Title

Investigation of pre-weaning mortality in a farrow-to-finish operation.

ID number: xxxxxxx

Introduction

Pre-weaning mortality in modern pork farms may result from variety of insults. These can be hereditary or congenital problems, nutritional deficiencies, errors in husbandry, and infectious agents. Piglets are particularly susceptible to infections due to their naïve immunological system, and their relative inability to develop appropriate pro-inflammatory responses that, together with a predominant hypo-inflammatory state, actively causes immunosuppression[1]. Pre-weaning infectious diarrhea is a major issue for pork production today, and many etiological agents have been described as significant[2-4]. Causes of suckling pig diarrhea includes TGEV (transmissible gastroenteritis virus), PEDV (porcine epidemic diarrhea virus), porcine rotavirus, *Clostridium perfringens* types A and C*, Clostridium difficile, Escherichia coli* (enterotoxigenic), *Strongyloides ransomi, Cystoisopora suis* and *Cryptosporidium* spp.

TGEV is a highly contagious coronavirus known to induce vomiting, diarrhea and high mortality in piglets less than 2 weeks of age. Older animals present with similar clinical signs, but mortality is decreased or absent. Lactating sows develop anorexia and agalactia, which can contribute to the piglet mortality[5]. Naïve herds develop epidemic TGE, where the disease spreads rapidly to pigs of all ages across the herd[6]. Endemic TGE occurs when the virus persists in the herd by a constant influx of susceptible animals. It is often a result of epidemic TGE in herds that have frequent farrowing, or commingling of pigs from a naïve source. Sows are frequently immune to TGEV and can transfer a variable degree of passive immunity. Mortality in piglets

24 older than 2 weeks of age is usually under 30%[7]. Post-mortem findings in affected piglets
25 include inflammation and thinning of the small intestinal wall. Microscopically, marked villous
26 atrophy in the jejunum and ileum is observed. Enterocyte necrosis leads to acute malabsorptive
27 diarrhea and dehydration[8]. Diagnosis based on the observation of clinical signs at both individual
28 and herd levels, together with gross post-mortem lesions is suggestive but not conclusive.
29 Confirmatory laboratory tests are necessary. Formalin-fixed intestinal segments of affected pigs
30 can be used for visualization of viral particles through immunohistochemistry or fluorescent
31 antibody imaging assays. PCR can be performed both in tissue or fecal samples of affected
32 animals for detection of viral nucleic-acid material[9]. Treatment for TGE is supportive, to
33 alleviate starvation, dehydration and acidosis. Parenteral delivery of fluids, electrolytes and
34 nutrients can be performed on individual pigs, but it is impractical from a herd perspective.
35 Prevention relies heavily on biosecurity, precluding the introduction of carrier pigs or TGEV-
36 contaminated fomites (boots, shoes, clothing, trucks, feed) to a naïve farm. Commercial vaccines
37 are useful in herds affected by endemic TGE only, as they fail to completely protect naïve
38 animals and should be used as booster[10].
39 PEDV is another coronavirus associated with pigs, similar to TGEV. In fact, PEDV
40 shares most of its clinical features with TGEV, including gross and microscopic lesions. The
41 agent was first detected in North America in 2013, during an outbreak in swine farms in the
42 USA[11]. Although clinical diagnosis is suggestive, final diagnosis relies on ancillary laboratory
43 tests such as PCR, ELISA or immunofluorescence[12]. Currently, no treatment is available, but
44 commercial vaccines can be used to prime sows and gilts, providing passive immunity. Stringent
45 biosecurity may slow viral spread between farms, especially through proper washing and
46 disinfection of trucks and other equipment potentially shared between premises. Intentional

47 exposure of sows to PEDV-contaminated feces or intestines can stimulate lactogenic immunity,
48 thus it is suggested to shorten clinical outbreaks[13].

49 Porcine rotavirus is a reovirus capable of causing diarrhea in the young of many animal
50 species, including suckling and recently weaned pigs[14]. Rotavirus serogroup A is more
51 commonly associated with diseased piglets than serogroups B or C[4]. However, in the past years,
52 serogroup C is suggested as an emerging and relevant cause of diarrhea in commercial swine
53 operations[14]. Rotavirus is transmitted through the fecal-oral route, and clinical signs are profuse
54 watery diarrhea, dehydration, lethargy, anorexia and rapid weight loss in suckling pigs[15-17].
55 Morbidity varies with herd immune status, but studies under controlled conditions reported
56 mortality rates below 20% when no other pathogens are present. Incubation period ranges
57 between 1-4 days, and diarrhea persists for 1-10 days[15, 17]. Once in the small intestine, rotavirus
58 is usually found replicating in superficial epithelial cells of the jejunum and ileum, leading to
59 villi blunting and atrophy[18, 19]. Viral proteins (VP) 1-3 form the inner capsid of the virion, while
60 VP6 makes up the middle capsid and VP7 and VP4 the outer capsid. VP4 specifically binds to
61 sialic acid, a receptor present on the apical site of enterocytes, leading to internalization of the
62 virus[20]. Clinical signs result from the loss of intestinal villi, subsequent deficiency in fluid
63 absorption characterizing malabsorptive diarrhea[21]. In addition, non-structural viral protein 4
64 (nsp4) disrupts intracellular Ca^{2+} regulation, leading to Cl^- secretion into the lumen through a
65 CFTR-independent mechanism and increased peristalsis[22, 23]. Eradication of rotavirus from swine
66 herds is impractical, since it is ubiquitous. Thus, maternal immunity is key to reduce mortality
67 and to minimize the impact of infection. While vaccines for rotavirus serogroup A are
68 commercially available, there are none for serogroups B and C[14]. A common field strategy to
69 control Rotavirus disease is the use of a controlled exposure program (i.e. feedback). This

strategy involves exposing gilts and sows to Rotavirus-contaminated material 4-6 weeks prior to farrowing, to induce a humoral immune response[24]. Herds where sows and gilts are properly exposed to the virus pre-farrowing seldomly report significant issues with rotaviral diarrhea in piglets.

C. perfringens is a ubiquitous bacterium that colonizes the gastrointestinal tract of pigs. It is a Gram negative bacillus, anaerobic, capable of sporulation. All *C. perfringens* can produce alpha toxin (CPA), which is the sole toxin secreted by type A, while *C. perfringens* type C produces both alpha and beta toxins (CPB). *C. perfringens* type A induces watery diarrhea and necrotizing enteritis, whereas type C induces neonatal hemorrhagic diarrhea and necrotic enteritis. Either syndrome may appear as early as 12 hours after birth, but are commonly found in piglets between 3-7 days of age[25]. Type A associated disease was not reliably reproduced under controlled conditions in pigs, posing a question to its true clinical significance[25]. Type C enteritis may reach 100% morbidity in litters from non-vaccinated sows, becoming endemic as they develop lactogenic immunity[26]. Type C can be associated with high mortality (50-70%), whereas type A infection alone is seldom fatal. Type C lesions may involve the entire small intestine. Acute cases will present as necro-haemorrhagic while chronic cases may develop a necrotic pseudomembrane. Type A infections lead to mild small intestine lesions, mostly inducing thinning of the intestinal wall. Microscopically, it is observed necrosis of the epithelial wall, with conflicting reports regarding villous atrophy[25, 27]. Type C diagnosis is frequently obtained through the observation of clinical signs, post-mortem lesions and culture followed by genotyping of isolates[25]. There are no established criteria for the definitive diagnosis of type A enteritis due to its commensalism with the pig gut. However, non-haemorrhagic diarrhea associated with large numbers of *C. perfringens* type A isolated from affected jejunum or ileum

