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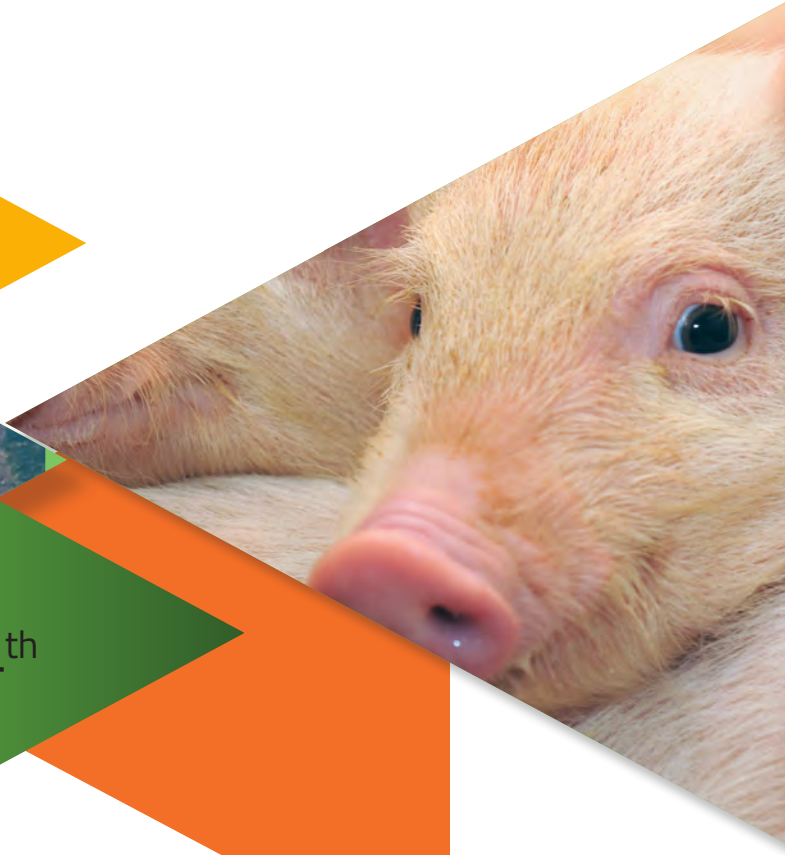

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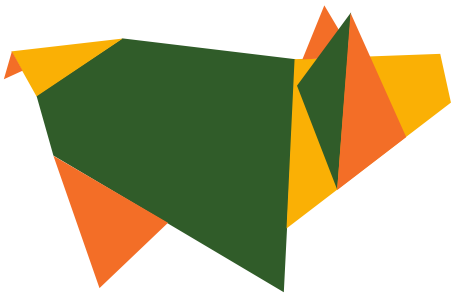

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Colonic innate immune defenses in swine dysentery caused by *Brachyspira hyodysenteriae*

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Introduction

Barrier function of porcine colon relies on organization of epithelial cells and mucus

The gastrointestinal tract lies at the interface between host and environment, absorbing nutrients and water into the body while sequestering foreign materials, toxins, pathogens, and commensal flora. As such, the pig's intestines form a crucial barrier against infection. This gut barrier is compromised during infectious colitis, allowing invading pathogens to colonize the intestines and trigger myriad host responses that attempt to clear the pathogen. Simplistically, intestinal host defenses may be divided into three broad categories: the epithelial and mucus barrier, innate immune responses, and the colonic microbiota (Figure 1).

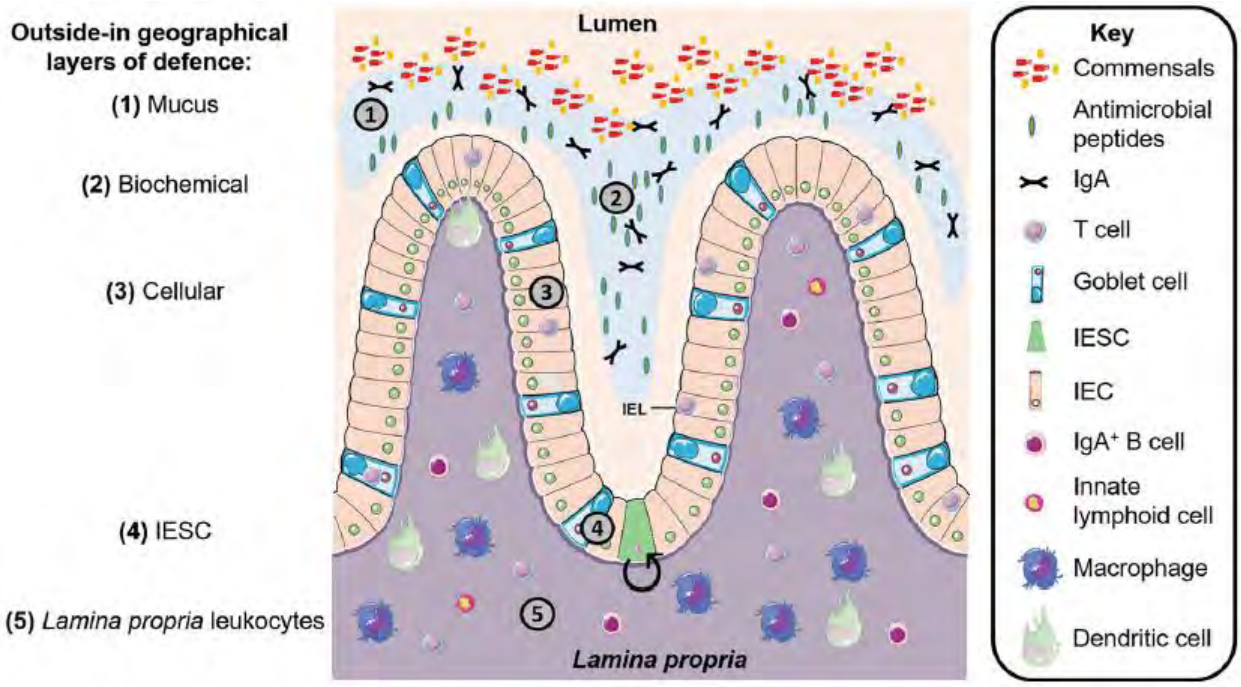


Figure 1. Intestinal host defenses. IESC: intestinal epithelial stem cell. IEC: intestinal epithelial cell. IgA: immunoglobulin A. Figure from previous publication (1).

The colon consists of folds of tissue (villi) separated by invaginations (crypts). Villi are composed of a monolayer of intestinal epithelial cells (IECs) and mucus-producing goblet cells, which derive from stem cells at the bottom of crypts and move toward the villus tip as they mature (2,3). IECs and goblet cells are connected by tight junctions that adhere each cell to its neighbours, forming a selectively permeable monolayer called the epithelium. The epithelium separates the intestinal lumen from the underlying tissue (lamina propria), where blood vessels and innate immune cells reside. Intestinal barrier function is compromised if the integrity of the epithelium is disrupted, allowing invasion of microorganisms into the lamina propria.

