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Application of probiotics for the treatment of neonatal diarrhoea in piglets

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1. Introduction

Neonatal diarrhoea in piglets poses a common challenge for many pig herds. Frequently the aetiology of the disease is of an infectious nature (like enterotoxigenic *Escherichia coli*, *Clostridium perfrigens* type A/C, *Clostridium difficile*, rota- and coronaviruses), which is often predisposed by factors like insufficient colostrum uptake, poor housing conditions (i.e. hygiene, air-conditioning, overcrowding) and environmental changes. *Isospora suis* has also been described as a causative agent of neonatal diarrhoea (Zimmerman et al. 2012).

In the past neonatal diarrhoea has often been treated with antibiotics, in order to prevent the spread of the disease to pen mates or bacterial co-infections in case of a primary viral infection. However, as the application of antibiotics is ineffective in the case of viral and parasitic infections and as it poses the risk of the development of bacterial resistance, which could potentially have a disastrous impact on the economy and public health, attempts have been made to find alternatives. These include pre- and probiotics, organic acids (Kreuzer et al. 2012) and essential oils, which also exhibit some antimicrobial activity (Lambert et al. 2001; Mahmoud et al. 2016). There are several studies that "show positive effects of probiotics against microbial infections" (Kreuzer et al. 2012) in pigs and that they even affect the shedding of enteric viruses.

In this study the effect of the combined application of essential oils, electrolytes, probiotics and vitamins in piglets suffering from neonatal diarrhoea is investigated and compared to an antibiotic treatment with respect to the duration of the disease, as well as the average daily gain of the animals, starting from the onset of clinical signs until weaning. The reasoning behind this approach is to improve the intestinal flora with the help of probiotics in order to prevent the overgrowth of pathogenic bacteria, as well as restoring the electrolytes lost as a side effect of the diarrhoea.

For this purpose, the preparations Entero-VET® Suis mini (PM VET, Jessen) containing essential oils, and BactoLyt-VET® Pecus (PM VET, Jessen), a combination of electrolytes, *Bacillus subtilis*, *Bacillus licheniformis* as well as Vitamin A, D & E were selected. Baytril® 1nject (Bayer AG, Leverkusen), which is a 100mg/ml solution of enrofloxacin with a long-acting adjuvants preparation, was chosen for the antibiotic treatment. It is a well-known and -established product on the market and was therefore chosen as a standard.

2. Literature review

2.1 Causative agents of neonatal diarrhoea in pigs

2.1.1 Clostridiosis

Clostridia are, with some exceptions, strict anaerobic Gram-positive, rod-shaped, spore-forming pathogenic bacteria. They can be found worldwide, residing in the environment and in the intestinal tract of mammals. Depending on the disease they cause, clostridia species can be divided into three groups: the gas-gangrene diseases, the enterotoxaemic diseases and the intoxication causing clostridia. In this thesis we will focus on the ones causing enteric infections, namely *C. perfringens* and *C. difficile*. *C. perfringens* is further divided into the five toxinotypes A-E, based on the production of major toxins (α-, β-, ε- and ι-toxin) (Zimmerman et al. 2012).

2.1.1.1 *Clostridium perfringens* type C

C. perfringens type C is the causative agent of the "Neonatal haemorrhagic and necrotic enteritis". It can be transmitted between the pen mates, however, as it is also present in the sow's intestinal flora, the usual infection happens via the faeco-oral route. Even though it is a primary pathogen, it can colonise lesions caused by other enteric diseases, such as the transmissible gastroenteritis, the porcine epidemic diarrhoea and rotavirus infection. Due to its short generation time *C. perfringens* is able to outcompete other bacteria present in the piglet's microflora. The organism first attaches to the villous apices of the jejunal epithelial cells, which are then desquamated. Proliferation towards the basal membrane and the production of β-toxin, a heat-labile, trypsin-sensitive exotoxin, ensue. β-toxin is responsible for the resulting extensive necrosis, which advances towards the crypts and, in severe infections, might even reach as far as the muscular layers. In that case perforation of the jejunum can occur, leading to peritonitis and emphysema in the muscle layers as well as the mesenteric lymph nodes. As a result of the necrosis, the bacteria adhering to the villi can be shed into the intestinal lumen along with cellular debris and blood. Sporulation can be occur and those spores will be shed via the faeces, leading to the infection of the pen mates (Zimmerman et al. 2012).

Due to the presence of protease inhibitors in the colostrum and a trypsin secretion deficiency, piglets under four days of age are especially susceptible to the β-toxin. Therefore, piglets usually are affected peracutely by *C. perfringens* type C within the first three days of life. They may develop haemorrhagic diarrhoea, are weak and reluctant to move, which increases the chances of being crushed by the sow, while also decreasing the milk uptake further weakening the piglets. As the condition deteriorates, the rectal temperature falls to 35°C and death can ensue within 12-36 hours even without developing diarrhoea. Usually necrotic lesions can be found in the jejunum and ileum, but can reach from a few centimetres' posterior of the pylorus to the proximal colon. Due to the haemorrhages as a side effect of the necrosis, bloodstained fluid can be observed in the abdominal cavity. During microscopic examination clostridial rods can be found covering the necrotised villi. Necrosis of the crypt epithelium and profuse haemorrhages within the mucosal and submucosal layer are observable as well (Zimmerman et al. 2012).

Should the piglets survive the first one to two days of the onset of clinical signs, they are regarded as acutely infected. These piglets may suffer from dehydration as a result from the diarrhoea, which appears "reddish-brown containing grey shreds of tissue debris" (Zimmerman et al. 2012). In the case of an acute *C. perfringens* type C infection the gross lesions are much more localised compared to the peracute phase. The emphysema appears sharply demarcated in the jejunum. Due to vast endothelial damage, caused by thrombosis, fluid accumulation and fibrinous peritonitis can be observed. Adjacent segments of the jejunum might adhere loosely to each other. The intestinal walls are thickened and appear yellow-greyish. Microscopic examination reveals a deeper invasion of Gram-positive bacteria into the intestinal wall (Zimmerman et al. 2012).

In the subacute phase of the infection, the affected piglets appear active and appetent, but due to the villous atrophy and the resulting malabsorption and maldigestion they become more and more emaciated. The diarrhoea is no longer haemorrhagic and may appear yellow at first, eventually becoming clear. Necrotic debris might also be present in the faeces. A necrotic membrane is covering the mucosal surface, to which it is adhering tightly (Zimmerman et al. 2012).

Pigs that suffer from a chronic infection frequently show signs of intermittent diarrhoea. The diarrhoea is of yellow-greyish colour and mucoid consistency. Even though the piglets usually show a good appetite, they still might succumb to the disease after several weeks. Additionally, the average daily gain is greatly decreased and as this has a great economic impact on the farm, they might be euthanized. Gross lesions in chronic cases are not as distinct as in the subacute phase. Locally a thickening of the intestinal wall can be observed, as well as adhesion of necrotic membranes to demarcated areas (Zimmerman et al. 2012).

Epidemiological data, clinical signs and the gross lesions are sufficient for a preliminary diagnosis. However, in order to distinguish type C enteritis from diseases that manifest similar clinical signs, especially type A enteritis, a bacteriological culture from intestinal contents and mucosal lesions has to be made. Other methods include the toxin detection and genotyping. Large Gram-positive rods can be seen during microscopic examination of the intestinal lesions (Zimmerman et al. 2012).

Due to the rapid course of the disease, there is no treatment available for animals already showing clinical signs. That is why prevention of the disease is the common approach. It can be done by vaccinating the sow with the anatoxin at insemination or midgestation again 2-3 weeks ante partum. So far toxoid vaccines have shown good results, usually leading to an elimination of the disease within one farrowing cycle. Colostrum uptake is of great importance, as the piglets will benefit from passive immunisation. For the following farrowing, it is enough to boost the sow 2-3 weeks before farrowing (Zimmerman et al. 2012).

2.1.1.2 *Clostridium perfringens* type A

In contrast to *C. perfringens* type C infection, which results in the production of two major toxins (α-, β-toxin), type A only produces the α-toxin (CPA) as its major toxin. The β2-toxin may act as a virulence factor, as its presence has been linked to the occurrence of neonatal diarrhoea in piglets, allowing for its encoding gene to “be used as a virulence marker in the diagnosis of CPA-associated diarrhoea” (Silva et al. 2013).

C. perfringens type A is present in the microflora of the sow and the environment. Therefore, the infection usually takes place within the first few days of age, however not all strains cause the disease. Only the presence of β2-toxin may possibly indicate the pathogenicity, as most of the strains that can be found either in the gut or the environment do not possess β2-toxin and are not pathogenic. The pathogenesis is thought to be multifactorial, but not yet fully understood. In contrast to type C infection, direct information about the role of the toxins is not available (Zimmerman et al. 2012).