93 is suggestive of type A-associated disease[27]. Given *C. perfringens* ubiquitous nature, vaccination
94 of sows with commercial CPA and CPB toxoids prior to farrowing, including a booster, is
95 suggested. This strategy has been proven to reduce mortality up to ten-fold[28].
96 *Clostridium difficile* is a gram positive, strict anaerobic rod. Similar to other Clostridia, it
97 is ubiquitous in the environment and is able to sporulate[29]. Shedding sows are the main source of
98 infection for piglets, which mostly develop clinical signs between 1-7 days of age (watery
99 diarrhea, abdominal distension and sudden death in severe cases). Gross lesions are non-specific
100 and mostly found in the cecum and colon. Mesocolonic edema and colitis characterized by
101 mucosal erosion and ulcers were reported[25, 30]. Microscopic lesions are characterized as
102 necrosuppurative or erosive typhlocolitis [30]. Lesions result from cellular damage by 3 toxins
103 (TcdA, TcdB and CDTa) secreted by *C. difficile*. Diagnosis is based on clinical signs, post-
104 mortem findings and the detection of genes encoding the 3 toxins in feces or colonic contents of
105 affected animals by PCR. Culture of *C. difficile* is not presumptive of disease, given its
106 commensal state[30, 31]. Control and prevention of this disease is poorly studied in pigs, but
107 immunoprophylaxis based on toxoids is likely to be successful, as it is for other Clostridia toxins.
108 Antibiotic therapy should follow *in vitro* antimicrobial susceptibility results on an isolate basis.
109 *E. coli* is a gram negative, flagellated, rod-shaped bacterium, often found as a
110 gastrointestinal tract commensal. Neonatal diarrhea in pigs is associated with enterotoxigenic
111 (ETEC) *E. coli* expressing a combination of the F5(K99), F6(987P), F41, F4(K88), F18 or AIDA
112 adhesins, and STa, STb, LT, EAST1, α-hemolysin toxins. ETEC strains colonize the apical
113 brush border of enterocytes, without invading cells, inducing the secretion of cyclic guanosine
114 monophosphate (cGMP, an intracellular messenger), which results in ion leakage into the
115 intestinal lumen and diarrhea[32]. ETEC neonatal diarrhea is observed between 0 to 5 days of age,

characterized by watery or creamy, yellowish feces. Mortality may reach up to 70% in affected litters. It is associated with lethargy, dehydration, dilation of the stomach, dilation and congestion of the small intestine walls and enlargement of enteric lymph nodes. Microscopically, large mats of rods covering the mucosa are observed, together with increased numbers of neutrophils and macrophages in the lamina propria and mild, infrequent villous atrophy[32, 33]. Diagnosis is based on the observation of clinical signs and small gram-negative rod-shaped bacteria colonizing the small intestine. Further confirmation is necessary, achieved through fecal or small intestinal samples used for bacterial culture, preferably from euthanized, untreated piglets. Pathotyping through PCR is key to differentiate commensal isolates from pathogenic ones[34]. Treatment of individual litters using antimicrobials reduces mortality, and should always be coupled with antimicrobial sensitivity tests. Supportive care is useful to treat dehydration and prevent the systemic accumulation of d-lactate. Management strategies to reduce piglet exposure to pathogenic *E. coli* and to increase immunity in sows and piglets are suggested to control the disease. Proper environmental temperature, free of drafts and excessive humidity, helps ensure low-weight piglets thrive and don't become a source of infection for littermates. Maternal vaccination is very effective in controlling clinical signs in piglets. Commercial vaccines in general include bacterins from strains producing F4, F5, F6 and F41 adhesins, given in two doses prior to parturition, parenterally[32, 33].

Strongyloides ransomi is a small (3-5mm) nematode, rarely present in modern indoor swine operations, that parasites the small intestine of pigs. Embryonated eggs hatch in the environment within a few hours of being excreted and can either become infective third-stage larvae (homogonic cycle) or free living (heterogonic cycle). In adult pigs, infective larvae penetrate the skin or the oral mucosa, reach the lungs through the bloodstream and are

swallowed. By contrast, the main route of infection for piglets is through the ingestion of contaminated colostrum[35]. Larvae accumulate in a hypobiotic state in the mammary fat of pregnant sows, which is mobilized for colostrogenesis. Larvae reach the mammary gland and are shed in the colostrum. Piglets develop a patent infection between 2-4 days of age. Clinical signs include poor weight gain, watery to haemorrhagic diarrhea and death due to systemic acidosis. Intestinal mucosa hyperemia can be identified in affected piglets, depending on parasite load. Diagnosis is based on the observation of embryonated eggs by fecal flotation or fluorescence microscopy, or the observation of parasites within mucosal scrapings from the small intestine only[36]. Treatment of pigs with anthelmintic drugs is a temporary solution, and all efforts should focus on breaking the parasite life cycle to prevent new infections. Control is based on sanitation of premises, the use of indoor facilities, and limiting exposure to potential sources of infection (e.g introduction of infected pigs to the herd).

Cryptosporidium suis and *C. scrofarum* are parasites of pigs worldwide. They are obligate intracellular protozoans, capable of infecting humans. Feces or food contaminated with oocysts leads to the invasion of enterocytes by sporozoites, which will develop into merozoites and, 2-9 days after ingestion, male or female stages. Oocysts are excreted in feces for 9-15 days. Clinical signs include vomiting, diarrhea, depression and inappetence[37]. Diagnosis must be coupled with the observation of lesions, since pigs can be colonized without developing disease. PCR has been successfully applied to detect and type *Cryptosporidium* spp.[38]. No drugs have been proven to effectively treat *Cryptosporidium* infection in pigs. Sanitation of premises using heat, proper drying of surfaces and exposure to sunlight inactivates oocysts, thus breaking the parasite life cycle and limiting spreading of the disease[39].

Cystoisopora suis is a protozoan parasite reported worldwide in different swine management systems[40]. Suckling and weaned piglets as young as 6 days-old are most commonly affected with a non-hemorrhagic, yellowish, watery diarrhea, leading to poor weight-gain and rough hair coat. Disease severity decreases as pigs age. However, pigs of any age may become infected following the 5 days pre-patent period. Infection occurs through the ingestion of sporulated oocysts from contaminated feces or the environment[41]. Sporocysts are released from the oocyst in the small intestine, and activated sporozoites invade epithelial cells (frequently found in the jejunum). An inflammatory response is observed grossly, and microscopic lesions are characterized by villi atrophy, fusion and blunting[42, 43]. Specific molecular mechanisms associated with the pathogenesis are not clear. As mature enterocytes are invaded and necrotize, they are replaced by immature cells uncapable of efficiently performing either secretion or absorption of ions and, consequently, water. Peak oocyst shedding is observed 2-3 days after clinical signs develop[42, 44]. Preventive therapy involves the use of anticoccidials such as toltrazuril. A single dose of 20 mg/kg given to 3-days old piglets was shown to reduce clinical severity[45]. Control of neonatal coccidiosis can be successfully achieved through proper premise sanitation in certain cases. However, depending on environmental factors and socking density, the use of toltrazuril may still be required to reduce shedding[43]. Sanitation must be coupled with the removal of organic matter from the premises, disinfection and steam cleaning. Proper internal biosecurity prevents the introduction of contaminated fomites to the farrowing room.