On the luminal side of the epithelium lies the colonic mucus, which forms a physical barrier designed to sequester luminal microorganisms away from host tissues. Mucus is formed mainly by glycoproteins called mucins that are composed of long protein chains covered in polysaccharides. Transmembrane mucins, particularly MUC1, 3, and 4 in pigs, are expressed on the apical surface of IECs. The luminal portion of these mucins forms a dense glycocalyx that protects the cells, and the transmembrane portions may facilitate intracellular signaling in response to luminal stimuli. Covering the glycocalyx are mucus layers composed of secreted gel-forming mucins that are produced, stored, and secreted by goblet cells. Unlike the monomeric transmembrane mucins, secreted gel-forming mucins homo-oligomerize into large polymers that attract water, thus forming a hydrogel that coats the intestine. In the colon, mucus is separated in two layers. The inner layer is tightly adhered to the epithelium. This dense layer is reported as sterile because microorganisms cannot penetrate its small pores (4,5) and are killed by the constitutive presence of antimicrobial peptides (AMPs) (6). Host and bacterial enzymes degrade polysaccharide residues on mucins of the inner layer, which allows expansion of the mucin polymer to form a loosely-attached outer mucus layer (4). This outer layer is where commensal microorganisms reside. In pigs, colonic mucus is mainly composed of MUC2 but expression of MUC5AC may be induced during infection (7). The organization of colonic mucus and glycocalyx serves to protect epithelial cells from luminal microorganisms and toxins. When the mucus barrier is compromised, pathogens may come into direct contact with the epithelium and activate inflammatory host responses.

Inflammation initiates the recruitment of innate immune cells that clear infection

The presence of pathogens in the colon triggers inflammation (colitis), which is a signaling cascade that brings innate immune cells to the site of infection. IECs are important initiators of inflammatory responses because they come into direct contact with invading pathogens when the mucus barrier is compromised. IECs may sense the presence of microorganisms by a variety of mechanisms, including cell-surface pattern recognition receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs) expressed by microorganisms (3). Transmembrane mucins may play a similar role in pathogen detection. PRR activation triggers intracellular signaling cascades within IECs that may result in secretion of antimicrobial peptides (AMPs) to kill the microbes in the lumen, and cytokines and chemokines to attract innate immune cells from the bloodstream into the lamina propria (3). Epithelial erosion may result from the pathogen itself or collateral damage from inflammatory responses (e.g., colitis caused by attaching/effacing pathogen *Citrobacter rodentium* in mice (8)). This may cause stem cells in the bottom of crypts to divide continuously (hyperplasia), which lengthens colonic crypts.

Neutrophils are considered the first innate immune cell to arrive at the site of infection with the primary goal of pathogen killing. Neutrophils destroy microorganisms using phagocytosis to engulf and digest pathogens, NETosis to trap them, and secretion of microbicidal compounds such as AMPs, myeloperoxidase (MPO) and reactive oxygen species (ROS) (9–12). Neutrophils also secrete chemokines and cytokines to further activate IECs and recruit more immune cells, thus perpetuating and amplifying inflammatory immune responses (13). Monocytes are recruited from the bloodstream then differentiate into macrophages inside colonic tissue, where they destroy pathogens using similar mechanisms to neutrophils (e.g., phagocytosis, ROS, cytokine production) (14). However, macrophages also participate in wound healing and tissue repair by phagocytosing dead cells and debris, and can initiate adaptive immune responses by antigen presentation to T and B cells in the lymph (14,15).

Colonic microbiota modulates host defenses by interacting with mucus, innate immune cells, and pathogens

The outer mucus layer is colonized by luminal commensal microorganisms that make up the colonic microbiome. These microorganisms use the mucus layer as a habitat, expressing lectins to attach to glycans on mucin molecules and finding food by consuming both glycans and ingested nutrients in the lumen. The repertoire of mucin glycosylation patterns possessed by the host may influence the composition of the microbiota and vice versa, which influences pathogenesis of infectious disease. Commensals strengthen the immune system by secreting metabolites such as butyrate, which promotes epithelial barrier integrity (16,17) and stimulates secretion of AMPs (18). Commensals may further strengthen host defenses by consuming nutrients and occupying space, thereby competing against pathogens. This is demonstrated by murine models that require antibiotic pre-treatment before infection with *Clostridium difficile* (17,18). Alternatively, commensals may contribute to pathogenesis by creating favorable environments for pathogen colonization (e.g., by secretion of metabolites as pathogen food sources or altering mucin glycosylation). Commensals normally sequestered in colonic lumen may even become opportunistic pathogens themselves when disruption of the mucus/epithelial barrier brings them into direct contact with host cells.

Swine dysentery as a model for colonic innate defenses against bacterial infection

Swine dysentery (SD) is an infection of grower-finisher pigs with the enteric anaerobic spirochete *Brachyspira hyodysenteriae*. The hallmark clinical sign of SD is mucohemorrhagic (bloody) diarrhea, often leading to poor feed conversion, stunted growth and up to 30% mortality in naturally infected pigs (19). Remarkably, evidence from experimental infection studies suggest that colonization with *B. hyodysenteriae* alone is not always sufficient to produce clinical signs of disease. Thus, interplay between pathogen, host, and environmental factors may be required to produce clinical SD, offering a unique opportunity to study colonic innate defenses in infectious colitis.

Although muco-hemorrhagic diarrhea is considered the hallmark clinical presentation of SD, literature reports that incidence of bloody diarrhea in experimentally infected pigs is variable and rarely reaches 100% (20). Most experimental inoculation studies report hemorrhagic diarrhea incidence ranging from 33% to 92% (21–27), while others fail to induce hemorrhagic diarrhea at all, producing only milder diarrhea without blood (20,24,28). Despite rigorously controlled experimental conditions, the incubation period between exposure and onset of clinical signs is also variable (1- 4 weeks) and some infected pigs may remain asymptomatic while still transmitting the pathogen by fecal shedding. This demonstrates that clinical presentation of SD is variable among pigs: some pigs may be particularly susceptible to *B. hyodysenteriae* infection whereas others are resilient. Research to date has often compared challenged and unchallenged pigs regardless of diarrhea severity and individual host responses. Thus, it remains unknown why some challenged pigs become diseased while others are non-diseased, and whether gut innate defenses influence clinical outcomes.

To understand host/intestinal factors that impact pathogenesis and their relation to disease severity, we conducted experimental infection of pigs with *B. hyodysenteriae*. Like literature, we showed a proportion of challenged pigs were diseased (developed bloody diarrhea, clinically affected with SD) but others were non-diseased (did not develop bloody diarrhea). Of the non-diseased pigs, some were colonized with *B. hyodysenteriae* while others were not, but all diseased pigs were colonized (Figure 2a). Comparing *non-diseased colonized* and *diseased colonized* pigs allowed us to identify host factors involved in pathogenesis of clinical SD. Diseased pigs displayed microscopic colonic lesions that were absent in non-diseased pigs regardless of *B. hyodysenteriae* colonization status (Figure 2b). They showed longer crypt depth (hyperplasia), erosion of the epithelium, hemorrhage in the lamina propria, and infiltration of immune/inflammatory cells in the colonic lumen and near the epithelial surface. These results indicate that pigs may be infected with *B. hyodysenteriae* yet remain asymptomatic and without histological colitis.

