

## DETECTION OF COMMON PATHOGENS AND THEIR ASSOCIATION WITH HUSBANDRY FACTORS IN PRE-WEANED PIGLETS WITH DIARRHOEA

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### SUMMARY

Pre-weaned piglet diarrhoea significantly impacts the swine industry. This study investigates common pathogens associated with this condition, including *Clostridium perfringens*, Coronaviridae, *Cystoisospora suis*, and *Salmonella* spp., and examines their correlation with husbandry factors. A total of 183 rectal samples from diarrhoeic piglets across 15 farms in Malaysia were analysed, revealing *Clostridium perfringens* (97.96%) and Coronaviridae (77.05%) as the predominant pathogens. Notably, 73.77% of samples exhibited co-infection by two to three pathogens, with significant correlations identified between co-infection rates and farrowing crate emptying time. Specifically, crate emptying time of 3-5 days was associated with a significantly lower percentage of co-infections ( $p < 0.05$ ) compared to 1 day and 7 days emptying time, providing actionable insights for farmers to enhance management strategies and control measures against these pathogens.

**Keywords:** pre-weaned piglet diarrhoea, *Clostridium perfringens*, *Salmonella* spp, Coronaviridae, *Cystoisospora suis*

### INTRODUCTION

Pre-weaned diarrhoea in piglets is one of the most frequent health and economic issues in modern production, which can be associated with high mortality up to 19%, slow growth rates, and rising medical expenses (Luppi et al., 2023), translating into substantial economic losses. This study focuses on detection of several common pathogens associated with diarrhoea in pre-weaned piglets, including enteric salmonellosis, clostridial enteritis, coccidiosis, and coronaviral gastroenteritis and their relationship to various husbandry factors. The selected common pathogens are *Cystoisospora suis*, *Clostridium perfringens*, Coronaviridae, and *Salmonella* spp. *Cystoisospora suis*, formerly known as *Isoospora suis* (Barta et al., 2005) is a significant parasite, causing swine neonatal coccidiosis that affects intensive pig farming worldwide (Mundt et al., 2005). *Clostridium perfringens* causing clostridial enteritis tends to occur mainly in non-immune piglets less than one week old (Henry Too, 2019). Coronaviridae causing viral gastroenteritis are highly contagious intestinal infections and clinically associated with occasional vomiting, profuse diarrhoea, and dehydration in piglets less than 2 weeks of age. Pigs of all ages are susceptible to enteric salmonellosis that is caused by *Salmonella* spp. (Burrough, 2021).

### MATERIAL AND METHODS

The study analysed 183 rectal samples collected from pre-weaned piglets with diarrhoea across 15 farms spanning Perak, Selangor, and Johor according to approved animal ethics methods (UPM/IACUC/AUP-U024/2023). Farm background parameters such as sow parity, litter size, farrowing unit type, farrowing crate emptying time and pre-weaned piglet mortality are taken from the farms. These samples were grouped into 61 pools based on individual litters ( $n=61$ ). Rectal samples that were collected previously were subjected to 3 different methods to detect different common pathogens. Samples were sent for (1) *Salmonella* spp. isolation via Rappaport-Vassiliadis (RV) enrichment broth and Xylose Lysine Deoxycholate (XLD) agar using a micropipette; (2) *Clostridium perfringens* isolation where direct selective culturing of samples was done on Tryptose Sulfite Cycloserine (TSC) agar; (3) Modified McMaster technique and egg per gram (EPG) was calculated for *Cystoisospora suis* detection; (4) extraction of RNA was done using innuPREP Virus DNA/RNA Kit and Nested RT-PCR (T100™ Thermal Cycler, Bio-rad, USA) for Coronaviridae family gene detection using primers and conditions according to Watanabe et al., (2010).

### RESULTS AND DISCUSSION

Pre-weaned piglet diarrhoea is often related to a complex interaction of factors, including a mixture of infectious agents and non-infectious factors such as husbandry practices, environmental temperature and humidity and passive immunity transferred by colostrum and milk. This factor, particularly passive immunity, plays a critical role in resisting infection in piglets and contributes to the manifestation of diarrhoea (Kylla et al., 2019). In our study, our results showed that 56 out of 61