Piglets suffering from the infection develop creamy diarrhoea. Also, the hair coat is rough, due to decreased nutrient absorption. Over the duration of the disease the diarrhoea eventually becomes mucoid and pinkish. In most cases recovery can be observed, however the affected piglets tend to develop worse than their healthy pen mates all the way through the growing and finishing phase (Zimmerman et al. 2012).

Pathological findings show thin walled, flaccid small intestines that are filled with gas and watery content but without blood. The mucosal surface is only mildly inflamed. Under microscopic examination the villi may appear superficially necrotized apically with fibrin accumulation, while they appear completely normal in other cases. Large amounts of bacteria can be found in the lumen, but occasionally the jejunal and ileal lesions are also heavily colonized. The large intestines are usually filled with creamy content and might be distended, but do not have lesions (Zimmerman et al. 2012).

The diagnosis of type A enteritis is rather complicated, as the differentiation between the normal gut flora and pathogenic strains is impossible (Silva et al. 2013). Clinical signs and bacterial isolation from jejunal and ileal contents may support a diagnosis. Genotyping reveals positivity for β2-toxin of *C. perfringens* type A in almost all cases. Another important factor for a distinct diagnosis would be the absence of other relevant pathogens (Silva et al. 2013). Using microscopic examination the bacterium might also be detected covering the jejunal lesions (Zimmerman et al. 2012).

Antibiotic treatment of type A enteritis is possible; however, it is best to prevent the disease altogether by reducing the number of enteric pathogens in the environment, which can be accomplished by regular, thorough disinfection and washing the sow before farrowing. Ensuring sufficient colostrum uptake is also highly important. Studies have been conducted on the use of autogenous toxins for the prevention, but the efficacy for the prevention of the disease was not evident (Silva et al. 2013).

2.1.1.3 *Clostridium difficile*

Due to its non-invasive nature *C. difficile* must colonise the caecum or colon and produce toxins in order to cause the disease. Normally colonisation is prevented by the established microflora, but in the case of antibiotic treatment, the colonic microflora is disrupted, allowing *C. difficile* to colonise the areas void of natural flora. This is why *C. difficile* infections are commonly linked to antibiotic-associated diarrhoea. Other factors like stress, mycotoxins in the diet and a high number of clostridial spores in the environment also serve as predisposing factors (Edwin H. Waters et al. 1998).

The disease typically affects piglets within the first week of life. This can be explained by the fact that the microflora of the piglet is still in development. Upon oral uptake and colonisation, *C. difficile* produces two major toxins - toxin A (TcdA), an enterotoxin and toxin B (TcdB), a cyto- and enterotoxin. So far, there is no evidence that shows the binding of toxin B to any tissue of neonatal pigs or producing lesions in the intestines, which is why toxin A is believed to be the sole mediator of the disease (Zimmerman et al. 2012).

Lesions usually include oedema formation in the mesocolon. This is due to the effect of toxin A, which allows extravasation of albumin, other plasma proteins and fluid by inducing endothelial retraction. As a result of albumin leaking into tissue spaces, plasma colloidal osmotic pressure decreases, while the tissue colloidal osmotic pressure increases, which can lead to the formation of hydrothorax, ascites and the aforementioned oedema (Edwin H. Waters et al. 1998). Microscopic examination frequently reveals the invasion of mononuclear inflammatory cells and neutrophils into the oedematous tissue. The colonic mucosa may show signs of segmental erosions and volcano lesions, which can be explained by the exudation of neutrophils and fibrin into the lumen. The faeces appear yellowish and has a party to watery consistency. Prevalence in individual herds can be as high as 100% with up to 50% affected animals per litter, but not all animals that are toxin positive necessarily develop clinical signs (Zimmerman et al. 2012).

The detection of TcdA and TcdB in faecal and colonic contents are used for the gold standard of the diagnosis of *C. difficile* associated disease in pigs with the reference method being the measurement of neutralizable cytotoxicity in monolayers of Chinese

hamster ovary tissue, but nowadays the vast majority of diagnostic institutes use enzyme immunoassays. Due to its strict anaerobic nature, culturing *C. difficile* proves to be more complicated than other clostridial species. Pathologic lesions are less pronounced than in the aforementioned species, but suppurative foci as described above can be observed during microscopic examination (Zimmerman et al. 2012). Taking the epidemiological data and anamnesis of the animals, i.e. prior antibiotic treatment, quality of feedstuff, exposure to stress, etc. into consideration is also helpful for the diagnosis.

2.1.2 *Escherichia coli*

Escherichia coli is a Gram-negative, facultatively anaerobic, rod-shaped bacterium, classified within the *Enterobacteriaceae* family. The species can be found as normal inhabitants of the mammalian gastrointestinal tract and can act as the causative agents of a broad variety of diseases, depending on the pathotype (Zimmerman et al. 2012).

Neonatal *E. coli* diarrhoea is caused by enterotoxigenic strains of *E. coli* (ETEC) and can usually be observed within the first four days of age. This can be explained by the fact that during the process of farrowing the piglets ingest large amounts of microbes from the sow's intestinal flora and are confronted with a heavily contaminated environment of the den, before they reach the sow's teats. Therefore, strict hygienic measures and regular disinfection of the den is paramount to prevent a high amount of pathogenic strains in the environment. In the presence of predisposing factors ETEC strains attach to specific receptors on the mucosal epithelial cells of the small intestine and produce two major types of enterotoxins: the heat-stable toxin (ST), which is further divided into STa, the predominant enterotoxin in the case of neonatal ETEC, and STb – based on their solubility in methanol and biological activity, and the heat-labile toxin (LT). The attachment is achieved by fimbrial adhesins, namely F4 (K88), F5 (K99), F6 (987P) and F41. The piglet's susceptibility to F5 and F6 is high during the first several days of life, but decreases with age. Inversely the susceptibility to F18 starts out low and increases with age, while the age is not at all connected to the susceptibility to F4. Other important predisposing factors include the number of the sow's gestation, as gilts produce lower levels of colostral antibodies than older sows. However, it is important to keep in mind that the sow will only produce specific antibodies – immunoglobulin G (IgG) and

immunoglobulin A (IgA) – which will prevent the adhesion of the adhesins developed by the pathogenic *E. coli* the sow has been exposed to. Therefore, colostral antibodies do not protect the piglets if they are exposed to *E. coli* that develops different adhesins. Chances of disease development are also increased in case of low ambient temperature (less than 25°C), due to the decreased peristaltic activity, effectively delaying the passage of bacteria and protective antibodies. Also, the presence of other enteric pathogens like TGEV, PEDV, rotaviruses or coccidial infections affects the piglets adversely. The average morbidity within an affected herd is variable. It usually ranges from 30-40%, but in some herds up to 80% of the animals are affected. Depending on the predisposing factors, mortality can be as high as 70%. Some of the animals may even succumb before developing clinical signs (Zimmerman et al. 2012).

STa activates guanylate cyclase, by binding to a guanylyl cyclase C glycoprotein receptor, stimulating the production of cyclic guanosine monophosphate (cGMP), which in turn increases electrolyte and fluid secretion. Diarrhoea develops if the excess fluid is not absorbed in the large intestine, resulting in dehydration, acidosis and eventually death in severe cases. Depending on the disease's severity the diarrhoea may be mild without evidence of dehydration, progressing to watery, profuse in more severe cases. The faeces may appear brown, whitish or watery (Zimmerman et al. 2012).

Over the course of the disease the animals may lose up to 40% of their body mass, the abdominal musculature becomes flaccid, and bone structures become more prominent. Due to the severe dehydration the skin may become parchment-like and take on a bluish-grey colour. Piglets suffering the severe course usually die. Inflammation of the skin around the anus and perineum can be observed in chronic, less severe cases, due to contact with the alkaline faeces (Zimmerman et al. 2012).

Pathological examination may reveal a distended stomach, which might still be filled with undigested milk curd. The small intestines are likely to be dilated and congestion of the small intestinal wall can be observed. During microscopic examination *E. coli* can be found adhering to mucosal epithelial cells in the crypts of Lieberkühn or covering the intestinal crypts and tips of the villi along the major part of the jejunum and ileum – in the case of F4 (K88)-positive ETEC – and the posterior jejunum and ileum in other cases. Mild villus atrophy, vascular congestion with haemorrhages into the intestinal lumen may sometimes be observed as well (Zimmerman et al. 2012).

During the diagnosis it may be helpful to determine the faecal pH in order to differentiate ETEC (alkaline faeces) from TGEV and rotavirus caused diarrhoea (acidic faeces). Next to considering the epidemiological data, clinical signs and pathology, faecal samples can be inoculated onto blood agar, MacConkey agar or other selective media for *Enterobacteriaceae*. Doing so allows differentiating between lactose-positive and lactose-negative Gram-negative bacilli. As *E. coli* is a normal inhabitant of the intestinal flora, virotyping (determining the virulence factors) achieves more definitive results than serotyping. Slide agglutination and ELISA can be used to test for the presence of adhesins. Other techniques include DNA hybridization and polymerase chain reaction (PCR) to detect genes that encode toxins and adhesins (Zimmerman et al. 2012).