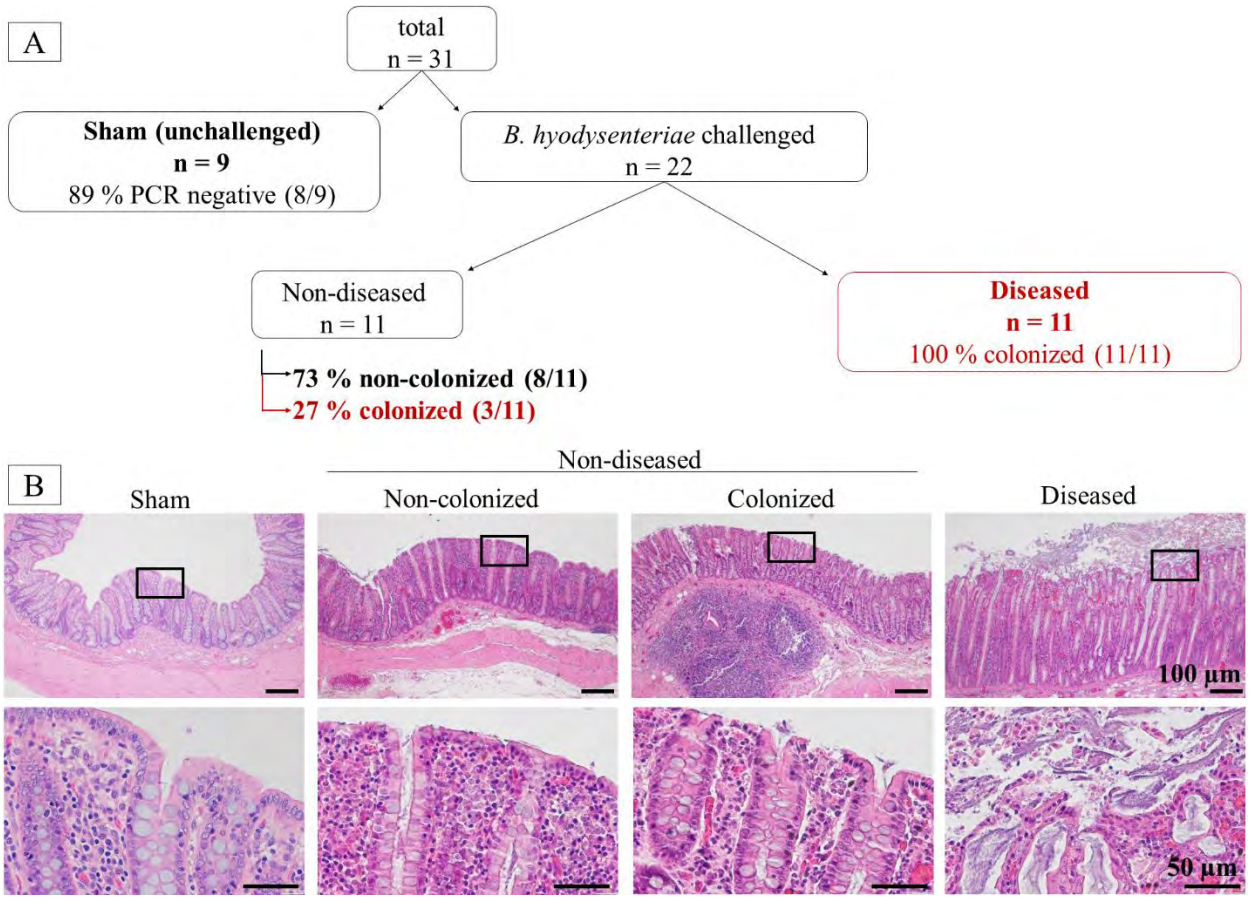


Figure 2. Pigs experimentally infected with *Brachyspira hyodysenteriae* show variation in severity of diarrhea and colitis. A) Breakdown of animal groups. Pigs that develop bloody diarrhea are "diseased". Pigs that are challenged with *B. hyodysenteriae* yet do not develop bloody diarrhea are "non-diseased". Detection of *B. hyodysenteriae* from colonic digesta by qPCR further divides non-diseased pigs into colonized and non-colonized groups. **B)** Representative images of H&E-stained colon sections.

Intestinal mucus alterations may promote *B. hyodysenteriae* colonization

SD causes mucus hypersecretion in the gut (21,29–31), unlike other colonic bacterial infections that degrade mucus. Mucus hypersecretion may function as a host defense mechanism to flush out pathogens. However, this strategy may be useless against SD because *B. hyodysenteriae* is well-adapted to thrive in a mucus-rich environment (32,33), illustrating the evolutionary arms race between pig and pathogen. Whether mucus hypersecretion in SD is driven by host or pathogen has been debated. In our study, diseased pigs showed alterations in the colonic mucus environment. Alcian blue staining showed fewer filled goblet cells, crypts filled with mucus, and excess mucus secretion into the lumen in colons of diseased pigs (Figure 3a). This corresponded with a trend in gene upregulation of MUC2 and MUC5AC (data not shown), and mobilization and increased presence of sialylated mucins into crypt and colonic lumens (Figure 3b). The finding that non-diseased pigs colonized with *B. hyodysenteriae* do not display mucus alterations infers such mucus changes are mostly host-driven rather than promoted by the pathogen itself. *B. hyodysenteriae* consumes sialic acid monosaccharides as a food source, and glycan patterns containing sialic acid were more abundant in pigs with SD than unchallenged pigs (34). Furthermore, there is greater variation in the repertoire of glycan patterns between individual unchallenged pigs than between pigs with SD (30). Taken together, these data indicate that host-driven changes in mucus composition and mucin glycosylation with greater sialic acid content may drive susceptibility to *B. hyodysenteriae* infection.