Here, we report a case of increased pre-weaning mortality associated with profuse, watery diarrhea and weight loss in suckling pigs.

Clinical Report

184 *Farm description*

185 Pre-weaning diarrhea was not observed or a cause of concern in a 2500-sow farrow-to-
186 finish commercial farm in Western Canada. Up to July 2017, pre-weaning mortality averaged
187 12.7% (January 2017 to June 2017). This operation was comprised of two sites (length of time
188 pigs were reared in parenthesis, Figure 1), Site 1: gilt development (220 days), breeding,
189 gestation, farrowing (0-3 weeks), nursery (4-8 weeks) and grower (4-12 weeks) pigs. Finisher
190 pigs (13 weeks to market) were housed in Site 2 located about 5 km northwest from Site 1. The
191 farm practiced weekly weaning with lactation length averaging 22 days. Site 1 had 13 farrowing
192 rooms: rooms A to J had 14 farrowing crates per room, and the larger rooms (M, L, K) had 36
193 crates each. All rooms were operated on an all-in/all-out basis per room. Gilt replacements were
194 supplied internally and from a high health multiplier (69.9% annual replacement rate). Females
195 were artificially inseminated, and boars used for estrus stimulation and detection only. Average
196 parity of sows farrowed during the 12 months previous to the start of the interventions described
197 herein was 4.1. External biosecurity measures included shower-in/shower-out, locked doors, on-
198 site composting of dead stock, and visual signs outside the farm to prevent erratic visitors.

199 This herd was serologically negative for porcine respiratory and reproductive syndrome
200 virus type 2 (PRRSV2) and *Mycoplasma hyopneumoniae*. Animals were vaccinated for porcine
201 circovirus 2 (PCV-2)[a]. Swine influenza A virus (IAV-S) has been identified in this herd, as a
202 H3N2 strain was detected until 2015. After 2015 until July 2017, a H1N2 strain was detected.
203 *Actinobacillus pleuropneumniae* (serotype 12), *Haemophilus parasuis* (untyped) and
204 *Streptococcus suis* (serotype 9) were previously isolated from diseased pigs in this herd. The
205 vaccination protocol implemented in this operation is provided in Table 1. To control the parasite

load, the breeding herd was purge-dewormed three times per year (pyrantel tartrate[b], 106 mg/kg,) via feed for 28 days.

