rough, non-haemolytic *E. coli, Klebsiella, Enterobacter, Pantoea, Proteus, Staphylococcus, Enterococcus, Lactobacillus* and *Bacillus* species. Colony forms on blood and MacConkey agars that had the appearance of rough *E. coli*, *Klebsiella* species, *Pantoea, Enterobacter, Proteus, Enterococcus, Lactobacillus, Staphylococcus* or *Bacillus* species are usually considered to be non-pathogenic and were therefore not further investigated. Although pre-enrichment and selection methods were used for *Salmonella* species, none were cultured. The *E. coli* was identified using the API10S test (BioMerieux, France) and smoothness determined by the fact that the lactose-fermenting colonies were mucoid and the colony edge was smooth and entire on MAC after 48 hours of incubation. The smooth *E. coli* were not serotyped.

The smooth *E. coli* was isolated from samples from the farms shown in Table 5.

Table 5: Farms with where smooth *E. coli* were isolated from the ileum.

Farm	Number of positive samples	% of positive samples
Farm B	1/4	25%
Farm G	0/1	0%
Farm I	0/3	0%
Farm H	3/11	27.2%
Farm W	3/10	30%

Anaerobic cultures

Anaerobic cultures were done to isolate any obligate anaerobes that cause disease in pigs and included *Clostridium perfringens, C. difficile* and *Fusobacterium necrophorum.* Of these bacteria only *C. perfringens* was cultured where it was cultured from 12 (40%) of the samples. The farms of samples where *C. perfringens* was cultured is shown in Table 6.

Table 6: *Clostridium perfringens* isolations from the different farms in the study

Farm	Number of samples	% of total number of samples per farm
Farm B	2/4	50%
Farm G	1/1	100%
Farm H	2/11	18%
Farm I	0/3	0%
Farm W	6/10	60%

Comparison of the culture of *C. perfringens* to the presence of clostridial-like bacteria observed on ileal smears is shown in Table 7.

Table 7: A comparison of *Clostridium perfringens* isolation to the presence of clostridial-like bacteria on the smears of the ileal mucosa.

C. perfringens		**Smear evaluation for clostridia-like bacteria**			
cultured	**not cultured**	**0**	**+**	**++**	**+++**
12		3	2	2	5
	17	2	7	2	6

Key:
0 No clostrium-like bacteria were observed
+ Up to 5 clostridium-like bacteria were observed per high power field
++ 6 to 30 -like bacteria were observed per high power field
+++ The smear consisted almost entirely of clostridium-like bacteria

Another organism that was associated with positive *Clostridium perfrigens* isolations was smooth *E.coli* (6/11).

Macroscopic pathology

The macroscopic pathology revealed the following: Typically it was a pig that was healthy before the onset of the disease. Invariably it was one of the best pigs in the pen. The skin was pale, but otherwise there were no other external lesions. The abdomen would be severely distended, in some cases the internal pressure was so much that the rectum protruded. This never occurred while the animal was still alive. When the carcass was opened, a severely distended intestinal tract filled the abdomen. The colour of the intestinal tract varied from a light rose colour to a blackish maroon, although the first 10 cm of the duodenum appeared to be unaffected. The intestinal content consisted of a foul smelling, reddish fluid. Only the stomach would be filled with food. The liver was very pale in all the cases. None of the cases had any sign of intestinal volvulus or stomach or duodenal ulceration. No other abnormalities were present.

Histopathology

“The intestines of 36 pigs that had died of haemorrhagic enteropathy were examined histologically. Samples from each pig were taken of the proximal duodenum, mid-jejunum and ileum, and fixed in 10% buffered formalin.

Transverse blocks were cut from the fixed tissues, embedded in paraffin wax, and H&E sections prepared, as previously described. The sections were examined microscopically and the lesions were noted and evaluated.

Advanced autolysis of the sampled tissues precluded histological evaluation of the following cases:

H9 – duodenum, jejunum and ileum.

H11 – duodenum.

W1 – duodenum and jejunum.

W3 – duodenum, jejunum and ileum

W4 – duodenum.

W7 – duodenum, jejunum and ileum.