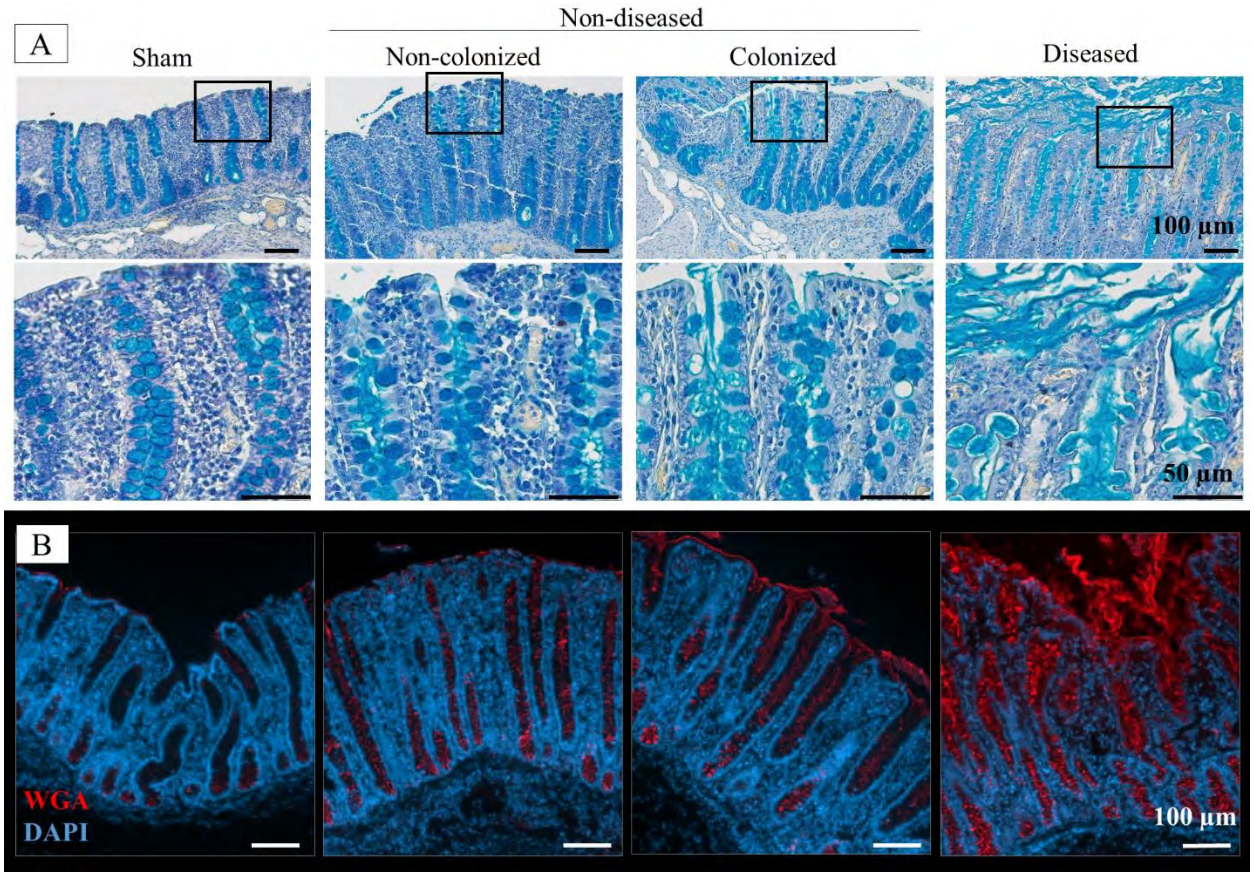


Figure 3. Diseased pigs display colonic mucus alterations not observed in non-diseased pigs. A) Representative images of Alcian blue-stained colons. **B)** Representative immunofluorescent images of colons stained with WGA lectin to detect N-acetylglucosamine and sialic acid (red), counterstained with DAPI (blue).

Neutrophils and macrophages did not clear *B. hyodysenteriae* despite AMP expression

Early recruited leukocytes are a key defense against infection, and neutrophilic infiltrates are commonly reported in colons of clinically affected pigs with SD (21,29). We showed neutrophils in diseased pigs localized in the lumen and near the epithelial surface (Figure 4). This is where neutrophils would encounter *B. hyodysenteriae,* since the pathogen localizes within colonic mucus by binding to sialic acid residues (29,34), attaching to epithelial cells (35), or internalizing within goblet cells (30). The fact that neutrophils are strongly detected in diseased but not in non-diseased pigs regardless of *B. hyodysenteriae* colonization status suggests that neutrophils are associated with clinical signs of SD. Gene expression of CXCL8, the classical chemokine for neutrophil recruitment (36), was similar among all groups (data not shown) indicating that neutrophil recruitment in SD is by other CXCL8-independent mechanisms or by secretion of pre-formed cytokine without transcriptional regulation.

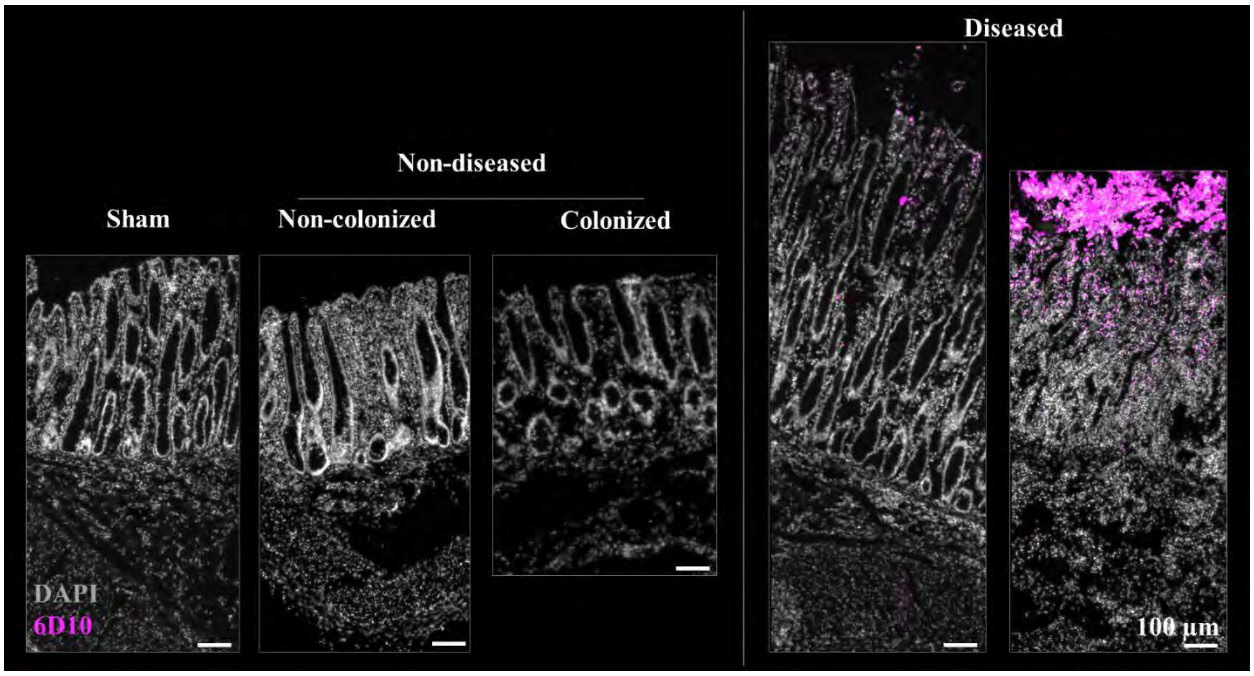


Figure 4. Neutrophils localize near colonic epithelium and lumen in diseased pigs. Representative immunofluorescent images of colon cryosections stained for the 6D10^{+} neutrophil marker (magenta) and counterstained with nuclear DAPI (gray).