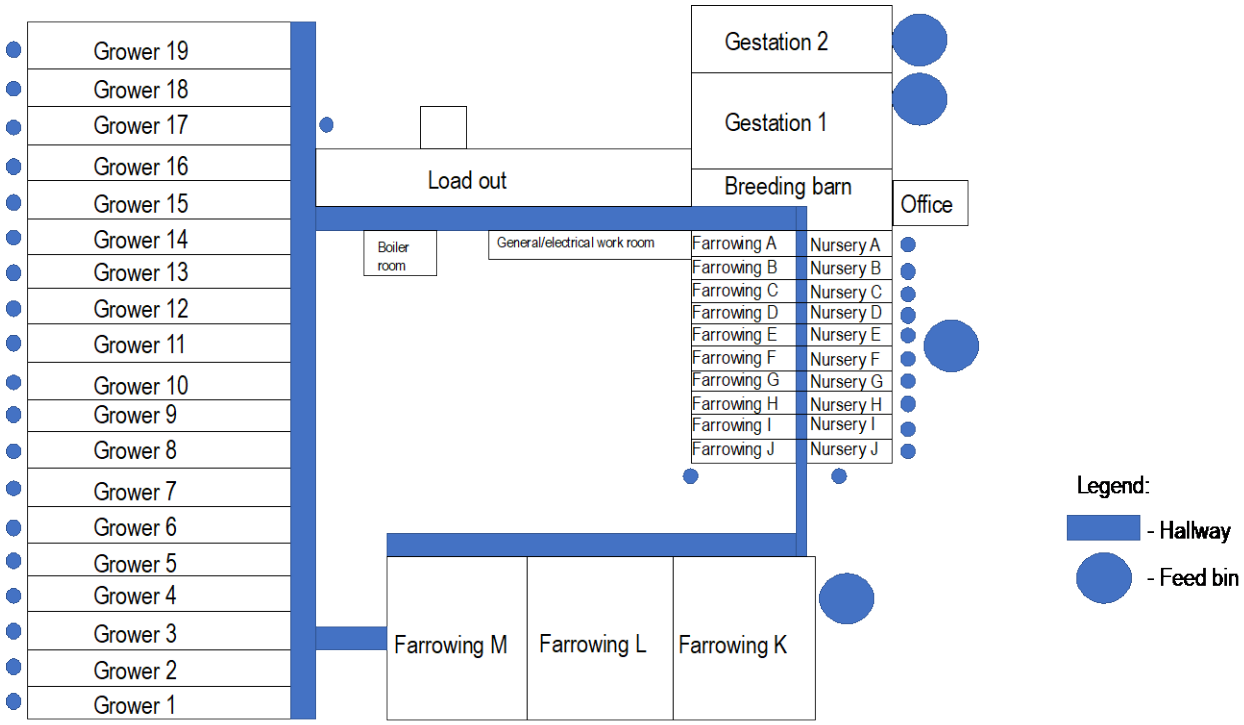


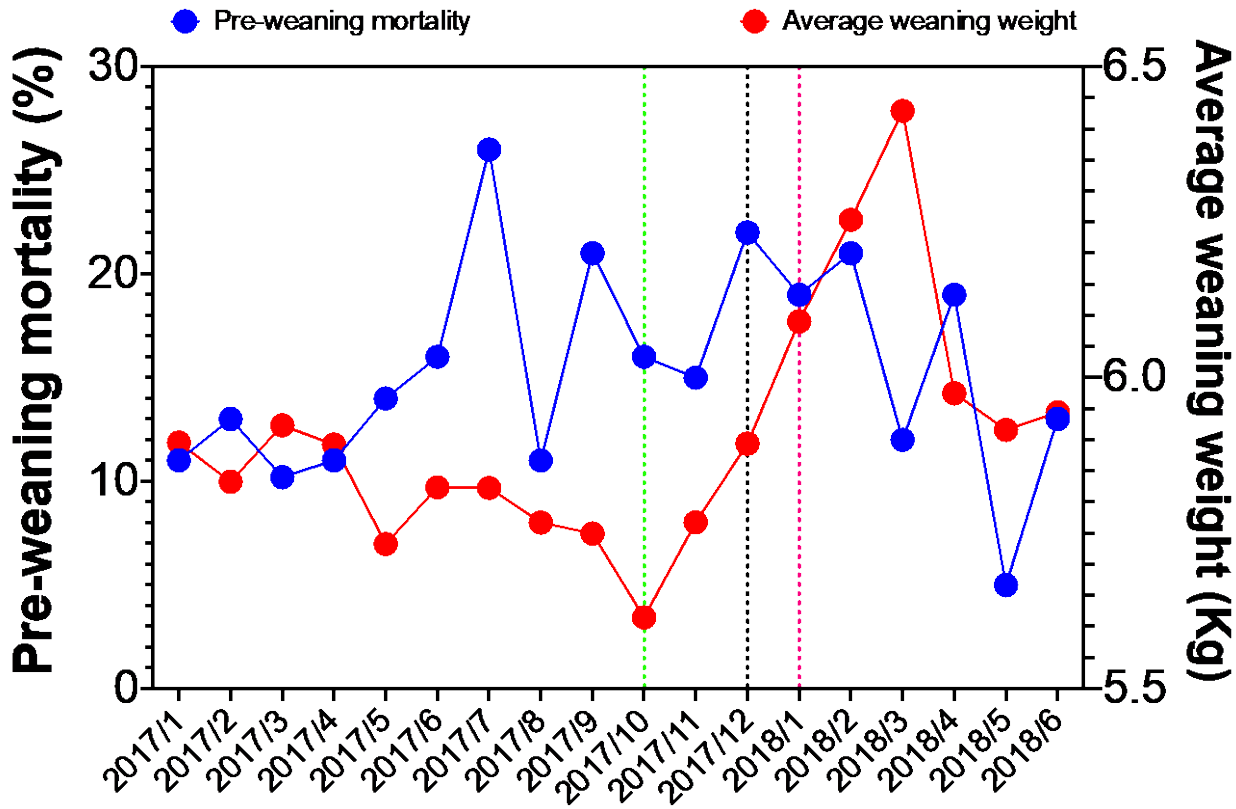
Figure 1 – Site 1 layout.

Table 1 - Vaccination protocol for all ages.

When{PRIVATE }	All gilts	Sows > First Parity	Gilts from multiplier	Boars	All Piglets
At multiplier, prior to delivery			PCV-2 *E. rhusiopathiae* *H. parasuis*		
On Entry (to breeding herd)	IAV-S at 160 and 190 days. PLE twice, on arrival and 3 weeks later.		*H. parasuis* and IAV-S on arrival. IAV-S boost 3 weeks later. PLE twice, 5 weeks and 2 weeks before breeding	PLE & IAV-S twice, 3 weeks apart	
Pre-Farrowing	*C. perfringens* and *E. coli* ETEC 6 & 3 wks before farrowing IAV-S & PLE 3 weeks before farrowing	*C. perfringens, E. coli* ETEC and *E. rhusiopathiae 3* weeks before farrowing.	*C. perfringens* and *E. coli* ETEC 6 and 3 weeks before farrowing IAV-S & PLE - 2 weeks before farrowing		
Once a year				*E. rhusiopathiae*	
At weaning					PCV-2

PLE[c] (Porcine Parvovirus, *Erysipelothrix rhusiopathiae*, and *Leptospira canicola, grippotyphosa, hardjo, icterohaemorrhagiae* and *pomona),* 5ml/pig, IM.
Erysipelothrix rhusiopathiae[d], 2ml/pig, IM.
C. perfringens[e] and *E. coli* ETEC[e], 2ml/pig, IM.
H. parasuis[f], 1ml/pig, IM.
IAV-S, 2 ml/pig, IM (autogenous[g]).
PCV-2[a], 2ml/pig, IM.

222 Although the farrowing rooms had dedicated staff, personnel from other sections were
223 frequently recruited to support their activities. Farrowing room husbandry included monitoring
224 sows every 30 minutes by dedicated staff, and split suckling to help disadvantaged piglets have
225 access to minimal colostrum amounts and improve weight gain. Low-viability piglets (weighting
226 less than 1 kg) were identified and euthanized within the first hours following birth. Piglet
227 processing took place at 5 days of age and included tail docking, castration of males, and iron
228 injection (IM, 200 mg/piglet, ferric hydroxide dextran complex[h]) coupled with meloxicam[i] (IM,
229 0.4 mg/kg) and ceftiofur[j] (IM, 3mg/kg). Teeth clipping was not routinely performed. Farrowing
230 rooms were maintained between 18°-20°C, while piglets had access to a water-heated floor pad
231 (water temperature kept between 36°C and 40°C). For the 12 months ending June 2017, the pre-
232 weaning mortality averaged 12.7%, and average wean weight was 5.96 kg.
233
234 *Problem definition, diagnostic investigation and interventions*
235 Starting July 2017, higher pre-weaning mortality rates were reported by the barn manager.
236 Between July and October 2017, pre-weaning mortality averaged 19.0%, peaking in July when
237 26% of the piglets born alive died before weaning (1723/6599, Figure 2).



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239 **Figure 2 –** Pre-weaning mortality by month (blue) and average weaning weight by month

240 (red) from January 2017 to June 2018. The dotted green line depicts when the first intervention

241 was performed. The dotted black line depicts when the second intervention was performed. The

242 dotted pink line depicts when the sanitation audit was performed.

243

A herd-health visit was conducted in October 2017. Pre-weaning mortality for September 2017 was 16.6% (1511/6873), with weaning weights 0.4 kg lighter than the targeted goal (6 kg). Upon examination, the main clinical observations were piglet huddling and piling, diarrhea (feces were soft to watery, yellow in color) affecting at least 30% of the piglets in the farrowing rooms (approximately 450/1500 piglets/batch) either during early lactation (0-3 days old) or later (14-17 days old), rough hair coat and low weight for their age. Coughing was also observed in piglets, mostly later in lactation (14-17 days old pigs) and affecting 10-15% of the piglets. About 50% of the piglets also presented with severe facial dermatitis, likely due to neonatal fights for teat access. During this visit, five suckling piglets presenting with diarrhea were necropsied, and a summary of findings is presented in Table 2. The main gross and microscopic lesions (reported by a board-certified veterinary pathologist) were related to the gastrointestinal tract, including villi atrophy, blunting and enteritis, colonic mucosal erosion and colitis. Differential diagnoses for enteritis in piglets of this age in this particular geographic location include: porcine rotavirus, TGE, *Strongyloides ransomi*, enterotoxigenic *Escherichia coli, Cystoisospora suis*, *Cryptosporidium* spp., *Clostridium perfringens,* and *C. difficile*. Respiratory lesions were described as suppurative bronchopneumonia. Differential diagnosis included *Haemophilus parasuis*, *Streptococcus suis, Pasteurella multocida* and IAV-S as the primary agent. Bacteriological investigation resulted in *E. coli* isolated from the intestine of in 2/3 piglets, and *Proteus* sp. isolated from lungs. Pathotyping of *E. coli* isolates indicated a toxin pattern suggestive of ETEC, limited to the presence of the STb gene only (Table 3). The pathotype isolated produced antigens different from the ones sows were vaccinated against, explaining the failure in inducing immunity.

Table 2 – Post-mortem findings from pre-weaning piglets with diarrhea – 1st diagnostic intervention.