In all of the remaining cases (30) the histopathological findings were essentially identical, varying only in degree of severity. The three basic lesions that were consistently found in most cases, and to varying degrees, were: necrosis of the intestinal mucosa, and congestion and haemorrhages in the intestinal wall. Rod-shaped bacterial bacilli were usually, but not always, present on and in the intestinal mucosa.

The following analyses were made from the scores recorded in Addendum 1:

Mucosal necrosis, if present, was invariably linear, i.e. parallel to the mucosal surface and was sharply delimited from the underlying non-necrotic mucosa. Necrosis varied from absent (0%) to full-thickness necrosis of the mucosa (100%) and was scored subjectively. The most severe lesions were in the ileum and the least severe lesions were in the duodenum. The severity of necrosis per site is depicted in Figure 2.

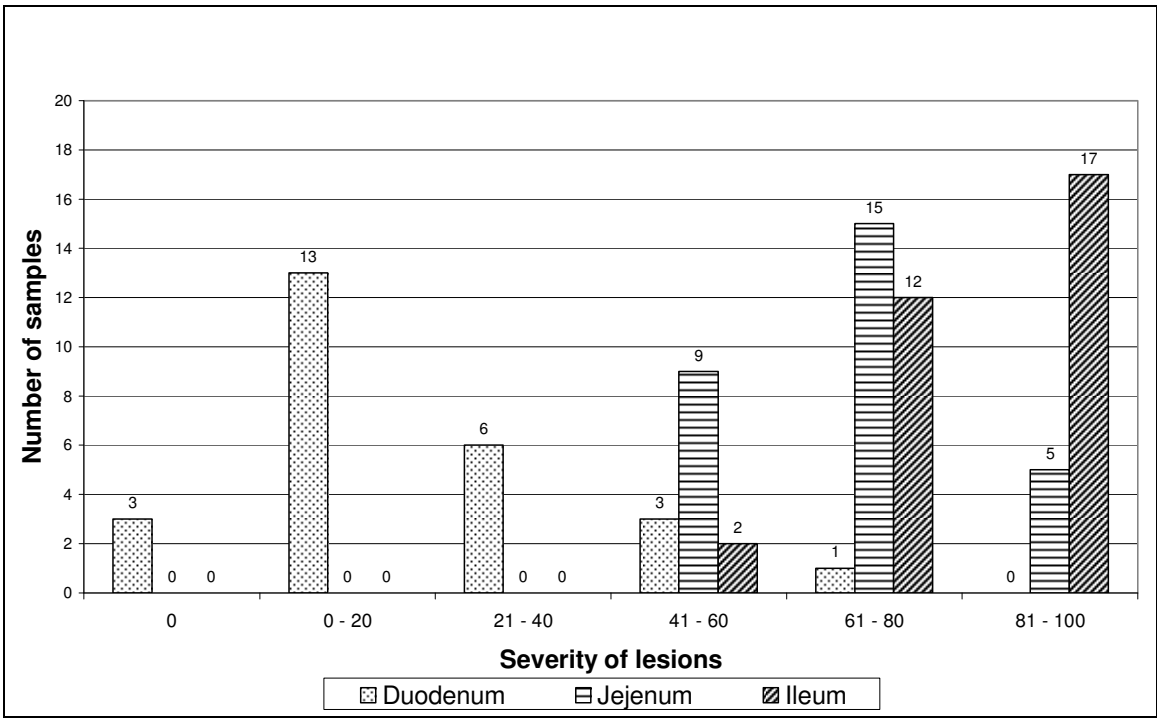


Figure 2: Severity of necrosis according to site. n Duodenum = 29, n jejunum = 32, n ileum = 33

The comparative severity of mucosal necrosis in the duodenum, jejunum and ileum was calculated by dividing the sum of the percentages of mucosal necrosis in each part of the intestine by the total number of cases (excluding autolysed samples). For the duodenum this was 19.4%, for the jejunum 69.4% and for the ileum 85.6%. It would thus appear that mucosal necrosis becomes more severe from the proximal to the distal end of the small intestine.

Congestion, if present, was noted as distended capillaries, filled with red blood cells, in the lamina propria of the intestinal mucosa as well as distended large blood vessels, filled with red blood cells, in the submucosa, muscular layers and serosa. Congestion was scored subjectively as none, mild, moderate or severe. The severity of congestion per site is depicted in Figure 3.