In order to prevent the disease, the amount of pathogenic *E. coli* in the environment has to be decreased. This can be achieved by regular disinfection of the premises (all-in/all-out farrowing), regular cleaning of the den and placing new animals in quarantine before introducing them to the herd. Ensuring a warm, dry resting area for the piglets and preventing their exposure to draught are also important factors to prevent the disease. After farrowing sufficient colostrum uptake is highly beneficial for the prevention of neonatal ETEC diarrhoea. However, as the sow only produces specific lactogenic antibodies against the fimbrial antigens that it has been exposed to, the dam should be vaccinated with the strains predominant in the given area. Antimicrobial treatment of new-born piglets is possible individually or on a litter basis either per os or parenterally, but in this case, it is important to confirm an *E. coli* infection by culture and perform sensitivity tests, as antibiotic sensitivity varies greatly and the resistance to antimicrobial agents is on the rise. A supportive treatment, i.e. fluid therapy, is helpful in order to treat dehydration and acidosis (Zimmerman et al. 2012).

2.1.3 Salmonella

Like *E. coli*, *Salmonella* is a genus of the *Enterobacteriaceae* family. As such it is a Gram-negative bacterium, that consists of two species: *Salmonella enterica* and *Salmonella bongori*. *S. enterica* is further divided into six subspecies, which have a wide variety of serotypes themselves. More than 2.500 serotypes have been identified so far and many possess a broad host spectrum, while others have adapted to a single host

species. There are two reasons why Salmonella infections are of importance, the first one being the clinical manifestation itself and the second is that pigs can be infected by many serotypes, that can also cause disease in humans and therefore raises public health concerns, which have economic consequences retrospectively (Zimmerman et al. 2012).

Salmonellae are present worldwide and infection of pigs is common. Outbreaks, however, are relatively rare and predominantly seen in farms, where pigs are reared intensively. Their reservoir is the intestinal tract of warm- and cold-blooded animals, but they are also able to survive in wet, warm environments. Due to frequent inapparent, long-term carriage and continuous or intermittent faecal shedding, the infection within a herd is sustained. In the presence of stress factors, like animal transport, heat stress, overcrowding, feed deprivation, or factors that have adverse effects on the immune system or the intestinal microflora, like an antibiotic treatment, mycotoxins in the feedstuff, concurrent diseases etc., the shedding of salmonellae is exacerbated. Pigs are usually infected by several serotypes simultaneously, but the disease is rarely caused by other serotypes than *S.* Cholerasuis, a host-adapted serotype that usually manifests as septicaemia, or *S.* Typhimurium, which is not host specific and causes enterocolitis (Zimmerman et al. 2012).

The manifestation of salmonellosis is normally seen in weaned piglets, while suckling piglets are commonly infected. The low incidence of salmonellosis in suckling piglets can presumably be explained with lactogenic immunity. An outbreak is usually linked to co-infections and the presence of stress factors. Due to a dynamic and complicated relationship between salmonellae, hosts and the environment, transmission and shedding are highly variable. However, *Salmonellae* are most likely transmitted via the faeco-oral route. Horizontal as well as vertical transmission is possible. Due to the ability to survive in the environment, pigs can be infected by salmonellae when they come into contact with contaminated material (Zimmerman et al. 2012).

About 10^7 organisms per gram of intestinal content are necessary to produce lesions in case of an infection with *S.* Typhimurium. The conditions for bacterial replication improve, when the gastric pH is increased and/or the intestinal flora or peristalsis are altered. During the invasion of the Peyer's patches, jejunal and ileal epithelial cells, new proteins are being synthesised. Once the salmonellae attach to the epithelial receptors, a microfilament-controlled uptake is triggered, forming a vacuole around the bacteria,

which allow them to be transported through the cell's cytoplasm to the basal membrane, where they enter into the lamina propria via exocytosis. During this transport the enterocytes are damaged mildly and transiently. Simultaneously an acute inflammatory response takes place and microvascular damage with thrombosis can be observed in the lamina propria and the submucosa. Caused by the production of cholera- and Shiga-like enterotoxins, the sodium resorption decreases, while chloride secretion increases, resulting in the onset of diarrhoea. Neutrophils are being stimulated by endotoxins start secreting prostaglandins, which may have an exacerbating effect. Due to the endothelial damage, the blood supply of the affected mucosal area decreases. Therefore, the area becomes progressively more anaemic, cyanotic and finally necrotic. The general clinical signs present in a *S.* Cholerasuis infection can be explained with the endotoxin effect. The endotoxins interact with the plasma and leukocytes to stimulate an inflammatory response and fever. During this phase the pigs are febrile, lethargic and inappetent and may develop respiratory signs, like a shallow, moist cough and mild expiration dyspnoea. Usually in the septicaemic form of salmonellosis, diarrhoea does not appear until the third or fourth day of the disease. When it does, the faeces are of watery consistency and have a yellowish colour. Furthermore, neurological signs can be present, as necrotising and histiocytic vasculitis can lead to encephalitis and/or meningitis. In the case of *S.* Typhimurium yellow, watery diarrhoea lasting for 3-7 days is the first clinical sign. The diarrhoea might reappear intermittently for several weeks. Along with the diarrhoea, the affected animals are usually febrile, suffer from secondary dehydration, but they usually recover completely. Some of the animals may develop a chronic form, resulting in an unthrifty, wasting appearance. Even after the disappearance of diarrhoea, some of the animals keep carrying and shedding salmonellae for at least five months (Zimmerman et al. 2012).

In the case of an *S.* Cholerasuis infection, the gross lesions are as follows: Cyanosis can be observed in the distal extremities, ears, tail and the ventral region of the abdomen during the acute infection. Enlargement of the lymph nodes, especially the gastrohepatic and mesenteric lymph nodes, can be seen. Similarly, the liver may be slightly enlarged and may have small necrotic foci on the surface. These so-called "paratyphoid nodules" can also be observed on the splenic surface, where they are randomly scattered. Due to fibrinoid thrombi in the venules, the gastric mucosa becomes congested. The gall bladder wall is oedematous and thickened. In the lungs diffuse histiocytic interstitial pneumonia

or in other cases suppurative bronchopneumonia are frequently observable. Enteric lesions caused by *S.* cholerasuis have the same appearance as the ones caused by *S.* typhimurium. In this case enterotyphlocolitis usually extends from the ileum, over the caecum and spiral colon, but may also reach as far as the descending colon and rectum. The wall of the affected intestinal segments is oedematous and thickened and, on the mucosa, multifocal erosions and/or even ulceration occurs. Grey-yellowish fibronecrotic debris, which may contain myriad opportunistic bacteria, covers the ulcers. Again, mesenteric lymph nodes are enlarged and have a moist appearance. On a microscopic level, necrosis of the crypt and surface epithelial cells can be observed. Over the course of the infection, the lamina propria and submucosa are first infiltrated by neutrophils and then by macrophages and few other lymphocytes as the time progresses. During the acute phase of the infection, the lymphoid patches of the submucosa are usually necrotic, but during pathological examination, lymphoid hypertrophy and regenerative hyperplasia have been described to be more common (Zimmerman et al. 2012).

For a definitive diagnosis of salmonellosis, bacterial isolation and identification has to be performed. At the same time, typical lesions should be present in the animal, as the isolation alone does not result in a reliable diagnosis, due to the fact that environmental salmonellae are widespread and frequently cause subclinical infection and faecal shedding (Zimmerman et al. 2012).

2.1.4 Porcine Coronavirus infection

The *Coronaviridae* family belongs to the *Nidovirales* order and has two subfamilies: the first one being the *Coronavirinae*, which in turn consist of the genera *Alpha-*, *Beta-* and *Gammacoronavirus*, and *Torovirinae*, which comprises the genera *Torovirus* and *Bafinivirus*. As the transmissible gastroenteritis virus (TGEV) and the porcine epidemic diarrhoea virus (PEDV) are of importance for this study, they will be discussed below. In general, coronaviruses are enveloped, pleomorphic viruses and measure from 60 nm up to 160 nm in diameter. Their single-stranded genomic RNA (~30 kDa) is of positive-sense polarity. In most cases, these viruses have a single layer of S proteins, which appear as club-shaped spikes and measure 12-25 nm in length. As the virus incorporates

phospho- and glycolipids that are derived from its host into the envelope, the composition of its envelope is dependent on the host cells (Zimmerman et al. 2012).