Pig ID	Age	Respiratory tract findings	Digestive tract findings	Other findings	Ancillary diagnostics
K3	20 days	Severe and acute suppurative bronchopneumonia, locally extensive.	Small intestine: Enteritis, severe villous atrophy, and myriads of intraluminal bacilli. Large intestine: Colitis, multifocal, with superficial enterocyte necrosis, erosion and intralesional bacilli.	Multiple abscesses in the liver, spleen, thymus, diaphragm, mediastinum, mesentery, and retroperitoneum. Facial dermatitis.	**Bacterial culture:** Lung, small intestine and swab from abscesses: *Proteus* sp. (1+). Intestine: negative for *Salmonella* spp. **PCR**: Negative for porcine rotavirus (A, B or C).
K31	16 days	Moderate to severe and acute suppurative bronchopneumonia, locally extensive.	Small intestine: Villous atrophy, multifocal, mild with multifocal superficial necrosis, erosion, and neutrophilic inflammation. Colon: Colitis, neutrophilic, multifocal, moderate with superficial enterocyte necrosis, erosion and intralesional bacilli.	Facial dermatitis.	**Bacterial culture:** Lung: *Streptococcus suis* (3+), *Haemophilus parasuis* (1+). Small and large intestine: *E. coli* (2+). **PCR**: Negative for porcine rotavirus (A, B or C).
K47	14 days	None	Same as K31.	None	Large and small intestines positive for *Escherichia coli* (4+). Intestinal samples negative for *Salmonella* spp, porcine rotavirus (A, B or C, PCR) and *Clostridium* spp.
K24	14 days	None.	Enlarged colon, filled with pasty, yellowish feces.	Facial dermatitis.	Not performed.
K28	16 days	None.	Enlarged colon, filled with pasty, yellowish feces.	None.	Not performed.

271 **Table 3 -** *E. coli* pathotyping – isolates from 1st & 2nd diagnostic interventions.

Virulence gene	Detection by PCR
AIDA-1	Positive
Eae	Negative
F4	Negative
LT	Negative
STa	Negative
STb	Positive
Stx2e	Negative

272

Microscopic lesions (villi blunting and fusion) did not substantiate the hypothesis of *E. coli* as the primary agent causing the small and large intestinal lesions. Simultaneously, fecal samples from various affected litters were submitted for fecal flotation testing (associated with fluorescence microscopy for enhanced detection). Samples pooled by litter (given that not all pigs would be shedding at the same time, thus improving the diagnostic value of the sample) were collected from pigs aged 9-19 days (n=10 litters, at least 3 pigs sampled/litter). Eggs of *Cystoisospora suis* were detected in 6/10 pooled samples, suggesting the coccidia as a possible underlying cause of necrotic enteritis and villi atrophy. Toltrazuril[k] (oral, 50 mg/kg at 2 days of age) was prescribed as a short-term control strategy. At this point, respiratory lesions were not further investigated given the histopathological and bacteriological findings, the herd's high health status (PRRSV negative, SIV-A vaccinated), and the severe gastrointestinal disease. The latter was prioritized, especially given the assumption that it could be indirectly influencing the observation of mild lung lesions through an general immunosuppression.

Pre-weaning mortality in the following month (November) did not decrease to acceptable values (expected <10%), reaching 15.5% (1090/7028, Figure 2). Thus, a second diagnostic intervention was performed in December 2017. Diarrhea and poor weight gain, associated with rough hair coat were still observed in 30% of piglets/batch. Given the poor performance of the animals and to rule out systemic viral infections, 30 four-week-old pigs (prior to vaccination for PCV-2, to show no exposure in the farrowing room, previous to vaccination) and 15 twelve-week-old pigs (to demonstrate that vaccination was effectively controlling the agent) were sampled by venipuncture of the external jugular (8-12 mL per animal, no anticoagulant used for serum collection). Sera was separated routinely and submitted for PCV-2 quantitative PCR (qPCR[l]). All samples were negative. To rule out PRRSV infection, a different cohort of 30 four-

296 week-old pigs were bled (venipuncture of the external jugular, 8-12 mL per animal, no
297 anticoagulant used for serum collection) and all tested negative for PRRSV antibody using an
298 enzyme linked immunosorbent assay (ELISA^{m}). Post-mortem findings from necropsied suckling
299 pigs during this second diagnostic intervention are summarized on Table 4, including ancillary
300 diagnostics. *E. coli* pathotyping revealed that strains isolated had the same virulence gene profile
301 as isolates obtained from the first diagnostic intervention (Table 3).
302

303 **Table 4 –** Significant post-mortem findings from pre-weaning piglets with diarrhea – 2nd

304 diagnostic intervention.

Pig ID	Age	Respiratory tract findings	Digestive tract findings	Other findings	Ancillary diagnostics
1	4 days	Mild, non-specific interstitial pneumonia.	Small intestine. Mild villous atrophy, blunting and fusion.	Dehydrated.	**Bacterial culture**: Small intestine: *Clostridium perfringens* (1+) and *E. coli* (1+). Negative for *Salmonella* spp. Liver: *Clostridium perfringens* (1+) and *E. coli* (1+). **PCR**: Small intestine: porcine rotavirus type C (Ct 28).
2	4 days	Mild, non-specific interstitial pneumonia.	Small intestine. Mild villous atrophy, blunting and fusion.	Dehydrated.	**Bacterial culture**: Small intestine: *E. coli* (1+). Negative for *Salmonella* spp. **PCR**: Small intestine: porcine rotavirus type C (Ct 26).
3	4 days	Mild, non-specific interstitial pneumonia.	Small intestine. Mild villous atrophy, blunting and fusion.	None	**Bacterial culture**: Small intestine: *Clostridium perfringens* (1+) and *E. coli* (1+). Negative for *Salmonella* spp. Liver: *Clostridium perfringens* (1+). **PCR**: Small intestine: porcine rotavirus type C (Ct 25).

305

306

307 The most concerning lesions contributing to the poor piglet performance and mortality
308 were found localized in the gastrointestinal tract, characterized as villi atrophy, blunting and
309 fusion. Tissue tested positive for porcine rotavirus C by PCR, which may be implicated as the
310 primary cause of diarrhea and weight loss. Given that no commercial vaccine is available, a
311 strategy for controlled antigen exposure orally was applied to the next batch of farrowing sows
312 and gilts. Staff was educated about the infection and oriented to harvest intestinal tissue from
313 affected piglets that were either laid-on, low-viability or euthanized. Diarrheic feces, when
314 possible, were also collected. Using a clean 18 L pail, this material was mixed with clean water
315 (1:1 ratio) and blended using an industrial blender until a homogenous, liquid mixture was
316 obtained. This preparation was fed to sows and gilts 6 weeks prior to farrowing.
317 To aid on preventing and controlling both *C. suis* and rotavirus environmental load, a
318 farrowing room sanitation audit was performed to identify critical control points during the
319 cleaning and disinfection process. Staff were not aware of the audit, and were informed that this
320 was a regular herd health visit to prevent possible procedural changes. The cleaning and
321 disinfection protocol performed began with the use of a high pressure hot-water system to wash
322 the room and remove soil and organic material. This was followed by soaking the room for 15
323 minutes using an alkaline chlorinated degreaser (sodium hypochlorite[n]). Next, manual scrubbing
324 with pads[o] imbedded in a combination of sodium tallowate, sodium carbonate and sodium $C_{10\text{-}16}$
325 alkylbenzene sulfonate was performed inside and outside crate walls. A second degreasing step
326 (same product) was performed as previously described, followed by a high-pressure washing
327 step to remove the degreaser. Lastly, a glutaraldehyde/quaternary ammonium[p] disinfectant was
328 sprayed in the room and let dry until the next batch of pigs was ready to be moved in.
329 Immediately before the room was repopulated, 2 hours after sanitation was concluded, different

surfaces and materials throughout the room were swabbed[q] and submitted for bacterial culture (aerobic and anaerobic) or rotavirus PCR (all swabs could not be tested using both methodologies since the transport media for bacterial culture is a PCR inhibitor, thus they were randomly allocated to a diagnostic workflow). The audit identified the following non-compliance issues:

- Degreaser: The water used in these premises was known to have high concentrations of calcium carbonate. Alternation between alkaline and acid detergents was suggested to facilitate the removal of soil, and impair the formation of biofilms.