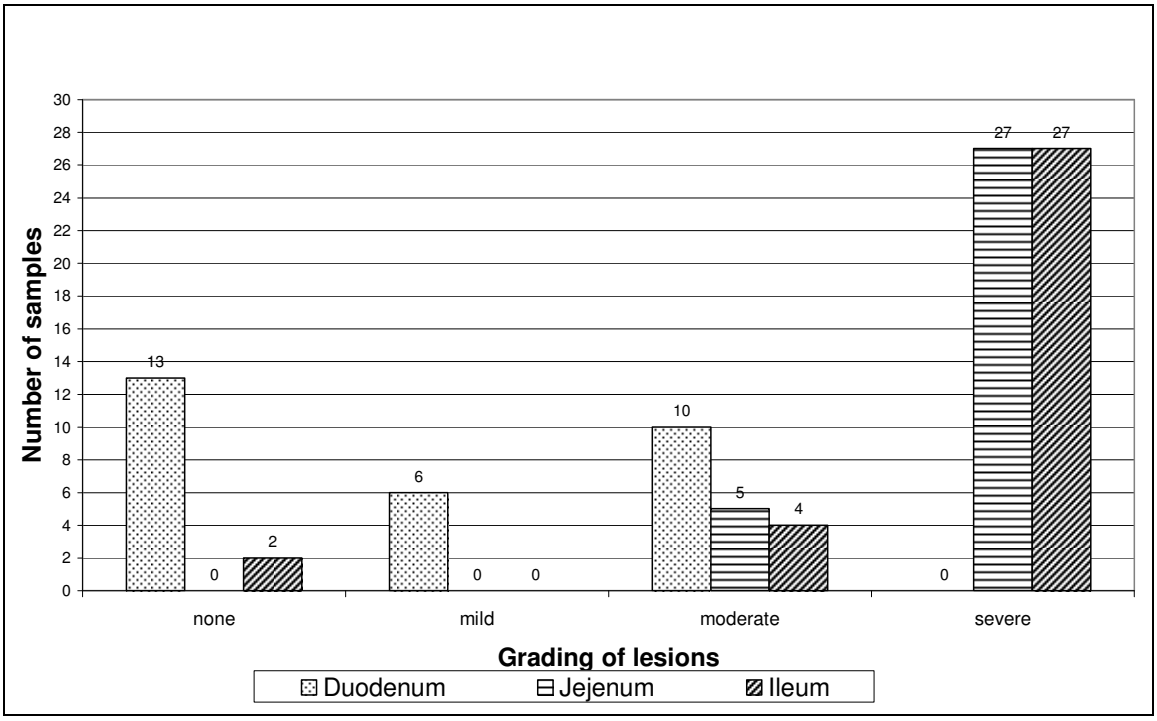


Figure 3: Severity of congestion according to site. n Duodenum = 29, n jejunum = 32, n ileum = 33

The comparative severity of congestion in the duodenum, jejunum and ileum was calculated by scoring the degree of congestion as 0 (= none), 1 (mild), 2 (moderate) and 3 (severe), summing these scores, then dividing this number by the number of cases (autolysed cases were excluded). For the duodenum this index was 0.9, for the jejunum 2.85 and for the ileum 2.71. The degree of congestion was therefore mildest in the duodenum and more severe, but similar, in the jejenum and ileum.

Haemorrhage, if present, was noted to be almost diffuse in the lamina propria of the mucosa, usually in association with the necrotic epithelium. Haemorrhage was

scored subjectively as none, mild, moderate or severe. The severity of haemorrhage in the different intestinal sites is shown in Figure 4