We analyzed whether macrophages may be providing bacterial clearance defenses in the colon. We observed macrophages located at the tops and bottoms of colonic crypts in both sham and diseased pigs **(Fig 5)** suggesting macrophages do not impact clinical SD. However, non-diseased non-colonized pigs tended to have macrophages localized throughout the colonic lamina propria. Furthermore, non-diseased colonized pigs varied greatly, from having macrophages distributed throughout the lamina propria (like non-diseased negative group) to very few macrophages detected whatsoever. Thus, the presence of macrophages in the colon may be associated with the reparative/wound healing phase of infection (as in the non-diseased non-colonized group), or indicative of early infection stages in pigs without mucohemorrhagic diarrhea (non-diseased colonized group).

It is important to note that both neutrophil and macrophage populations in this study were examined only at a single timepoint at the peak of clinical signs of SD (upon bloody diarrhea onset). Thus, efficacy of bacterial clearance by neutrophils and macrophages could not be determined since this would require detecting cell populations at different time points during infection to associate their presence with bacterial shedding. However, previous studies showed that circulating blood neutrophils and monocytes increase during the onset of clinical signs of SD, and remain elevated until recovery after clinical signs have ceased (22,23). The dysentery and recovery periods were not necessarily associated with pathogen clearance since some pigs continued to shed *B. hyodysenteriae* in feces during and after recovery (22). Thus, innate immune defenses by neutrophils and macrophages may drive clinical presentation (diarrhea) instead of pathogen clearance.

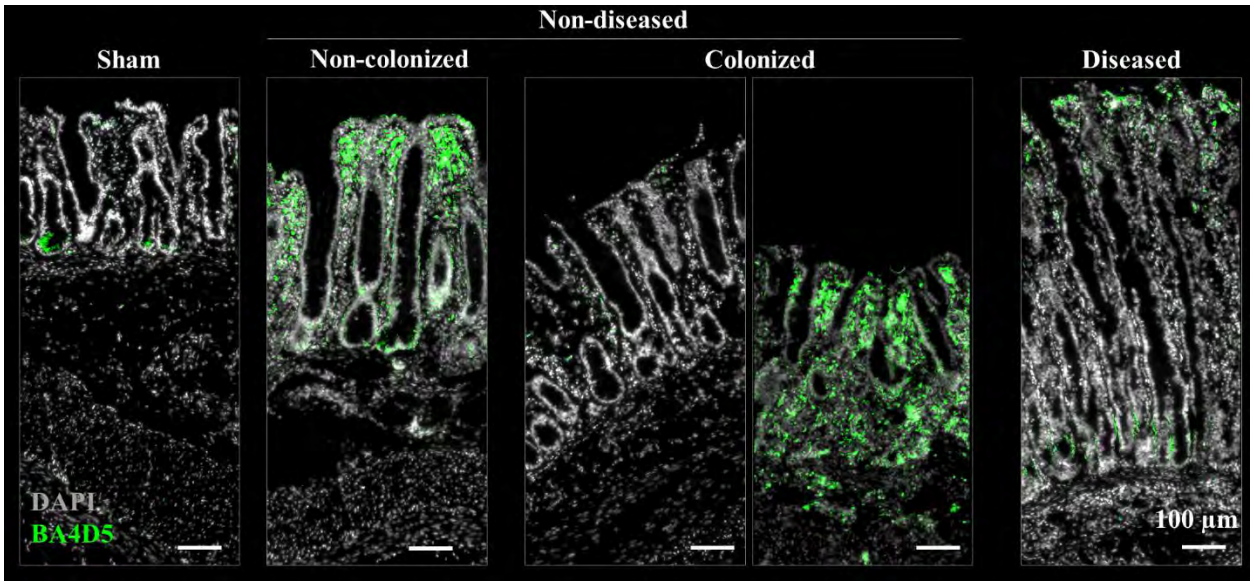


Figure 5. Colonic macrophages are distributed differently depending on clinical signs of disease. Representative immunofluorescent images of colon cryosections stained for the BA4D5^{+} macrophage marker (green) and counterstained with nuclear DAPI (gray).

We assessed antimicrobial effectors to further elucidate the ability of neutrophils to clear *B. hyodysenteriae* infection. Colonic myeloperoxidase (MPO), abundantly produced by neutrophils and macrophages, was quantified as a measure of degranulation (37). MPO activity did not differ between diseased and non-diseased groups. Colonic gene expression of cathelicidins, small antimicrobial peptides with immunomodulatory pro-inflammatory functions produced by neutrophils, were also assessed. Cathelicidins PMAP-37 was detected in 90% of diseased pigs compared to up to 67% in other groups, while PR-39 and protegrins 1-5 were not clearly associated with clinical signs of SD. Thus, PMAP-37 is activated as part of innate immune cell defenses (but not MPO or other cathelicidins) and is unable to clear *B. hyodysenteriae*.

Microbiome 16S sequencing indicates *B. hyodysenteriae* requires co-infection and is susceptible to competition from *Lactobacillus*

We explored the colonic microbiome associated with the mucosa and with the luminal contents (digesta) separately. In all parameters explored, the colonic mucosa showed more differences than colonic digesta, perhaps indicating invasion of commensals in the normally sterile inner mucus layer. In general, sham (normal microflora) and non-diseased groups showed similar microbiome profiles to each other but differed from diseased pigs. Diseased pigs showed dysbiosis in colonic mucosal microbiota, indicated by reduced alpha diversity. In colonic mucosa and digesta, beta diversity based on Bray-Curtis distance (a measure of diversity between samples) showed diseased pigs have an unique bacterial profile at genus level (Figure 6). A direct comparison of diseased and non-diseased pigs identified *[Acetivibrio] ethanolgignens* as characteristic in diseased pigs. *A. ethanolgignens* was originally discovered when isolated from pigs with SD (38,39) but has not been described since. It is possible that co-infection with this pathogen is required for clinical SD. *Lactobacillus* showed to be characteristic of non-diseased pigs, confirming similar findings in a previous study (40). This indicates promise for probiotic prevention strategies against SD. Bacterial taxa were matched to predicted functional categories in the KEGG database (41) which found differences in metabolic pathways among pig groups. Thus, changes in colonic microbiota composition may provide a mechanism to explain previous reports that diet affects SD susceptibility (42–49).