2.1.4.1 Transmissible gastroenteritis virus

As the TGEV is in the *Alphacoronavirus 1* species, it belongs to the *Alphacoronavirus* genus and the *Coronavirinae* subfamily. The disease is associated with severe diarrhoea, vomiting and a high mortality, which can reach as much as 100% of affected piglets. In pigs older than five weeks, the mortality decreases, although all age groups are susceptible. The resistance of the TGEV in the environment is rather weak, due to the fact that it is an enveloped virus. At low temperatures and even frozen, the virus is stable, while it becomes labile at room temperature or above. Hence a seasonal appearance from November to April is more common. Depending on the immunological status of the herd, the TGEV causes one of two epidemiological forms: A severe epidemic in susceptible herds, which is characterised by a rapid spread within the herd and serious clinical signs, as well as high mortality, or an endemic form, which is frequently observed in farms with high piglet production nowadays. In the case of the endemic TGE, the virus spreads slowly within the herd, usually affecting the replacement animals. Sows often are immune to the disease and will produce antibodies to a varying degree, which will be passed on to the offspring with the colostrum. The mortality is typically beneath 10% in piglets from day 6 of age until around two weeks after weaning. In the recent years, the presence of the Porcine respiratory coronavirus (PRCV), a variant of the TGEV, may have reduced the incidence of TGE in Europe in the past, due to cross immunity (Zimmerman et al. 2012).

Dogs, cats and foxes have been suggested to serve as carriers between herds, apart from pigs, which shed the virus with the faeces in high titres for two weeks. These animals are rarely exhibiting TGE-like clinical signs. Additionally, it was reported by Pilchard in 1965 that it was possible to detect the virus in the dropping of starlings (*Sturnus vulgaris*) for up to 32 hours after they had been ingested the virus. House flies (*Musca domestica*) may also serve as mechanical vectors, as they excrete the virus for up to three days (Zimmerman et al. 2012).

Upon oronasal infection, the virus will replicate in the jejunum and ileum, where it will cause severe necrosis in the enterocytes within the first 12 to 24 hours post infection. This leads to a decreased activity of enzymes (alkaline phosphatase, lactase, etc), which, in turn, results in a disruption of the digestive process, as well as the transport of nutrients and electrolytes. Maldigestion and malabsorption ensue. Due to the inability to properly digest the lactose present in the sow's milk, the osmotic pressure increases in the lumen of the intestines, therefore accumulating water within the lumen, causing dehydration of the affected piglets. Profuse, watery, yellowish diarrhoea is often described as a clinical sign, which is often preceded by emesis. Undigested milk can be seen in the faeces of suckling piglets. As the antibodies against TGEV are excreted to a varying degree, some litters may show milder clinical signs than others. Also, mortality in new-born piglets ranges up to 100% until they are about 10 days old, as they only have a diminished ability to replenish the damaged enterocytes. Co-infections with other enteric pathogens further exacerbate the effect of the enteritis. Due to the fact that the concentration of antibodies in the milk decreases with time, piglets that were protected during their first week of life might develop clinical signs around weaning. However, at this point the mortality is greatly decreased. Should the dam be infected during lactation, the viral replication takes place in the mammary glands of the sow, leading to a rapid spread of the virus. Additionally, agalactia is a common clinical sign in this scenario (Zimmerman et al. 2012).

During pathological examination, a distended stomach, filled with curdled milk is a common finding. The stomach wall may be inflamed and may have petechial haemorrhages. The same goes for the small intestines, which may also appear thin and translucent, due to the villous atrophy. During histopathological examination, the TGEV induced atrophy of the villi may be distinguished from the same lesion caused by rotaviral infection, as it is more severe in the case of the TGEV. Also, vacuolisation of the enterocytes and a massive infiltration of inflammatory cellular components can be observed. The extend of the lesions are highly dependent on the form of the disease (epidemic vs. endemic) (Zimmerman et al. 2012).

Due to the fact that TGEV causes similar signs to rotavirus, PEDV, *E. coli* and coccidia infections, the diagnosis relies on the detection of the viral antigen or nucleic acids in faeces or lesions. Alternatively, virus isolation or detection of TGEV- or PRCV-specific antibodies can be done (Zimmerman et al. 2012).

As the virus is heat sensitive, providing a dry, warm (above 32°C), draft-free environment to affected pigs and offer water or nutrient solution ad libitum, in order to treat the symptoms of dehydration. Antibacterial treatment is helpful for the prevention of bacterial co-infections. In case of the occurrence of TGEV on a farm, sows that are due to farrow in at least two weeks and have not been exposed to the virus can be inoculated orally in order to reduce losses of new-born piglets. Sows that have less than two weeks until the expected farrowing date, should be separated from the herd in order to prevent exposure to the virus until about three weeks post-farrowing. Again, applying the "all-in/all-out" principle for farrowing and nursery pens, as well as good sanitation and submitting new animals to quarantine helps reducing the risks of infecting unexposed animals (Zimmerman et al. 2012).

2.1.4.2 Porcine epidemic diarrhoea

PEDV causes a disease similar in appearance to TGE. As it exhibits similar genetic and antigenic criteria, it was placed in the *Alphacoronavirus* genus. The virus' main transmission is believed to be the direct or indirect faeco-oral route and is mainly brought to a farm by the introduction of infected pigs, which will shed the virus with the faeces for 7-9 days. Nonimmune animals usually develop the disease after an incubation period of 4-5 days. After the initial outbreak the virus usually disappears from the farm, as the pigs develop a strong immunity within 2-3 weeks post infection. Sows will excrete antibodies in the milk and will therefore be able to protect their offspring. In a susceptible herd up to 100% of the animals may develop clinical signs, but the course of the disease is usually less severe than in the case of TGE (Zimmerman et al. 2012).

The cytoplasm of the villous epithelium of the small intestine serves as the location for viral replication, which causes degeneration of the enterocytes. Viral replication in the proximal colon has been observed without causing any lesions. Due to the decreased villus length, the absorptive capacity decreases and a watery diarrhoea develops. This leads to an increased fluid loss and dehydration. Mortality due to dehydration averages at 50% in young piglets during their first week of life (Zimmerman et al. 2012).

Pathologic lesions are similar to those of TGE, however, only the top third of the villi are affected, which causes the milder clinical signs. The presence of PEDV or its antigens can be demonstrated by immunofluorescence. Additionally, several enzyme-linked immunosorbent assay (ELISA) techniques have been developed to detect faecal antigens (Zimmerman et al. 2012).

Due to insufficient significance in Europe, vaccines against the virus were not developed. Piglets suffering from PED should receive free access to water, in order to decrease the effect of dehydration. Due to the slow spread of the virus, it is possible to interrupt the transmission cycle, by immediately removing and relocating piglets post weaning (Zimmerman et al. 2012).

2.1.5 Porcine Rotavirus infection

Rotaviruses belong to the *Rotavirus* genus in the *Reoviridae* family. They are of icosahedral morphology, non-enveloped virus particles and have a triple-layered capsid structure, measuring about 75 nm in diameter. Removing the outmost layer by chemical or enzymatic means yields non-infectious particles of about 65 nm in diameter. The virus' double-stranded RNA is divided into 11 segments. Each of those segments encodes one of six structural or non-structural protein (NSP) with the exception being segment 11, encoding both NSP5 and NSP6, making genomic reassortment possible (Zimmerman et al. 2012).

Rotaviruses are a major cause of neonatal diarrhoea in multiple mammalian species. Based on the antigenicity of VP6, which is the most abundant viral structural protein, porcine rotaviruses are divided into the four subgroups A, B, C and E with group A being the predominant group accounting for roughly 90% of rotavirus diarrhoea in commercial pig farms. These are further divided into G serotypes and P serotypes/genotypes depending on the outer capsid proteins VP7 and VP4 respectively. Neutralizing antibodies are elicited by VP7 and VP4 independently (Zimmerman et al. 2012).

While rotaviruses are generally resistant and can endure in the environment for prolonged periods of time, they can be inactivated by repeated freeze-thaw cycles. Otherwise, they can tolerate a pH range from 3-9, chemicals and disinfectants, while retaining their infectivity (Zimmerman et al. 2012).

After transmission via the faeco-oral route, or the ingestion of contaminated water or feedstuff, the virus infects and multiplies in the enterocytes of the small intestine, causing villous atrophy. Especially jejunum and ileum are affected, while the duodenum remains intact for the most part. Depending on the age of the pig, as well as the strain and serogroup of the virus, the degree of cellular damage varies. Younger animals are more susceptible and group A and C rotaviruses tend to produce more severe lesions than the ones from group B and E. Again, villous atrophy decreases the absorptive capacity, resulting in malabsorption and maldigestion, which causes the occurrence of osmotic diarrhoea and dehydration. Additionally, the normal secretion of sodium and water from the crypts of Lieberkühn is disrupted by the impaired absorption at the villous tips, which is cause by decreased Na^+K^+-ATPase activity and glucose-coupled sodium absorption (Zimmerman et al. 2012).