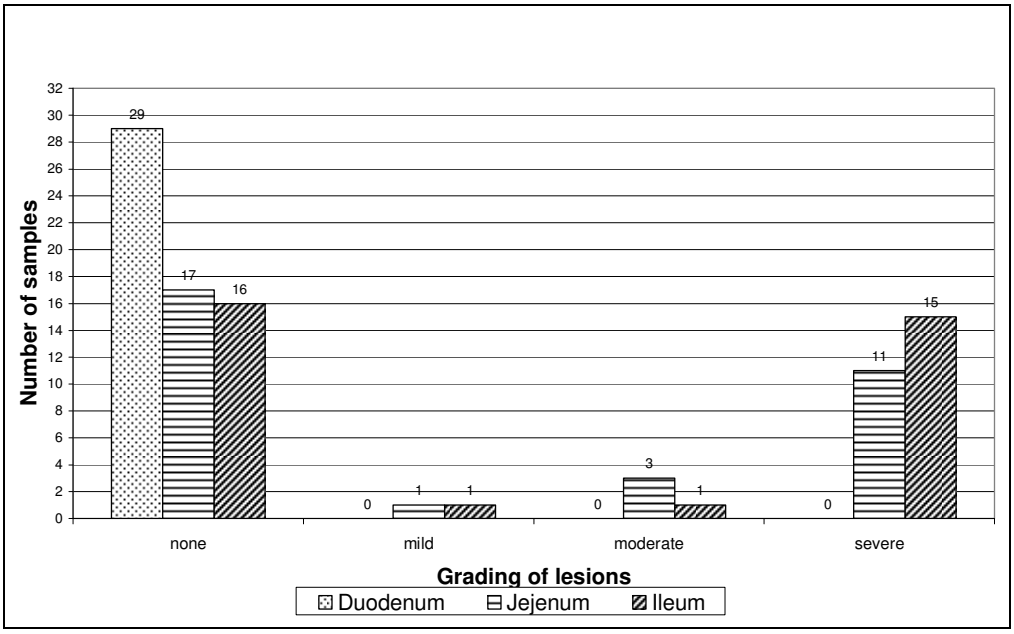


Figure 4: Severity of haemorrhage according to site. n Duodenum = 29, n jejunum = 32, n ileum = 33

The comparative severity of haemorrhage in the duodenum, jejunum and ileum was calculated by scoring the degree of haemorrhage as 0 (= none), 1 (mild), 2 (moderate) and 3 (severe), summing these scores, then dividing this number by the number of cases (autolysed cases were excluded). For the duodenum this index was zero, for the jejunum 0.94 and for the ileum 1.5. Thus, haemorrhage was absent from the duodenum, intermediate in the jejenum and most severe in the ileum.

Organisms were mostly found on the surface of the mucosa, in clumps of varying size. Such groups of bacilli were often embedded within sloughed or attached bits of necrotic mucosa. The morphology of the bacilli was typical of bacteria of the genus *Clostridium*, namely large brick-like organisms, which stained pale purple with H&E. The number of organisms present was scored subjectively as none, few, moderate or many. The distribution of organisms per site is depicted in Figure 5.