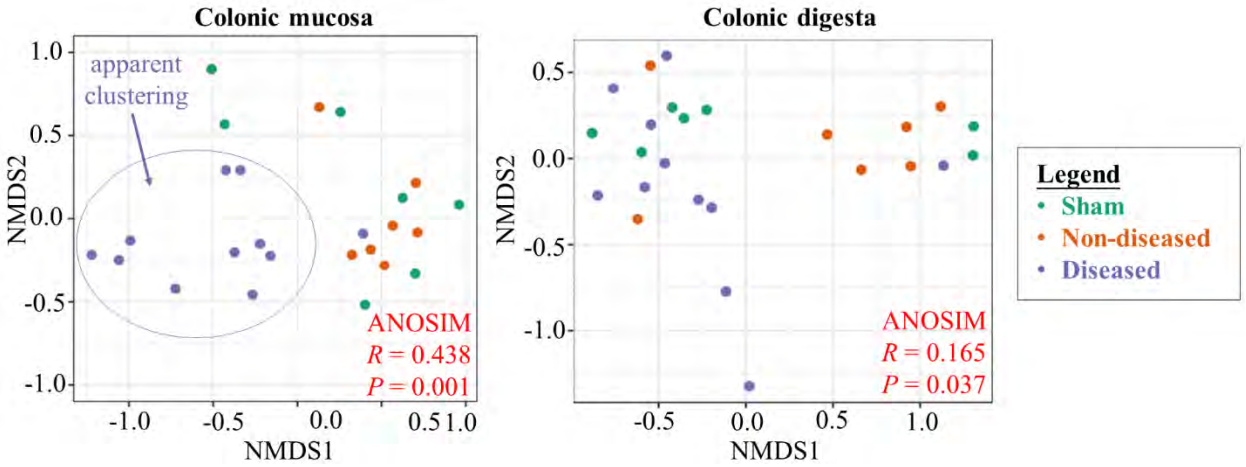


Figure 6. Beta diversity based on Bray-Curtis distance of bacterial profiles at genus level identified by 16S sequencing. Each data point represents one animal. Clustering of datapoints suggests similarity while dispersion suggests differences between animals. α = 0.05.

Cathelicidins are potential therapeutic modulators of colonic defenses in SD

Cathelicidins are a group of host defense peptides in mammalian innate immunity, secreted by leukocytes and differentiated epithelial cells at the surface of colonic crypts (50–53). All mammals express cathelicidins, but mammalian species like humans and mice contain a single gene for an alpha-helical peptide: LL-37 and CRAMP, respectively (54). Pigs are an exception and express nearly a dozen cathelicidins with a variety of structural conformations, including proline-arginine-rich 39-amino-acid peptide (PR-39), protegrins (PGs) -1 to -5, and porcine myeloid antimicrobial peptides (PMAPs)-23, -36 and -37 (50,51,54). Although not tested against *B. hyodysenteriae,* these peptides possess microbicidal activity against a variety of Gram-negative pathogens (54). The mechanism of microbicidal activity depends on the peptide and may be lytic (PGs, PMAPs) or non-lytic by internalizing into cells and blocking DNA synthesis (PR-39) (54). However, evolutionary evidence suggests that the function of cathelicidins is immunomodulation and not merely microbicidal activity. Peptide regions responsible for antimicrobial activity are not evolutionarily conserved among mammalian species (55). Conversely, the portions of cathelicidin precursors that are indispensable for immunomodulation are highly conserved and co-evolved with the pathogen recognition formyl peptide receptor (FPR2) (55). Thus, mammalian cathelicidins may be integral in innate defense against enteric infection and porcine cathelicidins regulate many aspects of host responses in colitis. The colonic mucus layer is regulated by cathelicidins, demonstrated when PG-1 attenuated murine DSS colitis by increasing MUC2 gene expression, preventing goblet cell loss and restoring the colonic mucus barrier (56). Furthermore, the most well-studied porcine cathelicidin, PR-39, promotes pro-inflammatory neutrophil and macrophage functions. PR-39 binds neutrophil DNA to protect NETs from enzymatic degradation (57) and is directly chemo attractive for neutrophils (58,59) but not mononuclear cells (59). PR-39 also promotes IL-8 and TNF-α secretion by immortalized porcine alveolar (3D4/31) macrophages (60). Thus, cathelicidins could mediate colitis by modulating innate immune cell and goblet cell function.

Cathelicidins have not been explored in SD. However, their immunomodulatory activities warrant their investigation as a possible therapeutic, which may reduce or replace antibiotic use against this increasingly resistant disease. It is possible that *B. hyodysenteriae* has become resistant to host defenses mediated by endogenous porcine cathelicidins, so we focussed on the murine cathelicidin CRAMP as a primary candidate for a novel therapeutic. CRAMP has never been administered to pigs, so we explored its safety in naïve pigs unchallenged to *B. hyodysenteriae*. A pilot study showed CRAMP intraperitoneal injection was well tolerated for over 2 weeks, with no signs of histological colitis and minimal mucus barrier alterations. Future exploration of doses, practical routes of administration, and efficacy in a *B. hyodysenteriae* infection model may elucidate abilities of CRAMP to strengthen mucus, innate immune and microbiome defenses against SD.