Clinical signs usually appear within 24-48 hours post infection and include emesis, a sudden onset of watery diarrhoea, dehydration, apathy and a loss of appetite. All of these contribute to a poor bodyweight gain. Mortality is low (frequently less than 20%) in most cases and usually related to co-infections with other pathogens. Younger animals are affected more severely (Zimmerman et al. 2012).

Due to the effect of maldigestion and malabsorption, undigested, liquid content can be found in the stomach and small intestine. The intestinal wall appears thin-walled, which is owed to the villous atrophy and the intestinal mucosa is inflamed (Zimmerman et al. 2012).

Several methods for the detection of rotaviruses are available: electron microscopy (EM), immune EM, immunohistochemistry, immunofluorescence, RT-PCR, Ag-ELISA, etc. (Zimmerman et al. 2012).

Supportive fluid therapy to alleviate the effects of dehydration and the prevention of secondary bacterial infections with the application of antibiotics are options for the treatment. As there are no specific antivirals against rotaviruses and as they are resistant

in the environment, a regular disinfection of the premises is important in order to reduce a build-up of rotaviruses in the environment. Ensuring a sufficient colostrum intake by the piglets can also help preventing the disease, as immune sows produce maternal antibodies, which will then be ingested by the piglets. Furthermore, an ambient temperature of 35°C has been reported to reduce the losses due to rotaviral infection (Zimmerman et al. 2012).

2.2 Pre- and probiotics used as feed additives

Neonatal diarrhoea is frequently caused by the colonisation of the intestinal mucosa by several pathogenic bacterial agents, which then produce a variety of toxins, as described above. The beneficial effects of probiotic bacteria lie in the competitive colonisation of the available mucosal surface, temporarily modulating the intestinal microflora and the ability to regulate or stimulate the immune system (Zhang et al. 2010; Kreuzer et al. 2012; Ritter et al. 2018; Taras et al. 2006).

Taras (2006) suggests a decreased occurrence of diarrhoea in piglets, as well as less severe clinical signs, especially if the application of the probiotic treatment starts early in the piglets' life. Additionally, he points out that by supplementing sow feed with probiotics after confirmation of gestation, the "probiotic bacteria can be conferred to the piglets by contact with maternal faeces", as a long-term probiotic supplementation would not only modify the sow's intestinal flora, but the faecal microbiota as well (Taras et al. 2006).

Modulating the intestinal and faecal microbiota by supplementing *Lactobacillus rhamnosus*, has also been described to decrease the faecal shedding of *E. coli*. Furthermore, the study states that the probiotic treatment improved the intestinal mucosal resistance to *E. coli*, by stimulating intestinal mucosal IgA responses (Zhang et al. 2010).

Most probiotics used nowadays are associated with the genera *Lactobacillus* and *Bifidobacterium*. Another group of probiotic bacteria is constituted by the *Bacillus* genus. While many species are associated with toxin production and causing foodborne illnesses and therefore raising safety issues, *Bacillus subtilis* is a species often regarded as safe. As a member of the *Bacillus* genus, *B. subtilis* is able of forming highly resistant

endospores, which drastically improves its shelf life. Ritter (2018) suggests that some *B. subtilis* strains exhibit a broad spectrum of antibacterial activity against Gram-positive bacteria and fungi. In addition, *B. subtilis* is deemed to be able to be of interest, as it is able to survive in low pH environments and in the presence of bile salts, which allows it to reach the small intestine and colon in order to modulate the intestinal microbiota (Ritter et al. 2018).

In a study, regarding the safety and efficacy of *B. subtilis* PB6 as a feed additive for sows, which was requested by the European Commission, Rychen (2017) concluded that "the active agent fulfilled the requirements of the Qualified Presumption of Safety (QPS)" (Rychen et al. 2017). It was also stated in the report, that while there was "no significant treatment effect for piglet mortality at birth, or from birth to weaning" (Rychen et al. 2017), the piglets, which were subjected to the *B. subtilis* treatment had a significantly increased bodyweight at weaning, when compared to the control group.

2.3 Dehydration and electrolyte imbalance as cause of death

Dehydration occurs mostly due to two reasons. The first one being inadequate water intake, which may be caused by inaccessibility of water or the inability to drink, due to oesophageal obstruction, neuropathy, etc., or a decreased sensation of thirst, as a result of a toxaemia. The second one is caused by excessive loss of fluids. This can happen through excessive sweating, polyuria, vomiting and, most predominantly, diarrhoea. A frequent complication and result of dehydration, is the loss of electrolytes (Radostits et al. 2007).

In case of dehydration, fluid from the tissues is withdrawn in order to maintain normal blood volume as an initial response. This fluid is mostly contributed by connective tissue, muscle and skin, while essential organs contribute little. The decreased water content in those tissues results in a loss of elasticity in the skin, dry mucus membranes, as well as a volume reduction and retraction of the eyeballs. In an ongoing negative water balance, the blood volume is decreased, leading to an increased viscosity of the blood, which in turn has an exacerbating effect on the circulatory failure in the peripheric tissues. Mental depression, that may have been present before, due to toxaemia or acidaemia, becomes

more apparent. As the kidneys try to balance the faecal fluid loss, urine excretion decreases, which in turn leads to a higher concentration of the urine, having a detrimental effect on the kidneys itself if an adequate water and electrolyte uptake is not granted. In another effort to increase the water content, fat, carbohydrates and finally proteins are broken down under relatively anaerobic conditions, resulting in the production of acid metabolites and therefore the development of a metabolic acidosis. Protein breakdown and urine retention lead to moderately increased levels of nonprotein nitrogen in the blood (Radostits et al. 2007).

The maintenance of the osmotic pressure within the extracellular fluid is controlled by sodium, which is the predominant ion in it. Enteropathies are a common reason for sodium loss. During an *E. coli* infection, the sodium secretion is increased to the degree, that the sodium concentration of the intestinal content almost equals to one to the plasma. As a consequence, hyponatraemia (hypotonic dehydration) develops and becomes more and more severe, the longer the diarrhoea persists. In an attempt to correct the ion balance to a physiological level, renal water excretion is increased, which, in case of diarrhoea exacerbates the symptoms of dehydration. When treating animals with hypotonic dehydration, great care should be taken to ensure the replacement of electrolytes. Sodium-free or 5% dextrose solutions need to be supplemented with sodium, otherwise the hyponatraemia only worsens (Radostits et al. 2007).

3. Material & method

3.1 Enrolment of piglets

The experiment was carried out in a farm in the eastern part of Saxony-Anhalt. Nine litters of Piétrain (PIC®408) sows were selected, six of which exhibited clinical signs, while the other three were healthy and served as control groups.

The six litters showing clinical signs were further divided into two groups of three litters each, which were to be treated with Baytril® 1nject (AB - antibiotic) and a combination of Entero-VET® Suis mini and BactoLyt-VET® Pecus (PB - probiotic) respectively.

It was decided that at least three piglets should show clinical signs in order to ensure, that the cause was due to infectious aetiology. Once a litter had been identified, the age, number and weight of the piglets was documented, as well as the parity of the sow. Out of the three litters treated with AB, two originated from gilts, while one was from a third parity sow. Similarly, two of the PB litters were first parity litters, but the third one was from a seventh parity sow. In order to be able to compare the AB and PB litters adequately, two gilts and their litters were selected for the control litters. However, all further litters originating from gilts also developed clinical signs during the following days of the experiment and had therefore to be exchanged by the litters of a third, fourth and seventh parity sow.

3.2 Laboratory analyses

Two swab samples from ten piglets of the AB and PB litters were collected rectally. Additionally, one pooled sample per litter was taken. One of the swab samples was used for the diagnosis of *C. difficile*. The FASTest® C. diff 2T (MEGACOR Diagnostik GmbH, Hörbranz) was used to do so. According to the instructions this test allows the qualitative detection of Glutamate Dehydrogenase (GDH) and the Toxin A/B in the faeces of dogs, cats, horses and pigs. It is based on an immunochromatographic principle. First, the faecal sample (75 mg) is homogenised in a buffer diluent containing sample tube. After the homogenisation, the sample tube is then placed in a revolver test tube,

where the GDH or Toxin A/B will react with mobile monoclonal antibodies, which are bound to red latex particles and form antigen-antibody complexes. The revolver test tube is then placed on a horizontal surface for 15 minutes. During this time the formed complexes will migrate along the membrane with the help of the lateral flow and are then bound to fixed specific monoclonal antibodies against GDH or Toxin A/B, resulting a red test line and a blue control line in the case of GDH or two red test lines in the case of Toxin A/B. In case of a negative result only the blue control line will appear (*Figure 1*).