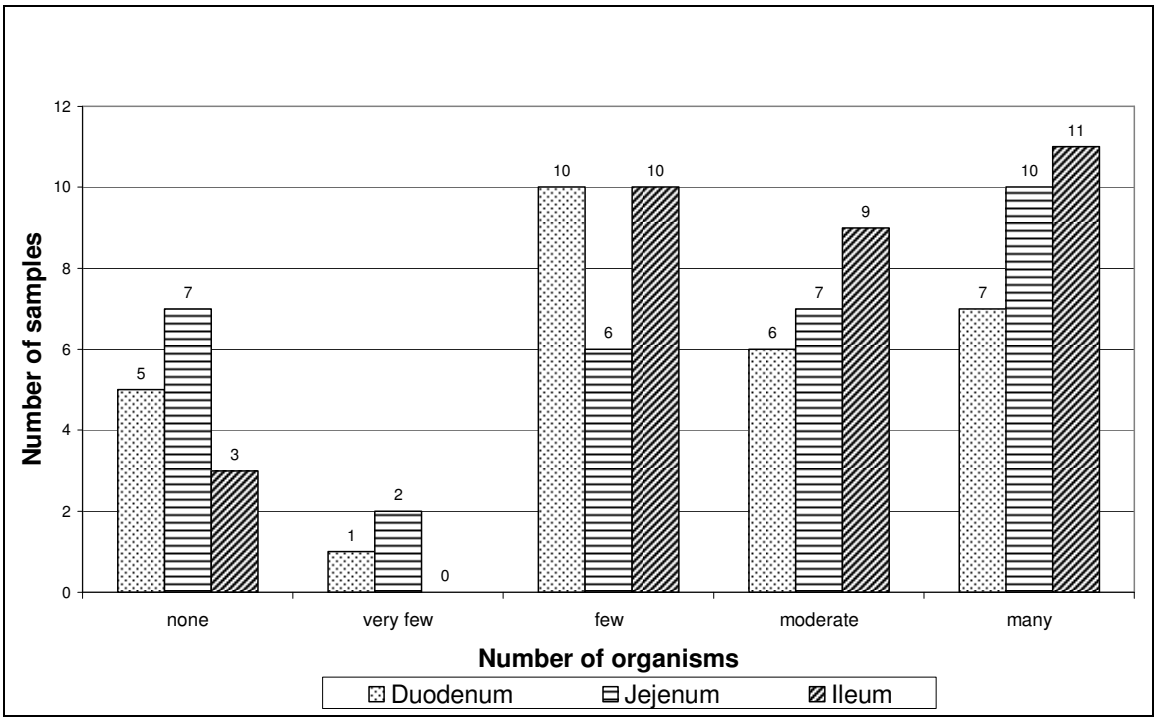


Figure 5: Number of organisms per site. n Duodenum = 29, n jejunum = 32, n ileum = 33

The comparative number of clostridial organisms in the duodenum, jejunum and ileum was calculated by scoring the number of organisms as 0 (= none), 1 (few), 2

(moderate numbers) and 3 (many), summing these scores, then dividing this number by the number of cases (autolysed cases were excluded). For the duodenum this index was 1.4, for the jejunum 1.62 and for the ileum 1.77. There is a tendency for the numbers to increase from the proximal to distal small intestine, but the differences are not significant. 84% of the submitted samples did have clostridial like organisms on the mucosal surface.

Necrosis of the intestinal mucosa, congestion and clostridial organisms were present in every case but could not be evaluated in three pigs (H9, W3, W7) because of advanced autolytic changes in the tissues. Haemorrhage was noted in 20 of the 37 non-autolysed cases but was absent in the samples of 12 of the pigs with “haemorrhagic enteropathy”.

CHAPTER 5: DISCUSSION

Previously HBS accounted for only 2% - 5% of grower mortality, but in the past 5 years it has become much more prevalent. Analysis of on-farm records revealed that up to 60% of all mortalities during the growing phase were attributed to HBS. Pigs from 9 – 22 weeks of age were affected with the majority between 15 and 20 weeks of age. This is the period when pigs are growing at their fastest. This syndrome seldom affects a group of pigs from the same pen or age group. Usually mortalities are spread over different age groups and it always appears to be the fastest growing pig in the pen that is affected. Prior to onset of the first clinical signs, such as vocalising and abdominal distension, the animal has been in perfect health. These clinical signs are very similar to those seen in other species such as feedlot cattle and dairy cows that were found to have suffered from a clostridal infection secondary to environmental and behavioural factors[9,19,41]. It also contrasts to PHE which usually affects more than one pig in a pen and where the first clinical sign is usually profuse bloody diarrhoea instead of a dead pig[18].

The macroscopic pathology also revealed a different picture from that usually seen when a pig dies because of acute PHE. The carcasses had severely distended abdomens, which on incision revealed intestines filled with a reddish, very watery content. Severe gas accumulation was responsible for the distension. The entire intestinal tract was discoloured red, of which the intensity of the discolouration

could vary between cases. The stomach was always filled with food with little or no food in the small or large intestines indicating that the death was peracute. A typical PHE case presents as a pale carcass with little or no abdominal distension. When the carcass is opened, the intestinal tract is filled with a tarry content[18]. The volume of food in the stomach is, however, usually less in cases of PHE than what was described for the pigs in the study.

Although the farms in this area all have a high seroprevalence of ELISA antibodies to *Lawsonia intracellularis*[15] and this bacterium is considered as a possible cause of HBS by the literature[31], the histology findings of this study paint a different picture.