- SOS Pads: The following locations were not scrubbed: inside heat lamps, sow gate (joints not open), and crate walls (top scrubbed, bottom usually not within arm length reach). This step was perceived an unnecessary burden by the staff.

- Post-washing and disinfection: Organic material was not completely removed from the room (including feed and feces), being identified on crate floor, sow feeders, on crate gates and walls.

- Finally, minimal time between the rinsing step, drying and repopulation of the room, as suggested by the manufacturer, was not observed (4 hours instead of overnight).

A summary of the laboratory findings from the swabs taken is presented on Table 5. Results from the audit were presented to the barn manager and staff, followed by a discussion of points to be improved while welcoming their input. It became clear that manually scrubbing the crates with pads was unpractical, and was not yielding the expect results. Staff was educated about the importance of removing organic material, and the protocol was adjusted to include the use of an acid degreaser in the 2nd degreasing step (peracetic acid solution[r]) to replace the manual SOS Pad scrubbing step. Furthermore, a boot-dip was placed by the door of each room, filled

353 with a disinfectant[s] (1% w/v) and staff were instructed to use it whenever entering or leaving the
354 farrowing rooms, and to change the solution following the last room check of the day.
355

356 **Table 5 –** Sanitation audition laboratory findings.

Sample	Bacteriology	Porcine rotavirus PCR
Sow feeder 12	*C. perfringens*: few *E. coli:* 1+	Not performed.
Feces crate 14	*C. perfringens*: few *E. coli:* 3+	Not performed.
Feces crate 15	Bacillus spp.: 2+ *E. coli:* 2+	Not performed.
Feces crate 18	*Enterococcus hirae*: 1+ *E. coli:* 1+ *Pseudomonas*: 1+	Not performed.
Crate 2 - Bar	N/A	Serogroup C: Ct 30
Crate 13 - Lamp	N/A	Serogroup C: Ct 31
Crate 16 – Heat pad	N/A	Serogroup C: Ct 34
Crate 6 – Wall	N/A	Serogroup C: Ct 33

357

358 The presence of mild interstitial pneumonia identified from fresh lung tissue obtained
359 during the post-mortem procedures conducted during the second intervention (Table 4) was
360 further investigated. Immunohistochemistry did not detect PCV-2 in any of the samples. Given
361 the lesions observed (mild, diffuse interstitial pneumonia and multifocal atelectasis) and the
362 absence of PRRSV in the herd, 5 litters (4 pigs each, from 5 different farrowing rooms) between
363 13-18 days of age were sampled (nasal swab[t]) and tested for presence of IAV-S by PCR[u]. All
364 samples were positive (mean Ct 27 ± 4), and typed by PCR as H3N2. Viral isolation and
365 subsequent sequencing of the HA gene revealed that the H3N2 strain detected was similar to a
366 strain that circulated in this herd until 2015, but that was not detected using the same
367 methodology between 2015-2017 (Figure 3). Although no classic epithelial necrosis lesions were
368 observed, histopathology results, associated with the isolation of a new strain from swabs of
369 healthy and coughing pigs, and the absence of PCV-2 suggest that H3N2 was the primary
370 pathogen in this case. The isolate was submitted to a commercial laboratory for autogenous
371 vaccine production. The entire sow herd was vaccinated once the vaccine was available, in May
372 2018.

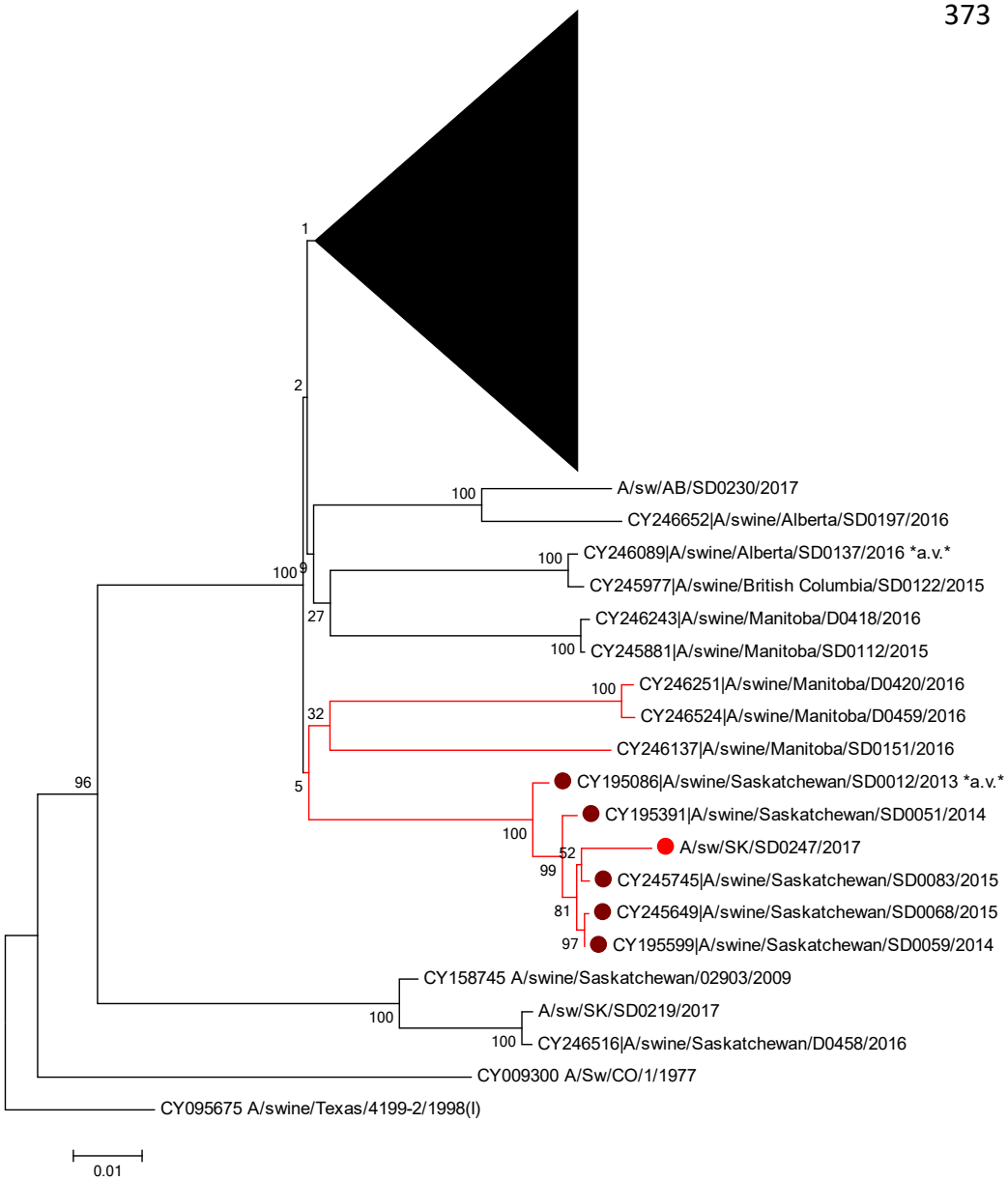

Figure 3 – IAV-S phylogenetic tree based on sequences of the HA gene (inferred using the Neighbor-Joining method). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method. Evolutionary analyses were conducted in MEGA6[46]. The new H3N2 sequence from October 2017 (red circle) is the same H3N2 virus that was present on this farm before and went undetected since 2015 (brown circle).