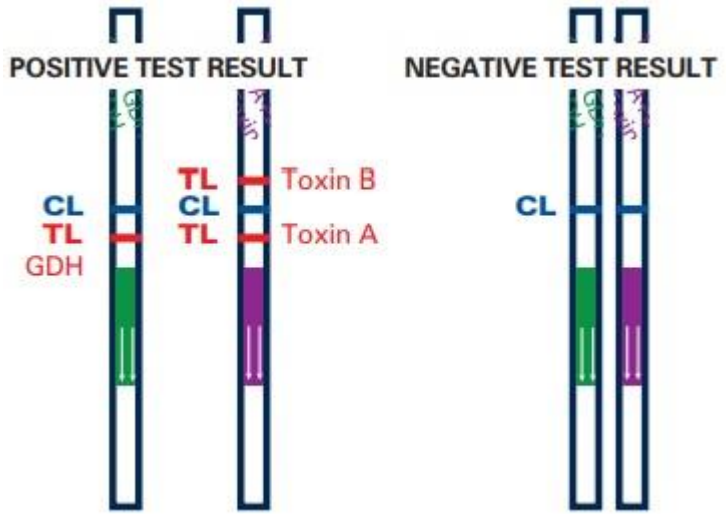

Figure 1

The second swab sample was used for culturing the bacteria present in the faeces on agar plates. Two Colombia agar (OXOID GmbH, Wesel) plates with 5% sheep blood were inoculated and incubated at 36°C under aerobic (24 hours) and anaerobic (18 hours) conditions for the diagnosis of *E. coli* and *C. perfringens* respectively. In the case of *C. perfringens* sharp haemolytic areas can be observed around the colonies.

Furthermore, the Schaedler anaerobe agar with 5% sheep blood (OXOID GmbH, Wesel) was used for the *C. perfringens* culture. The inoculum was incubated at a temperature of 36°C for 48 hours.

The last agar used was the Gassner medium (OXOID GmbH, Wesel), which is used for the detection and isolation of *Enterobacteriaceae*.

Finally, the pooled sample was used for the diagnosis of the antigens of PED-, TGE- and Rotavirus (group A) with the help of the WITNESS® PED-TGE-Rota test kit (Zoetis Deutschland GmbH, Berlin). Like the FASTest® C. diff 2T, WITNESS® PED-TGE is a chromatographic immunoassay.

Additionally, the SureTect™ Salmonella Species PCR-Assay (ThermoFisher Scientific, Schwerte) was used. Here the sample (25 g) was added to buffered peptone water as a medium, homogenised thoroughly and then incubated at 37°C for 24 hours. At the end of the incubation period 10 µL of the enriched sample was transferred to a Lysis tube, which was first allowed to reach room temperature by letting it rest for 10 minutes and was then filled with 10 µL of Proteinase K. A negative control was prepared by adding 10 µL of sterile enrichment media to another Lysis tube. The tubes were then sealed and the samples incubated using heating blocks at 37 ± 2°C for 10 minutes and at 95 ± 2°C for 5 minutes and then rested 2 minutes at ambient temperature.

For the preparation of the PCR the PCR tubes are first placed into a tube rack and brought to room temperature (letting it stand for approximately 5 minutes) and it is made sure that the PCR pellets are positioned at the bottom of the tubes by tapping the rack, should that not be the case. The Lysis tubes are then opened and 20 µL of the lysate are filled into PCR tubes in order to rehydrate the pellets. It should be taken care of that the lysate is collected from the top half of the Lysis tubes as to prevent the transfer of lysis particles into the PCR tube. For the next step, the PCR tubes are firmly sealed with optical PCR caps and mixed thoroughly for 15 seconds. Before the PCR tubes are placed into the PCR instrument (QuantStudio™ 5 Instrument), the liquid needs to be at the bottom of the tube. The analysis can then be initiated and is fully automated. The results can be displayed on the screen of the PCR instrument.

3.3 Treatment

The piglets designated for the AB treatment were weighed before every application, the individual dosage calculated according to the manufacturer's instructions, which was then injected intramuscularly into the neck muscles behind the ear. Reapplication was performed after 48 hours for as long as clinical signs were present. When the clinical

signs disappeared, the piglets were weighed again after 48 hours following the last injection as to determine the average bodyweight of the piglets within the litter. The duration of the treatment was documented and the average daily gain (ADG) of the given litter was calculated.

Before the PB treatment the solubility of BactoLyt-VET® Pecus was tested. The manufacturer suggested dissolving 125 g in one litre of water. However, as the dosage turned out to exceed the saturation concentration and a thick layer of precipitate formed at the bottom of the glass, the amount of BactoLyt-VET® Pecus was halved to 62.5 g/l, which resulted in a negligible amount of precipitation.

Just like in the AB treatment, the piglets receiving the PB treatment were weighed when the clinical signs first appeared, as described above. Then BactoLyt-VET® Pecus was dissolved in one litre of warm water and 5 ml of Entero-VET® Suis mini was added to the solution. The solution was then filled into a bowl, which was placed next to the resting area of the piglets. In order to accustom the piglets to the treatment, a 5 ml syringe was filled and offered orally to the piglets. As the treatment was designed to be offered ad libitum, the bowl was refilled, whenever it was deemed necessary.

When the clinical signs disappeared, the PB treatment was withdrawn and the piglets were weighed again. The treatment's duration was documented and the ADG calculated.

After both treatments had commenced, the three control litters were selected according to the average initial age of the treatment groups. Just as in the case of the other litters, all of the piglets were weighed. After the conclusion of the treatments, the average treatment duration of both groups was calculated and the control group was weighed accordingly.

All of the groups were then observed until weaning and weighed one last time. Again, the ADG per litter was calculated.

4. Result and discussion

4.1 Laboratory results

Out of the six litters sampled, five of them tested positively for *C. difficile's* GDH to varying degrees. Two piglets from the first AB litter and five of the second were positive. In the third AB litter, the test result was negative. As for the PB litters, the results were as follows: six, six and two of piglets were tested GDH positively in the first, second and third litter respectively. Toxin A was absent in all the samples, but there were two piglets, originating from the same litter (PB 1), that had positive results for Toxin B.

Culturing revealed moderate to high infestation of *C. perfringens* in all of the faecal samples after an incubation period of 24 hours on the Colombia agar as well as the Schaedler agar after a 48-hour incubation period. Similarly, *E. coli* was present in all litters.

The results PCR-Assay for salmonellae in the pooled faecal samples was positive for the first litters that received anti- and probiotic treatment. However, culturing was not possible.

The WITNESS® PED-TGE-Rota test revealed the presence of rotaviral antigens in every pooled faecal sample. Additionally, the third litters of both treatment groups were tested positively for antigens of the TGEV. Therefore, another faecal sample was collected and sent to the Institute of Virology of the department of Veterinary Medicine at Universität Leipzig, where a RT-PCR test was performed in order to detect the presence of coronaviruses. This test proved to be negative.

4.2 Weight development

In order to be able to compare the weight development of the litters appropriately, the litters were arranged depending on the age of the piglets at the onset of the clinical signs, as well as the sow's parity, due to the fact that the sow's productivity increases from the third to the fifth farrowing cycle, before it starts to decrease again (Carney-Hinkle et al.

2013; Ferrari et al. 2014). Therefore, the first two compared litters receiving AB and PB treatment respectively originated from gilts. The data is displayed in *Table 1*.

The piglets chosen for the AB treatment were five days old, when the clinical signs first appeared. The gilt had farrowed 14 living piglets, out of which two had been removed and added to another litter by the workers before the start of the experiment. This was done to ensure an optimised milk uptake. The average bodyweight per piglet amounted to 2.270 kg. Application of the treatment was repeated twice. Clinical signs disappeared on the sixth day after the initial treatment. Weighing the piglets on the seventh day resulted in an average bodyweight of 3.491 kg. The ADG for the treatment duration was calculated and amounted to 174 g/day. The piglets were weaned at the age of 21 days and the average bodyweight at weaning was 5.467 kg, resulting in an ADG of 200 g/day from the start of the experiment until weaning.

The litter, that was to receive the PB treatment first showed clinical signs, when the piglets were eight days old. In this litter 15 living piglets had been farrowed and two had been removed as well. Here, the average bodyweight was about 3.104 kg. The piglets recovered from the diarrhoea after four days of treatment, at which point the average bodyweight was 4.085 kg. Therefore, the ADG for the treatment period was around 245 g/day. At weaning, which was done when the piglets were 20 days old, the average piglet weighed 7.015 kg, resulting in an ADG of 326 g/day for the duration of the experiment.

The first control group originated from a fourth parity sow. 13 live piglets had been farrowed, one stillbirth and one mummy. The piglets were weighed when they were five days old and their average bodyweight was 2.640 kg. After six days, the piglets were weighed again and the average bodyweight was 4.037 kg, meaning that the ADG was about 233 g/day. Four days before weaning, one of the piglets was found dead, crushed by the sow. The average weaning weight of the piglets at the age of 19 days turned out to be 6.617 kg, hence the ADG for the whole duration was 284 g/day.