The histopathological lesions were not those typically ascribed to PHE i.e. elongation of infected crypts with a proliferation of the large immature epithelial cells. Often there is cellular and proteinous debris in affected crypts and *L. intracellularis* organisms are found in the cytoplasm of infected epithelial cells. The shape of the organism is curved. Furthermore there are acute inflammatory changes such as interstitial oedema, accumulation of neutrophils lymphocytes, mononuclear cells and macrophages in the lamina propria immediately surrounding the affected crypts[10]. The lesions are usually restricted to the mucosa of the ileum[18]. The histopathology done on samples from this study revealed 3 basic lesions that were found consistently in most cases only varying in degree between different cases as well as per site. Generally the ileum was the most

severely affected and the duodenum least affected. The three lesions were necrosis of the intestinal mucosa and congestion with haemorrhages in the intestinal wall. Rod-shaped bacterial bacilli were usually, but not always, present on and in the intestinal mucosa. This type of lesion confirms that *L. intracellularis* is unlikely to be the primary cause of HBS. Instead this type of histopathology is usually seen when a *Clostridium* spp. is the cause of death[34].

Because *E. coli* is considered as a cause for HBS in some literature[36], this study investigated the importance of the bacterium in the HBS disease complex. *Escherichia coli* was only isolated from 25% of the cases. Neither the clinical signs nor the age group affected were typical for an *E. coli* infection. Enteric *E. coli* is usually manifested by diarrhoea. In severe cases one would see dehydration and death. Neonates and newly weaned pigs are usually affected by *E. coli* infections. Pathogenic *E. coli* can colonise the intestinal tract of older pigs, but it usually does not cause disease. When older animals are affected, they are usually immune compromised as pigs build up an immunity against *E. coli*. Histopathological lesions seen from *E. coli* cases may include vascular congestion in the lamina propria with some haemorrhages into the lumen with some villous atrophy. Although this is similar to the histopathology seen in this study, the overall presentation of HBS does not correlate with what is seen during an *E. coli* infection[6].

The bacteriological and histopathological findings suggest that one of the *Clostridium* species might be involved in the pathogenesis of HBS. *Clostridium perfringens* was isolated from 40% of submitted intestinal mucosal scrapings. Histopathology revealed organisms on the surface of the mucosa in 84% of the submitted samples. These organisms had morphology typical of the genus *Clostridium*, namely large brick-like organisms which stained light purple with H&E. Although *C. perfringens* is not generally a difficult organism to culture (although it is easily overgrown by commensal bacteria), the toxins, especially alpha toxin, is broken down a short period after death[21]. The fact that *Clostridium*-like organisms were seen on some of the histopathological slides, but cultures from the intestinal content of that animal were negative for *Clostridium* spp, suggests that one will have to re-evaluate the preservation methods of possible necropsies as well as the sampling methods. Selective mediums must also be used to culture specifically for *Clostridium* spp. Because *C. difficile* is difficult to recognize on culture, but it would be present on slides it would be best to identify toxins to differentiate between the different *Clostridium* spp.

The most likely cause of HBS in this study is *Clostridium perfringens*. For some reason this aetiology has never been reported in pigs as a definite cause of HBS. Modern genetics has resulted in a commercial pig with improved average daily gain (ADG), feed conversion ratio (FCR) and leaner meat but maybe because of this they are more predisposed to metabolically induced diseases such as HBS. Pigs are probably pushed to their physiological limit from an early stage, which

would explain why 4 pigs weighing less than 50kg were also affected. Furthermore these pigs cannot tolerate mistakes in management nor feeding[4], especially when rations are high in carbohydrates, which under certain circumstances can lead to the proliferation of *C. perfringens*[26]. Although the pig farms where the study has been performed are well managed farms, one cannot ignore the impact of out-of-feed events. It may not be actual out-of-feed events, but because of the extremely high temperatures experienced in summer on all of these farms, pigs might not be feeding as they should during the day, and only start feeding properly at night. This could lead to over engorgement which in turn could lead to enteric microflora proliferation, intestinal stasis or mechanical torsion. This is supported by the fact that most of the deaths are discovered first thing in the morning with mortalities probably occurring early (4am) in the morning. The above theory might explain why HBS is more prevalent in summer. Further research will be necessary to determine whether virulent strains of *C. perfringens* displace normal intestinal *C. perfringens* as was shown in the case of poultry NE. If that is the case, factors enhancing the growth of virulent strains will have to be identified in order to manage the disease. Since the main ingredients of the diets fed on all the farms is maize-soya, one can also speculate on the importance as to the β2-toxin in the aetiology of this disease. Are affected pigs hypersensitive to soya, causing a decrease in trypsin activity? As mentioned earlier, trypsin renders the β2-toxin inactive. Under South African circumstances, in-feed medication with chlortetracycline does lower the incidence of the disease although it does not stop

it completely. This antimicrobial probably prevents over proliferation of clostridial species.