Here, poor sanitary conditions in farrowing rooms associated with the emergence of a different IAV-S strain lead to multiple suckling-pig infections associated with *C. suis*, rotavirus serogroup C, *E. coli* AIDA-I/STb and IAV-S H3N2. This combination culminated with a clinical syndrome characterized by watery diarrhea, poor weight gain and cough. A decrease in pre-weaning mortality was observed following the execution of the diagnostic interventions described above. Between July 2017 and October 2017, average pre-weaning mortality was high (21.3%) when compared to 12.0%, achieved after the suggested management and sanitary changes were implemented (March 2018 to June 2018). In addition, average weaning weight increased from 5.61 kg (during the outbreak period, July 2017 to October 2017) to 6.01 kg following the interventions (March 2018 to June 2018). A holistic approach, encompassing preventive (farrowing room sanitation, new IAV-S autogenous vaccine, sow feedback) and therapeutic (toltrazuril) recommendations, was taken. Antibiotics were not recommended, given that primary pathogens were viral or protozoan. Sows exposed to feedback material began farrowing in late January 2018. Suckling pig diarrhea affected less than 5% of animals per batch during a visit in May 2018. An autogenous IAV-S vaccine based on the newly isolated H3N2 strain was administered to the sow herd on May 2018.

Discussion

This case report describes a multi-factorial disease challenge in suckling piglets resulting from the combination of multiple intestinal primary pathogens and further complicated by secondary, opportunistic microorganisms. Poor pre-weaning weight gain and performance was associated with the diagnosis of diarrhea and coughing in piglets. The initial detection of *C. suis* in feces from diarrheic piglets eclipsed concurrent rotavirus type C infection, an emerging pathogen in North America. Gross and microscopical lesions caused by either pathogen are

similar; enteritis associated with villi blunting and atrophy, and were likely potentiated by the concomitant presence of AIDA-1/Stb *E. coli*[47]. An aggravating factor was that, despite the use of all-in/all-out strategy, sanitation procedures did not reduce environmental contamination to prevent transmission between batches. In addition, piglets were further challenged by an IAV-S strain that the dams were not vaccinated against. Pre-weaning mortality decreased once all primary agents were identified and control and prevention measures were applied, providing evidence of the success of the interventions proposed.

The most prominent clinical sign observed upon entering the affected farrowing rooms was watery diarrhea. Approximately 30% of the piglets were affected per room, which required immediate investigation. Intestinal and fecal samples collected at different times tested positive for *Cystoisospora suis* and porcine rotavirus serogroup C. Although Koch's postulates have not yet been experimentally fulfilled, this virus is commonly associated with neonatal diarrhea, being regarded as an emerging pathogen based on reports from North American investigations[2]. The incubation period can be as short as 24 hours, and piglets infected before 7 days of age will have diarrhea for 1-10 days[17, 48]. *Cystoisospora suis*, differently from rotavirus, requires at least 5 days from ingestion to shedding in feces, and another 1-3 days in the environment to sporulate[49]. Once recovered from infection, pigs become protected from re-infection and excrete no or very few oocysts[50]. Thus, it is possible that by the time piglets were necropsied and samples collected during the first intervention, they were no longer experiencing rotavirus infection or had minimal pathogen load. This, however, doesn't exclude the possibility that piglets were infected earlier in life. Regarding *C. suis,* severity of infection is directly related to the infectious dose, while protection is not related to sow immune status, transfer of passive immunity or serum antibodies. It mainly relies on cellular immune response following exposure to the pathogen[51, 52]. It is most

likely that pigs shed the pathogen at different ages, since they may have been infected at different ages as the pathogen was shed by other piglets. The fact that both pathogens lead to similar microscopical lesions (villi atrophy and fusion and necrosis of the epithelium) and the same type of diarrhea (malabsorptive) added another level of challenge when identifying the primary agent of infection[14, 53]. The lesions induced by both pathogens lead to a common outcome: disruption of intestinal epithelial integrity. Thus, the presence of either pathogen transforms the intestine into an easy *port d'entrée* for other microorganisms.

Post-mortem examination of piglets revealed necrosis of the superficial epithelium, enteritis and colitis. Such lesions are more severe than what is expected from rotavirus or *C. suis* infection alone. It has been suggested that rotavirus potentiates *C. suis* lesions and reduces its prepatent period, findings that could help explain the severity of the lesions described here[54]. In parallel, we consistently detected *E. coli* isolates negative for F4 (not tested for F5, F6 or F41), positive for AIDA-I (Table 4). It is known that neonatal diarrhea caused by *E. coli* is related to genes coding the F4, F5, F6 and F41 adhesins, not AIDA-I (adhesin involved in diffuse adherence I)[32]. F18 receptors are not expressed by enterocytes at this age, thus it was not tested. AIDA-I positive *E. coli* alone is not associated with intestinal lesions in pigs, even when delivered in large numbers through oral gavage[55]. Its significance in this case is challenging to define. The administration of ceftiofur parentally during piglet processing could play a role. It has been extensively documented that parenteral antibiotic administration affects the gut microbiome[56, 57]. In addition, the specific use of ceftiofur can disturb *E. coli* populations in the pig gastrointestinal tract, potentially selecting for virulent strains[58]. The ability of the pathotype isolated here to form biofilm and adhere to the mucus layer likely contributed to the severity of lesions observed, following loss of epithelial integrity due to rotavirus and/or *C. suis* invasion,

but its sole presence cannot explain the lesions found post-mortem: enteritis, villi atrophy and blunting[47, 55]. It has been previously documented that rotavirus-induced diarrhea allows the overgrowth of opportunistic bacteria[59]. This is likely due to the increased iron availability. Epithelial death leads to release of cytoplasmic contents into the lumen, including iron (binded to proteins or not)[60]. *E. coli* inherently produces siderophores, potent iron-chelating proteins that can harvest iron due to its extremely high affinity[61]. As iron is an absolute requirement for *E. coli* growth, this mechanism corroborates our hypothesis that the *E. coli* strain (specifically adapted to thrive following an initial injury) is a successful opportunistic pathogen.