References

1. **Thoo L, Noti M, Krebs P.** Keep calm: the intestinal barrier at the interface of peace and war. *Cell Death Dis* [Internet]. 2019;10:849. Available from: http://dx.doi.org/10.1038/s41419-019-2086-z
2. **Mahapatro M, Erkert L, Becker C.** Cytokine-mediated crosstalk between immune cells and epithelial cells in the gut. *Cells*. 2021;10(1):111.
3. **Pardo-Camacho C, González-Castro AM, Rodiño-Janeiro BK, Pigrau M, Vicario M.** Epithelial immunity: Priming defensive responses in the intestinal mucosa. *Am J Physiol Gastrointest Liver Physiol.* 2018;314(2):G247–55.
4. **Hansson GC.** Role of mucus layers in gut infection and inflammation. *Curr Opin Microbiol* [Internet]. 2012;15(1):57–62. Available from: http://dx.doi.org/10.1016/j.mib.2011.11.002
5. **Hansson GC.** Mucus and mucins in diseases of the intestinal and respiratory tracts. *J Intern Med.* 2019;285(5):479–90.
6. **Blyth GAD, Connors L, Fodor C, Cobo ER.** The network of colonic host defense peptides as an innate immune defense against enteropathogenic bacteria. *Front Immunol*. 2020;11:965.
7. **Quintana-Hayashi MP, Padra M, Padra JT, Benktander J, Lindén SK.** Mucus-pathogen interactions in the gastrointestinal tract of farmed animals. *Microorganisms*. 2018;6(55):1–21.
8. **Koroleva EP, Halperin S, Gubernatorova EO, Macho-Fernandez E, Spencer CM, Tumanov A V.** Citrobacter rodentium-induced colitis: A robust model to study mucosal immune responses in the gut. *J Immunol Methods* [Internet]. 2015;421:61–72. Available from: https://doi.org/10.1016/j.jim.2015.02.003
9. **Saha P, Yeoh BS, Xiao X, Golonka RM, Singh V, Wang Y, et al.** PAD4-dependent NETs generation are indispensable for intestinal clearance of Citrobacter rodentium. *Mucosal Immunol* [Internet]. 2019;12(3):761–71. Available from: http://dx.doi.org/10.1038/s41385-019-0139-3
10. **Hofman P, Piche M, Far DF, Le Negrate G, Selva E, Landraud L, et al.** Increased Escherichia coli phagocytosis in neutrophils that have transmigrated across a cultured intestinal epithelium. *Infect Immun*. 2000;68(2):449–55.
11. **Moghadam ZM, Henneke P, Kolter J.** From flies to men: ROS and the NADPH oxidase in phagocytes. *Front Cell Dev Biol*. 2021;9:628991.
12. **Othman A, Sekheri M, Filep JG.** Roles of neutrophil granule proteins in orchestrating inflammation and immunity. *FEBS J.* 2021;1–22.
13. **Tsai C-Y, Hsieh S-C, Liu C-W, Lu C-S, Wu C-H, Liao H-T, et al.** Cross-talk among polymorphonuclear neutrophils, immune, and non-immune cells via released cytokines, granule proteins, microvesicles, and neutrophil extracellular trap formation: A novel concept of biology and pathobiology for neutrophils. *Int J Mol Sci.* 2021;22(6):3119.
14. **Ruder B, Becker C.** At the forefront of the mucosal barrier: The role of macrophages in the intestine. *Cells*. 2020;9(10):2162.
15. **Lazzaretto B, Fadeel B.** Intra- and extracellular degradation of neutrophil extracellular traps by macrophages and dendritic cells. *J Immunol.* 2019;203(8):2276–90.
16. **Huang X, Oshima T, Tomita T, Fukui H, Miwa H.** Butyrate alleviates cytokine-induced barrier dysfunction by modifying claudin-2 levels. *Biology (Basel).* 2021;10(3):205.
17. **Fachi JL, Felipe J de S, Pral LP, da Silva BK, Corrêa RO, de Andrade MCP, et al.** Butyrate protects mice from Clostridium difficile-induced colitis through an HIF-1-dependent mechanism. *Cell Rep.* 2019;27(3):750–61.
18. **Hayashi A, Nagao-Kitamoto H, Kitamoto S, Kim CH, Kamada N.** The butyrate-producing bacterium Clostridium butyricum suppresses Clostridioides difficile infection via neutrophil- and antimicrobial cytokine–dependent but GPR43/109a-independent mechanisms. *J Immunol.* 2021;206(7):1576–85.
19. **Hampson DJ.** Brachyspiral colitis. In: Zimmerman J, Karriker L, Ramirez A, Schwartz K, Stevenson G, editors. *Diseases of swine* [Internet]. 10th ed. Hoboken New Jersey: John Wiley & Sons Inc.; 2012. p. 680–96. Available from: http://researchrepository.murdoch.edu.au/16962/
20. **Hyatt DR, ter Huurne AAHM, van der Zeijst BAM, Joens LA.** Reduced virulence of Serpulina hyodysenteriae hemolysin-negative mutants in pigs and their potential to protect pigs against challenge with a virulent strain. *Infect Immun.* 1994;62(6):2244–8.
21. **Wilberts BL, Arruda PH, Kinyon JM, Madson DM, Frana TS, Burrough ER.** Comparison of lesion severity, distribution, and colonic mucin expression in pigs with acute swine dysentery following oral inoculation with “Brachyspira hampsonii” or Brachyspira hyodysenteriae. *Vet Pathol.* 2014;51(6):1096–108.
22. **Jonasson R, Andersson M, Råsbäck T, Johannisson A, Jensen-Waern M.** Immunological alterations during the clinical and recovery phases of experimental swine dysentery. *J Med Microbiol*. 2006;55:845–55.
23. **Jonasson R, Johannisson A, Jacobson M, Fellström C, Jensen-Waern M.** Differences in lymphocyte subpopulations and cell counts before and after experimentally induced swine dysentery. *J Med Microbiol.* 2004;53(4):267–72.
24. **La T, Phillips ND, Coiacetto F, Hampson DJ.** An atypical weakly haemolytic strain of Brachyspira hyodysenteriae is avirulent and can be used to protect pigs from developing swine dysentery. *Vet Res* [Internet]. 2019;50(1):47. Available from: https://doi.org/10.1186/s13567-019-0668-5
25. **Jacobson M, Lindberg R, Jonasson R, Fellström C, Jensen Waern M.** Consecutive pathological and immunological alterations during experimentally induced swine dysentery - a study performed by repeated endoscopy and biopsy samplings through an intestinal cannula. *Res Vet Sci.* 2007;82(3):287–98.
26. **Kruse R, Essén-Gustavsson B, Fossum C, Jensen-Waern M.** Blood concentrations of the cytokines IL-1beta, IL-6, IL-10, TNF-alpha and IFN-gamma during experimentally induced swine dysentery. *Acta Vet Scand.* 2008;50(1):32.
27. **La T, Phillips ND, Wanchanthuek P, Bellgard MI, O’Hara AJ, Hampson DJ.** Evidence that the 36kb plasmid of Brachyspira hyodysenteriae

contributes to virulence. *Vet Microbiol* [Internet]. 2011;153:150–5. Available from: http://dx.doi.org/10.1016/j.vetmic.2011.02.053