In *Figure 2* the weight development is visualised. The AB treatment group is portrayed by the blue, the PB treatment group by the green and the control group (C) by the black graph. The first point on every graph symbolises the onset of clinical signs, the second one the time of recovery and the third one the time of weaning. The trendlines do not indicate the actual bodyweight, rather an estimate. There are two important things to

consider during the observation of the weight development: Firstly, the piglets from the PB treatment were three days older, when the diarrhoea first appeared. This is important, as the intestinal flora is likely to be further developed than in the case of the piglets that received the AB treatment. As described in the second chapter, an impaired intestinal flora often acts as a predisposing factor for bacterial diseases, which means, that the basic conditions for the PB treatment group was better compared to the AB treatment group. Secondly, as mentioned above, both treatment groups originated from gilts, while the control group was from a fourth parity sow. Normally this means, that the milk-yield as well as the immunoglobulin quality of the sow is increased, due to the already concluded development of the sow itself, which is not yet complete in the case of first and second parity sows.

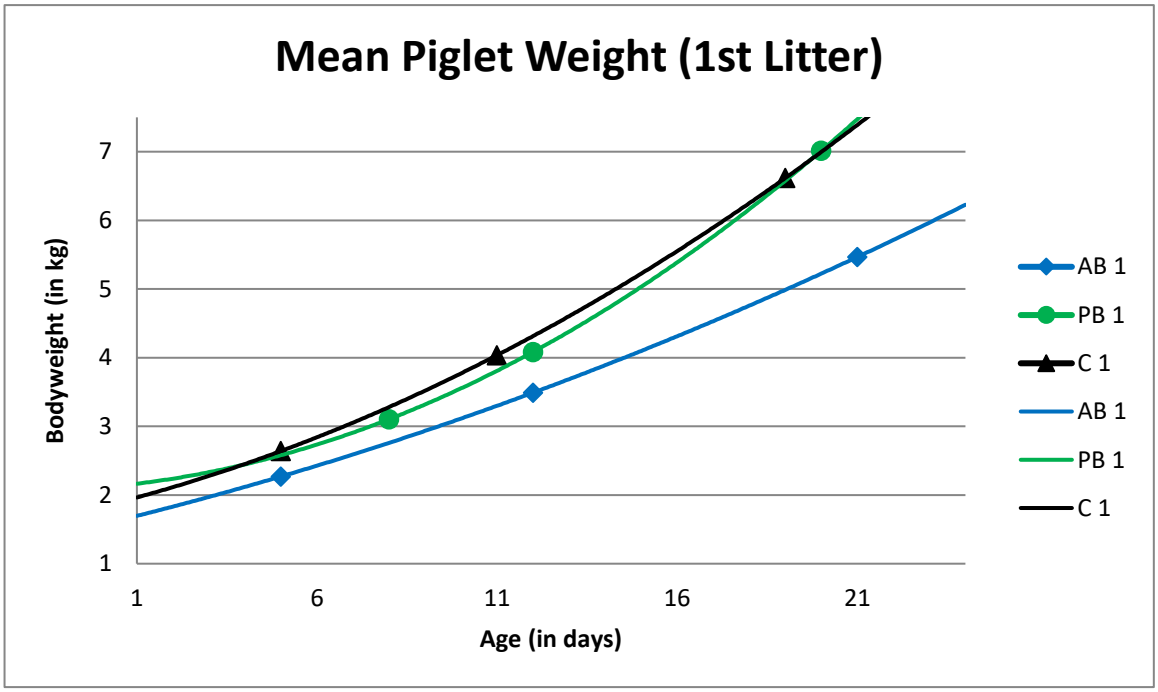

Figure 2

The weight development of the second litters (*Figure 3*) proved to be more homogenous: Again, both treatment groups originated from gilts and both groups developed clinical signs on the fourth day of life. The initial average bodyweight of the AB treatment group was 1.976 kg, which increased over a treatment duration of four days to 2.649 kg,

resulting in an ADG of 168 g/day. At weaning, performed on day 24 of the piglets' life, the average piglet weighed 6.900 kg and had gained about 246 g/day.

In the litter of the second PB treatment group 17 live piglets had been farrowed and four of them had been removed before the experiment. It performed as follows: the average bodyweight was 2.065 kg initially. After a seven-day treatment the average piglet weight was 3.531 kg, meaning that the ADG was 209 g/day. One of the weaker piglets was crushed by the sow on the second day of the treatment. The piglets were weaned when they were 20 days old and weighed 5.991 kg on average. This equals an ADG of 245 g/day. A second piglet was found dead beneath the sow three days before weaning.

The second control group was from a seventh parity sow. Ten living piglets had been farrowed along with three still-born. Three piglets were added before the experiment. Two of the piglets were crushed by the sow, before the start of the experiment. The initial weight was noted, when the piglets were five days old. It amounted to 2.359 kg on average. Six days later, weighing resulted in an average bodyweight of 3.994 kg, which equals an ADG of 272 g/day. At weaning (age: 19 days) the average piglet weighed 6.173 kg, meaning the ADG was constant at 272 g/day.

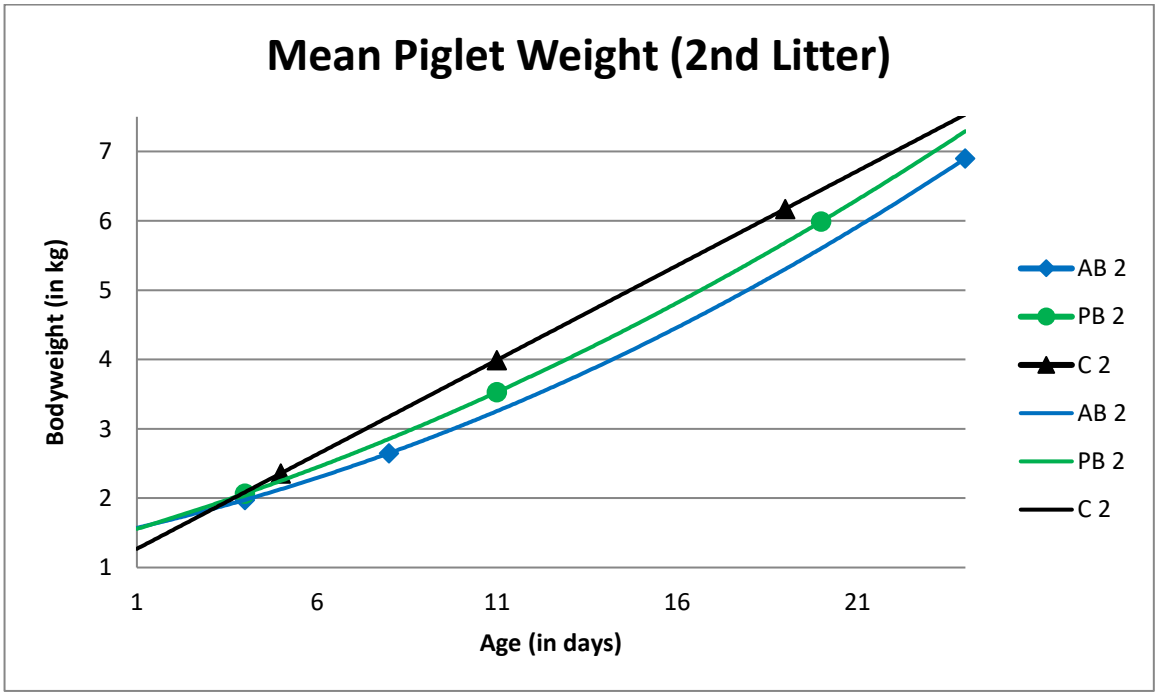

Figure 3

For the third AB treatment a litter from a third parity sow was used. 16 living piglets had been farrowed and one was stillborn. Two of the piglets were removed immediately after farrowing. In this case the onset of clinical signs was on the first day of life. The piglets weighed 1.274 kg on average and were treated for 6 days. Two lethargic, dehydrated piglets were crushed by the sow during the treatment period. At recovery the bodyweight had increased to 2.339 kg, resulting in an ADG of 213 g/day. Weaning was done on day 20 at which point the average bodyweight was 6.258 kg. Therefore, the ADG over the entire period amounts to 262 g/day.

In the case of the PB treatment, a seventh parity sow was used. 12 Piglets had been farrowed and two more were added to the litter. Diarrhoea also appeared on the first day of life. The average bodyweight was 1.586 kg at the start of the treatment and it increased to 2.503 kg over the next six days, when the clinical signs disappeared. This results in an ADG of 153 g/day. On day 10 a crushed piglet was removed from the litter. At the age of 20 days, the piglets were weaned. The average bodyweight was 5.785 kg, meaning the piglets gained 221 g/day.

The third control group was from a third parity sow as well. 13 piglets were farrowed and the litter was left in its original state without adding or removing piglets. The first time the litter was weighed was on the first day. The average bodyweight was 1.455 kg, which increased to 2.389 kg over the next 5 days, resulting in an ADG of 187 g/day. At weaning, which was done on day 20, the average piglet weighed 6.285 kg, amounting to an ADG of 254 g/day over the entire period.