Other agents that can cause such lesions at this particular age (4 to 18 days) include TGEV, PEDV (porcine epidemic diarrhea virus) and *C. perfringens*. Either virus, if present, were expected to incur very high pre-weaning mortality rates (>80%) and diarrhea in pigs at all ages, rapidly spreading throughout a naïve herd such as the one studied here[11]. That was not observed, as sows in close contact with affected piglets never developed diarrhea. In parallel, one study reported that only a small proportion of pigs affected by *C. perfringens* type A may develop villi blunting and fusion[62]. In addition, *C. perfringens* has been previously described to colonize the gastrointestinal tract of healthy pigs without causing disease, thriving only following lesions caused by other primary agents[63]. We have found, through anaerobic culture, small amounts of *C. perfringens* in the intestine of 2/3 piglets necropsied during the second intervention. This suggests its secondary role or post-mortem growth in both cases.

Environmental contamination was another determinant of disease in this farm. The detection of pathogens following a complete washing and disinfection cycle highlights one of the major biosecurity challenges in modern swine farming: aligning production flow needs with disease prevention measures. Residual organic matter found in the farrowing room audited can

reduce the efficacy of disinfectants. A previous study has shown that the presence of organic matter renders glutaraldehyde/quaternary ammonium-based products 10^6-times less efficient in inactivating rotavirus, when compared to samples without organic matter[64]. This same observation was described for *C. suis*[65]. Sanitation has been described as a key aspect to control neonate coccidiosis, including the removal of all soil and organic matter[43]. Taken together, these findings by previous authors corroborate with the strategy implemented in this farm. They support our working hypothesis that cleaning and removal of all organic matter from the farrowing room, instead of the choice of disinfectant or manually scrubbing the crates, was a critical control point for controlling both pathogens and pre-weaning diarrhea in this herd.

We have observed the emergence of an IAV-S strain (H3N2) following vaccination for a different strain (H1N2) in this herd. Suckling piglets are more likely to be positive for IAV-S by PCR than gilts or sows, which may explain why clinical signs were not noted in nursery pigs[66]. In this report, piglets were positive by PCR and viral isolation was also performed, which shows that H3N2 virions were actively circulating in this population and being shed in nasal secretions. At the time of sampling, the herd has been immunized for 2 years using an autogenous vaccine based on a previous H1N2 isolate. Until 2015, a H3N2 strain was the only type circulating in this farm. Based on sequencing of the HA gene, it is believed that the virus isolated in 2017 is the same one from 2015 (Figure 3). Suckling pigs could act as a maintenance population, given their immune status. Different studies have shown that a certain degree of cross-protection between HA lineages can be observed following vaccination[67, 68]. Passive immunity against the H1N2 strain could be playing a role in keeping the H3N2 virus from inducing severe lesions, leading to the mild, non-specific interstitial pneumonia described. Here, it remained to be further explored which lineage was circulating in grower and finisher pigs, and what impact the vaccine

496 strain discrepancy had in the overall herd health and performance. The presence of *Proteus* sp.
497 isolated from diseased piglets likely reflects contamination during sample collection, or an
498 opportunistic infection following immunosuppression by multiple enteric infections and loss of
499 epithelial integrity in the respiratory tract induced by IAV-S.

500 The series of actions reported here resulted in reduced pre-weaning mortality. However,
501 we have identified limitations in the diagnostic and therapeutic workup described. Although
502 there was evidence to associate the lung lesions with IAV-S, lung tissue was not collected to
503 submit samples for immunohistochemistry (only swabs were collected). This information would
504 have directly associated IAV-S with the lesions observed, providing further confirmation of the
505 diagnosis. Regarding the gastrointestinal lesions, the administration of ceftiofur intramuscularly
506 to piglets should be discontinued. This practice very likely impacts the gut microbiome and *E.*
507 *coli* load, as discussed above. Finally, an antimicrobial susceptibility test of the *E. coli* isolates
508 obtained should have been performed. Results could have provided further information regarding
509 the role of ceftiofur in selecting for the STb/AIDA-1 strain found here, serving as direct
510 justification to stop the administration to piglets during processing, and suggesting that it was
511 inducing intestinal dysbiosis.

512 This report presents a challenging, multi-factorial case of increased suckling pig mortality
513 associated with highly prevalent diarrhea and mild respiratory lesions. Following the
514 interventions reported, pre-weaning mortality reached acceptable rates, by dropping > 20% to
515 approximately 12%. It is not possible to completely refute the hypothesis that this drop was
516 seasonal or coincidental, but it is also unrealistic to include a control group in the commercial
517 setting described. Nevertheless, the combination of infectious agents found were very likely
518 contributing to this problem, as evidenced by the data and outcomes presented. While mortality

519 was still above the goal of 10% pre-weaning by the end of the studied period, some of the
520 changes suggested were not implemented until after this period (e.g. new H3N2 IAV-S
521 autogenous vaccine).
522
523 **Summary**
524 This report illustrates a common challenge in swine medicine that may be oversimplified
525 through a training gap: Koch's postulate and the association between a single agent with a single
526 syndrome. Multi-etiological infections damaging different organ systems are common in
527 intensive pig rearing, and may be neglected if not further investigated. This includes situations
528 where not all agents are typically characterized as primary pathogens, such as the AIDA-I/Stb *E.*
529 *coli* described here. This was likely associated with the injudicious use of antimicrobials in
530 piglets, disturbing the gut microbiome and likely contributing to the virulence of a commensal-
531 like *E. coli*. Furthermore, we demonstrated how the use of molecular methods, beyond PCR,
532 greatly improves our ability to elucidate disease etiology. DNA sequencing is the standard in
533 swine practice and proper interpretation of such data should be part of the veterinarian toolbox.
534

Endnotes

[a]Fostera Gold PCV, Zoetis, Kirkland, QC, Canada.

[b]PRO-banminth, Phibro Animal Health, Teaneck, NJ.

[c]Elanco, Guelph, ON, Canada.

[d]ER Bac Plus, Zoetis, Kirkland, QC, Canada.

[e]LitterGuard LTC, Zoetis, Kirkland, QC, Canada.

[f]Parasail, Merial, Burlington, ON, Canada.

[g]Gallant Custom Laboratories, Cambridge, ON, Canada.

[h]Dexafer-200, Vetoquinol, Lavaltrie, QC, Canada.

[i] Metacam, Boehringer Ingelheim, Burlington, ON, Canada.

[j] Excenel, Zoetis, Kirkland, QC, Canada.

[k]Baycox, Bayer Animal Health, Mississauga, ON, Canada.

[l] Bio-vet Swinecheck PCV2/PCV3 PCR, Bio-vet, Barneveld, WI, USA.

[m]Bio-vet Swinecheck MP PRRSV type 1 and type 2 indirect, Bio-vet, Barneveld, WI, USA.

[n]Premise degreaser, WestPenetone, Montreal, QC, Canada.

[o]SOS Pads, Clorox, Oakland, CA, USA.

[p]Gluquat 2, WestPenetone, Montreal, QC, Canada.

[q]BD Culture swab, BD, Mississauga, ON, Canada.

[r] Hyper O, WestPenetone, Montreal, QC, Canada.

[s] Virkon, Vetoquinol, Lavaltrie, QC, Canada.

[t] BD BBL™ CultureSwab™, liquid Stuart medium, single plastic applicator, Becton, Dickinson and Co., Sparks, MD, USA.

[u]VetMAX™-Gold SIV Subtyping Kit, Life Technologies, Austin, TX, USA.

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