28. **Jacobson M, Fellström C, Lindberg R, Wallgren P, Jensen-Waern M.** Experimental swine dysentery: comparison between infection models. *J Med Microbiol*. 2004;53(4):273–80.
29. **Quintana-Hayashi MP, Mahu M, De Pauw N, Boyen F, Pasmans F, Martel A, et al.** The levels of Brachyspira hyodysenteriae binding to porcine colonic mucins differ between individuals, and binding is increased to mucins from infected pigs with de novo MUC5AC synthesis. *Infect Immun.* 2015;83(4):1610–9.
30. **Venkatakrishnan V, Quintana-Hayashi MP, Mahu M, Haesebrouck F, Pasmans F, Lindén SK.** Brachyspira hyodysenteriae infection regulates mucin glycosylation synthesis inducing an increased expression of core-2 O-glycans in porcine colon. *J Proteome Res.* 2017;16:1728–42.
31. **Enns CB, Harding JCS, Loewen ME.** Decreased electrogenic anionic secretory response in the porcine colon following in vivo challenge with Brachyspira spp. supports an altered mucin environment. *Am J Physiol Gastrointest Liver Physiol*. 2019;316(4):G495–508.
32. **Casas V, Vadillo S, Juan CS, Carrascal M, Abian J.** The exposed proteomes of Brachyspira hyodysenteriae and B. pilosicoli. *Front Microbiol.* 2016;7:1103.
33. **Bellgard MI, Wanchanthuek P, La T, Ryan K, Moolhuijzen P, Albertyn Z, et al.** Genome sequence of the pathogenic intestinal spirochete Brachyspira hyodysenteriae reveals adaptations to its lifestyle in the porcine large intestine. *PLoS One.* 2009;4(3):e4641.
34. **Quintana-Hayashi MP, Venkatakrishnan V, Haesebrouck F, Lindén S.** Role of sialic acid in Brachyspira hyodysenteriae adhesion to pig colonic mucins. *Infect Immun*. 2019;87(7):e00889-18.
35. **Gömmel M, Barth S, Heydel C, Baljer G, Herbst W.** Adherence of Brachyspira hyodysenteriae to porcine intestinal epithelial cells is inhibited by antibodies against outer membrane proteins. *Curr Microbiol.* 2013;66(3):286–92.
36. **Russo RC, Garcia CC, Teixeira MM, Amaral FA.** The CXCL8/IL-8 chemokine family and its receptors in inflammatory diseases. *Expert Rev Clin Immunol.* 2014;10(5):593–619.
37. **Strzepaa A, Pritchard KA, Ditte BN.** Myeloperoxidase: a new player in autoimmunity. *Cell Immunol.* 2016;317:1–8.
38. **Robinson IM, Ritchie AE.** Emendation of Acetivibrio and description of Acetivibrio ethanolgignens, a new species from the colons of pigs with dysentery. *Int J Syst Bacteriol.* 1981;31(3):333–8.
39. **Robinson IM, Whipp SC, Bucklin JA, Allison MJ.** Characterization of predominant bacteria from the colons of normal and dysenteric pigs. *Appl Environ Microbiol.* 1984;48(5):964–9.
40. **Burrough ER, Arruda BL, Plummer PJ.** Comparison of the luminal and mucosa-associated microbiota in the colon of pigs with and without swine dysentery. *Front Vet Sci.* 2017;4:139.
41. **Kanehisa Laboratories. KEGG** pathway database [Internet]. 2020 [cited 2020 May 7]. Available from: https://www.genome.jp/kegg/pathway.html#genetic
42. **Siba PM, Pethick DW, Hampson DJ.** Pigs experimentally infected with Serpulina hyodysenteriae can be protected from developing swine dysentery by feeding them a highly digestible diet. *Epidemiol Infect.* 1996;116(2):207–16.
43. **Mølbak L, Thomsen LE, Jensen TK, Bach Knudsen KE, Boye M.** Increased amount of Bifidobacterium thermacidophilum and Megasphaera elsdenii in the colonic microbiota of pigs fed a swine dysentery preventive diet containing chicory roots and sweet lupine. *J Appl Microbiol.* 2007;103(5):1853–67.
44. **Hansen CF, Phillips ND, La T, Hernandez A, Mansfield J, Kim JC, et al.** Diets containing inulin but not lupins help to prevent swine dysentery in experimentally challenged pigs. *J Anim Sci*. 2010;88(10):3327–36.
45. **Hansen CF, Hernández A, Mansfield J, Hidalgo Á, La T, Phillips ND, et al.** A high dietary concentration of inulin is necessary to reduce the incidence of swine dysentery in pigs experimentally challenged with Brachyspira hyodysenteriae. *Br J Nutr.* 2011;106(10):1506–13.
46. **Bilic B, Bilkei G.** Effect of highly fermentable dietary fiber on pig performance in a large unit infected with endemic swine dysentery. *Acta Verinaria Beogr.* 2003;53(4):229–38.
47. **Pluske JR, Durmic Z, Pethick DW, Mullan BP, Hampson DJ.** Confirmation of the role of rapidly fermentable carbohydrates in the expression of swine dysentery in pigs after experimental infection. *J Nutr.* 1998;128(10):1737–44.
48. **Thomsen LE, Knudsen KEB, Jensen TK, Christensen AS, Møller K, Roepstorff A.** The effect of fermentable carbohydrates on experimental swine dysentery and whip worm infections in pigs. *Vet Microbiol.* 2007;119:152–63.
49. **Durmic Z, Pethick DW, Mullan BP, Accioly JM, Schulze H, Hampson DJ.** Evaluation of large-intestinal parameters associated with dietary treatments designed to reduce the occurrence of swine dysentery. *Br J Nutr.* 2002;88(2):159–69.
50. **Sang Y, Blecha F.** Porcine host defense peptides: Expanding repertoire and functions. *Dev Comp Immunol.* 2009;33(3):334–43.
51. **Kościuczuk EM, Lisowski P, Jarczak J, Strzałkowska N, Jóźwik A, Horbańczuk J, et al.** Cathelicidins: family of antimicrobial peptides, A review. *Mol Biol Rep.* 2012;39(12):10957–70.
52. **Iimura M, Gallo RL, Hase K, Miyamoto Y, Eckmann L, Kagnoff MF**. Cathelicidin mediates innate intestinal defense against colonization with epithelial adherent bacterial pathogens. *J Immunol [Internet].* 2005;174(8):4901–7. Available from: http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.174.8.4901
53. **Hase K, Eckmann L, Leopard JD, Varki N, Kagnoff MF.** Cell differentiation is a key determinant of cathelicidin LL-37/human cationic antimicrobial protein 18 expression by human colon epithelium. *Infect Immun.* 2002;70(2):953–63.

54. **Tomasinsig L, Zanetti M.** The cathelicidins - structure, function and evolution. *Curr Protein Pept Sci.* 2005;6(1):23–34.

55. **Zhu S, Gao B.** Positive selection in cathelicidin host defense peptides: adaptation to exogenous pathogens or endogenous receptors? *Heredity (Edinb).* 2017;118(5):453–65.

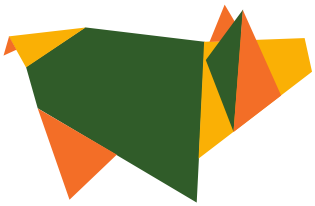
56. **Huynh E, Penney J, Caswell J, Li J**. Protective effects of protegrin in dextran sodium sulfate-induced murine colitis. *Front Pharmacol.* 2019;10(FEB):1–12.

57. **de Buhr N, Reuner F, Neumann A, Stump-Guthier C, Tenenbaum T, Schroten H, et al.** Neutrophil extracellular trap formation in the Streptococcus suis-infected cerebrospinal fluid compartment. *Cell Microbiol.* 2017;19(2):e12649.

58. **Djanani A, Mosheimer B, Kaneider NC, Ross CR, Ricevuti G, Patsch JR, et al**. Heparan sulfate proteoglycan-dependent neutrophil chemotaxis toward PR-39 cathelicidin. *J Inflamm.* 2006;3(14):1–5.

59. **Huang HJ, Ross CR, Blecha F.** Chemoattractant properties of PR-39, a neutrophil antibacterial peptide. *J Leukoc Biol* [Internet]. 1997;61:624–9. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-0030976983&partnerID=tZOtx3y1

60. **Veldhuizen EJA, Schneider VAF, Agustiandari H, Van Dijk A, Tjeerdsma-van Bokhoven JLM, Bikker FJ,** et al. Antimicrobial and immunomodulatory activities of PR-39 derived peptides. *PLoS One.* 2014;9(4):1–7.



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