In this study, contrary to the initial hypothesis, the indication is that *C. perfringens* can play a key role in HBS. The non-contagious nature of the disease as well as the fact that mainly pigs in good condition indicate that trigger factors are required to stimulate the overgrowth of C. perfringens which as a result of rapid growth and toxin production leads to acute deaths. This may be as a primary pathogen or with other trigger mechanisms. The influence of genetics, management and nutrition on each other in relation to HBS will have to be explored further. Although the pigs in this study were of the same genetic make-up, the growth rates experienced on these farms are of the highest in the country. Generally farms with lower growth rates do not have very high mortalities due to HBS, regardless of the genetics used on the farm. The ability to grow fast certainly plays a role, but to get the full benefit of fast growth, management needs to be very good. Further studies need to be done to investigate possible toxins, as well as other trigger factors that might predispose a pig to HBS.

Recommendations

Since this was a preliminary study with an unexpected outcome, in that *C. perfringens* may well be a cause of HBS in these pigs, it is critical that certain facets of the disease are explored further.

It is essential that the clostridial toxin-types and toxins involved in these cases are fully elucidated as this will open up the possibility of preventing the disease by vaccination. Once the toxin types have been determined a vaccine trial should be done to determine the protectiveness of the current vaccines.

To achieve this, the preservation and culturing techniques will have to be modified to improve the isolation of clostridia. This would incorporate the use of selective media for the isolation of this bacterium as well as ensuring that bacteria remain persevered in an anaerobic state. Another sample that would assist in the diagnosis would be frozen intestinal content for the ELISA test for *C. perfringens* toxin type determination. A PCR test on samples that have undergone an overnight enrichment step should also be considered. A direct PCR test on the samples would be too insensitive as the distribution of *C. perfringens* can be patchy on the intestinal mucosa.

Since it is believed that environmental, managemental and possibly genetic factors play a predisposing role in HBS[36], a year long case-control study should be done to compare farms that suffer from a high prevalence of HBS to those that do not have it or have it at a very low level.

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Addendum 1: Lesions in the intestines of affected pigs

The three suffixes for each S number (histopathology laboratory numbers) are A = duodenum; B = jejenum; C = ileum.

PIG NO.	S NO.	Necrosis	Congestion	Haemorrhage	Organisms
B1	2726.07A	none	none	none	moderate
	2726.07B	50%	severe	severe	moderate
	2726.07C	100%	severe	severe	many
B2	2727.07A	10%	none	none	few
	2727.07B	50%	severe	severe	few
	2727.07C	50%	severe	severe	few
B3	2728.07A	10%	mild	none	few
	2728.07B	50%	severe	severe	moderate
	2728.07C	75%	severe	severe	many
B4	2729.07A	10%	none	none	few
	2729.07B	50%	severe	severe	very few
	2729.07C	75%	severe	severe	moderate
G1	2730.07A	20%	none	none	many
	2730.07B	75%	severe	none	many
	2730.07C	75%	severe	severe	moderate
G2	2731.07A	75%	moderate	none	many

PIG NO.	S NO.	Necrosis	Congestion	Haemorrhage	Organisms
	2731.07B	100%	moderate	none	many
	2731.07C	100%	moderate	none	many
H1	2732.07A	10%	moderate	none	v. few
	2732.07B	75%	severe	none	few
	2732.07C	80%	severe	none	few
H2	2733.07A	autolysed	none	none	many
	2733.07B	75%	severe	none	few
	2733.07C	100%	severe	none	many
H3	2734.07A	25%	moderate	none	moderate
	2734.07B	50%	severe	none	moderate
	2734.07C	100%	severe	none	moderate
H4	2735.07A	10%	mild	none	few
	2735.07B	75%	severe	none	few
	2735.07C	90%	severe	none	few
H5	2736.07A	33%	mild	none	moderate
	2736.07B	50%	severe	none	none
	2736.07C	100%	severe	severe	moderate
H6	2737.07A	10%	none	none	few
	2737.07B	75%	severe	mild	many
	2737.07C	100%	severe	none	moderate
H7	2738.07A	10%	mild	none	few