Again, when examining the weight development (*Figure 4*), the differing parity of the sows should be kept in mind. *Figure 5* displays the mean weight development of the combined AB-, PB- and C-groups.

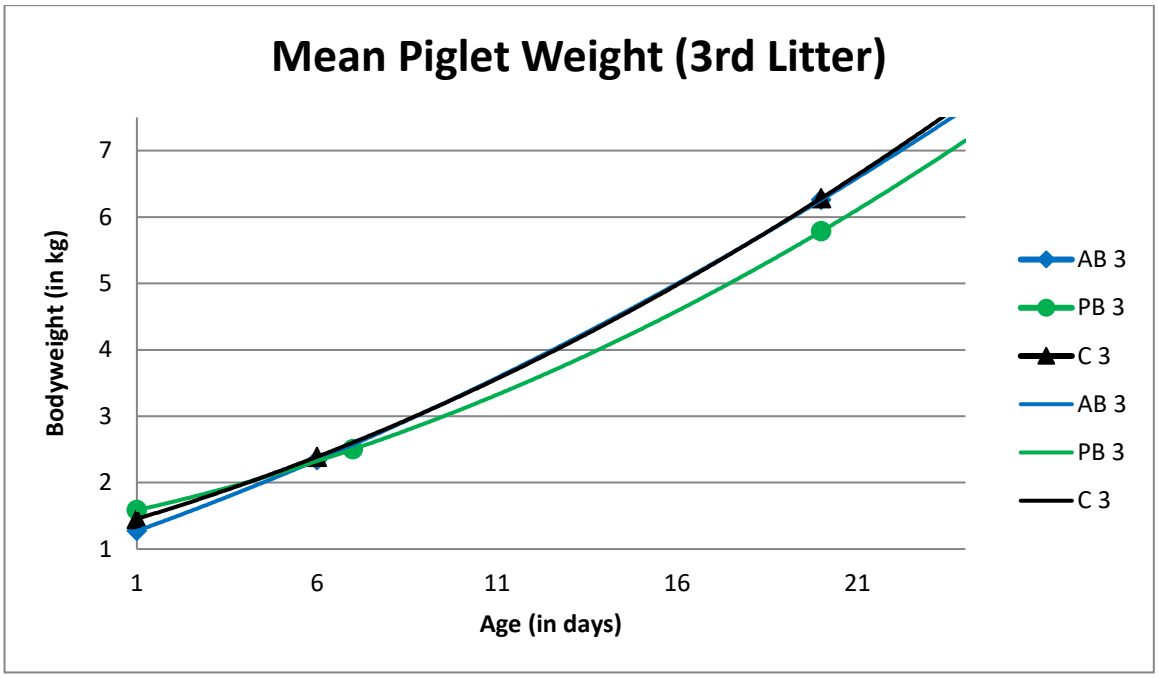

Figure 4

Figure 5

Treatment	Parity of the sow	Number of piglets – Onset	Average piglet BW – Onset (kg)	Number of piglets – Recovery	Average piglet BW – Recovery (kg)	Number of piglets – Weaning	Average piglet BW – Weaning (kg)	ADG – Recovery (kg)	ADG – Weaning (kg)
Baytril	1st	12	2.270	12	3.491	12	5.467	0.174	0.200
	1st	12	1.976	12	2.649	12	6.900	0.168	0.246
	3rd	14	1.274	12	2.339	12	6.258	0.213	0.262
Entero-VET	1st	13	3.104	13	4.085	13	7.015	0.245	0.326
	1st	13	2.065	12	3.531	11	5.991	0.209	0.245
	7th	14	1.586	14	2.503	13	5.785	0.153	0.221
Control	4th	13	2.640	13	4.037	12	6.617	0.233	0.284
	7th	11	2.359	11	3.994	11	6.173	0.272	0.272
	3rd	13	1.455	13	2.389	13	6.285	0.187	0.254

Table 1

The mean treatment duration was similar for both groups (5.33 days and 5.67 days for the AB- and PB- group respectively). When comparing the mean ADG of the two treatment groups to the one of the control group, we get the following results: Assuming that the ADG of the control group is 100%, the mean ADG of the AB treatment during the application amounts to 80.3% of the control groups ADG, while the mean ADG of the PB treatment groups is at 87.8%. Similarly, the mean of the total ADG for the AB treatment group reaches 87.4% of the control groups mean total ADG and the PB treatment amounts to 97.7%.

The ADG during the treatment and the total ADG of all the piglets were analysed with the ANOVA test in respect of the treatment they received. There was no significant increase of the ADG during the treatment period (AB: 0.180 ± 0.035 (*n=36*), PB: 0.197 ± 0.074 (*n=39*), p = 0.196), but the ADG over the entire duration – from onset of clinical signs to weaning – was increased significantly for the group that received the PB treatment (AB: 0.235 ± 0.034 (*n=36*), PB: 0.261 ± 0.062 (*n=37*), p = 0.026). Furthermore, when comparing the ADG of the PB treatment group to the C group, it turned out that the ADG of the PB treatment group was not significantly lower by the time the piglets were weaned (PB: 0.261 ± 0.062 (*n=37*), C: 0.269 ± 0.036 (*n=36*), p = 0.534). In the case of the AB treatment group the ADG was significantly lower, when compared to the C group at the time of weaning (AB: 0.235 ± 0.034 (*n=36*), C: 0.269 ± 0.036 (*n=36*), p = 0.000087).

5. Conclusion

Considering the fact, that the number of the piglets used in the experiment ($n_{AB}=36$, $n_{PB}=37$, $n_C=36$) is rather small to give a definite answer about the efficiency of the probiotic treatment. It is, however, possible to observe a tendency, suggesting that the probiotic treatment of neonatal diarrhoea in piglets is in fact more beneficial than the treatment with enrofloxacin.

However, there are a few things to consider: First of all, the application of the enrofloxacin via an IM injection allows individual treatment of the piglets and ensures that the piglet receives the treatment. In the case of a probiotic treatment through supplementation, the intake is not guaranteed. During the experiment this was especially evident when offering the preparation to the piglets on their first day of life, as they were not interested in the preparation and would not touch it for the first 48 hours, which makes sense, as the digestive system during the first 10 days is designed for milk digestion. The piglets that were already 8 days old, when the clinical signs first appeared, started consumption of the preparation within 24 hours. The uptake increased over the duration of the treatment.

One might expect that, due to the voluntary uptake, the losses would be higher in the case of the probiotic treatment, but while there were three piglets that died during the experiment in the group receiving the probiotic preparation, only one of them died during the treatment period, as opposed to two piglets in the group subjected to the antibiotic treatment.

According to Taras (2006), the problem regarding the insufficient probiotic intake could be circumvented by subjecting the gestating sows to a probiotic supplementation, as the faecal microbiota would be conferred to the offspring. Assuming a similar outcome to the trial performed by Zhang (2010), this approach would also help reducing the amount of pathogenic bacteria in the environment and therefore be an important factor for the prevention of neonatal diarrhoea in piglets.

In a trial, where he compares the performance of piglets receiving *Enterococcus faecium* as a probiotic supplement to a control group between day 0 and 56 of age, Taras (2006) suggests, that while there is no apparent difference in the development of bodyweight

between the two groups, the effects of an increased weight gain might have been more apparent if the environmental and nutritional challenges had been exacerbated. Even if the trial performed by Taras was of a different nature, compared to the one in this study, the statement seems to be coherent with the results of this study, as the animals of the PB group only have an insignificantly decreased ADG compared to the C group ($p = 0.534$).

6. Summary

In order to evaluate the efficacy of the application of probiotics for the treatment of neonatal diarrhoea in piglets in regards to treatment duration and piglet performance, three litters have been treated with a combination of probiotics, electrolytes, essential oils and vitamins, while three more litters were subjected to the conventional enrofloxacin treatment. Both groups were compared to a control group consisting of three healthy litters. While there was no apparent difference in the duration of the treatment (from the onset until the disappearance of clinical signs), which lasted 5-6 days in both groups, the ADG of the group subjected to probiotic treatment was significantly higher by the time of weaning compared to the group that received the antibiotic treatment ($p = 0.026$). This may be explained by the supportive effect probiotics exert on the intestinal microbiota, as well as the ability to regulate or stimulate the immune system. The effect of enrofloxacin on the other hand has the opposite effect. Due to its bactericidal activity and broad spectrum, not only pathogenic bacteria will be killed but part of the microbiota of the intestine as well, which means that after recovering from the disease, the microflora of the intestinal tract has to be regenerated before the piglet is able to digest and absorb nutrients efficiently. As an additional complication, the area on the intestinal mucosa void of bacteria may serve as a colonisation site for pathogenic bacteria like *B. difficile*.

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8. References

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