PIG NO.	S NO.	Necrosis	Congestion	Haemorrhage	Organisms
	2738.07B	75%	severe	none	many
	2738.07C	100%	severe	severe	few
H8	2739.07A	10%	mild	none	few
	2739.07B	75%	severe	severe	moderate
	2739.07C	100%	severe	severe	moderate
H9	2740.07A	autolysed	autolysed	autolysed	autolysed
	2740.07B	autolysed	autolysed	autolysed	autolysed
	2740.07C	autolysed	autolysed	autolysed	autolysed
H10	2741.07A	10%	none	none	many
	2741.07B	100%	severe	none	many
	2741.07C	100%	none	none	moderate
H11	2742.07A	autolysed	autolysed	autolysed	autolysed
	2742.07B	75%	severe	none	many
	2742.07C	100%	severe	none	few
I1	2743.07A	25%	none	none	few
	2743.07B	75%	moderate	none	many
	2743.07C	75%	severe	none	moderate
I2	2744.07A	25%	moderate	none	few
	2744.07B	50%	severe	severe	few
	2744.07C	50%	severe	severe	none (yeasts)
I3	2745.07A	10%	none	none	moderate

PIG NO.	S NO.	Necrosis	Congestion	Haemorrhage	Organisms
	2745.07B	50%	moderate	none	many
	2745.07C	75%	severe	severe	many
I4	2746.07A	10%	moderate	none	moderate
	2746.07B	75%	severe	severe	few
	2746.07C	100%	severe	severe	moderate
I5	2747.07A	25%	moderate	none	few
	2747.07B	75%	severe	severe	none (yeasts)
	2747.07C	75%	severe	severe	none (few yeasts)
I6	2748.07A	50%	moderate	none	many
	2748.07B	75%	severe	severe	moderate
	2748.07C	50%	none	none	moderate
I7	2749.07A	25%	moderate	none	none
	2749.07B	50%	severe	severe	none
	2749.07C	75%	severe	severe	few
I8	2750.07A	0%	none	none	many
	2750.07B	50%	severe	severe	none
	2750.07C	80%	severe	severe	few
W1	2752.07A	autolysed	autolysed	autolysed	few
	2752.07B	autolysed	autolysed	autolysed	many
	2752.07C	100%	severe	none	many
W2	2753.07A	50%	none	none	none

PIG NO.	S NO.	Necrosis	Congestion	Haemorrhage	Organisms
	2753.07B	90%	moderate	none	none
	2753.07C	100%	moderate	none	many
W3	2754.07A	autolysed	autolysed	autolysed	autolysed
	2754.07B	autolysed	autolysed	autolysed	autolysed
	2754.07C	autolysed	autolysed	autolysed	autolysed
W4	2755.07A	autolysed	autolysed	autolysed	none
	2755.07B	80%	severe	none	none
	2755.07C	90%	severe	none	many
W5	2756.07A	20%	none	none	many
	2756.07B	50%	severe	moderate	many
	2756.07C	90%	severe	severe	few
W6	2757.07A	0%	none	none	none
	2757.07B	50%	moderate	none	moderate
	2757.07C	75%	severe	none	few
W7	2758.07A	autolysed	autolysed	autolysed	autolysed
	2758.07B	autolysed	autolysed	autolysed	autolysed
	2758.07C	autolysed	autolysed	autolysed	autolysed
W8	2759.07A	50%	none	none	moderate
	2759.07B	75%	severe	none	moderate
	2759.07C	75%	moderate	none	many
W9	2760.07A	10%	moderate	none	none

PIG NO.	S NO.	Necrosis	Congestion	Haemorrhage	Organisms
	2760.07B	75%	severe	moderate	few
	2760.07C	75%	severe	mild	none
W10	2761.07A	20%	mild	none	moderate
	2761.07B	100%	severe	none	many
	2761.07C	100%	moderate	none	few
W11	2762.07A	10%	moderate	none	none
	2762.07B	90%	severe	moderate	none
	2762.07C	90%	severe	moderate	moderate