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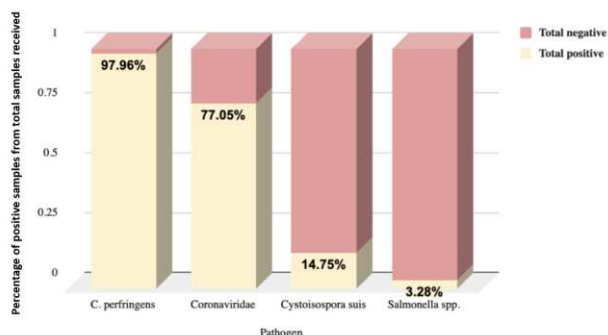
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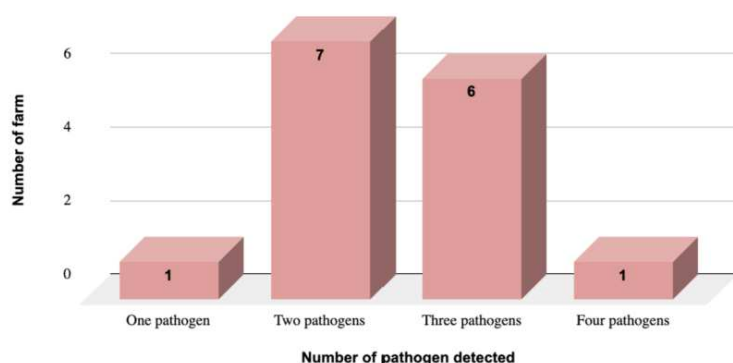


(91.8%) litters were positive to at least one of the examined enteric pathogens (*C. perfringens*, *Salmonella* spp., Coronaviridae and *C. suis*). 11 out of 61 (18.0%) litters were positive for only one of these pathogens, 45 out of 61 pathogens were positive for more than one pathogen and finally, only 5 out of 61 (8.2%) were negative for all the examined pathogens. The percentage of total positive sample for each pathogen are 97.9% of *C. perfringens*; 77.1% of Coronaviridae; 14.8% of *Cystoisospora suis* and 3.3% of *Salmonella* spp. (Figure 1).

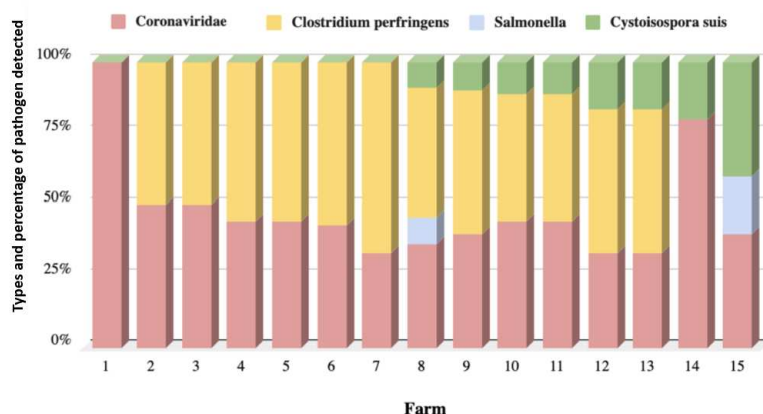


**Figure 1: Total Percentage of positive samples for each pathogen**

The other finding shows most of the farms are having co-infection with 2-3 pathogens (Figure 2-4). There is only



**Figure 2: Total number of farms with number of pathogens detected**



**Figure 3: Types and percentage of pathogens detected in each farm**

1 farm with only 1 pathogen (Coronaviridae) detected, 7 farms with 2 pathogens detected, 6 farms with 3 pathogens detected and 1 farm with 4 pathogens detected (Figure 2). This is similar to pre-weaned piglet diarrhoea in Spain where samples were also co-infected with either bacterial, viral or protozoal with *C. perfringens* for all cases (Mesonero-Escuredo et al., 2018). Besides, the rectal samples that were only taken from piglets with pre-weaned diarrhoea, 49.2% are from the first and second parity of sow (Figure 5). Moreover, 63.4% of rectal samples from diarrhoeic piglets having litter size higher than 11 due to competition of sow milk among piglets (Figure 6). Subsequently, the only two rectal samples with *Salmonella* spp. detected are both from the first parity of young sows (Figure 5), which may suggest that younger sows produce lower quality milk compared to the rest of the herd. In terms of farrowing crate emptying time, samples with emptying time of 3 to 5 days have significantly lower percentage of co-infections compared to samples with shorter or longer emptying time ( $p < 0.05$ ). To be more specific, the pathogens causing co-infection are mostly Coronaviridae, followed by *C. perfringens* (Figure 3), which suggests that farrowing crate emptying time of 3-5 days effectively lowers the entry and transmission of enteric pathogens within litter by allowing sufficient downtime and disinfection process. There is a positive weak correlation ( $r = 0.586$ ,  $p < 0.05$ ) between detection of *C. perfringens* and Coronaviridae.

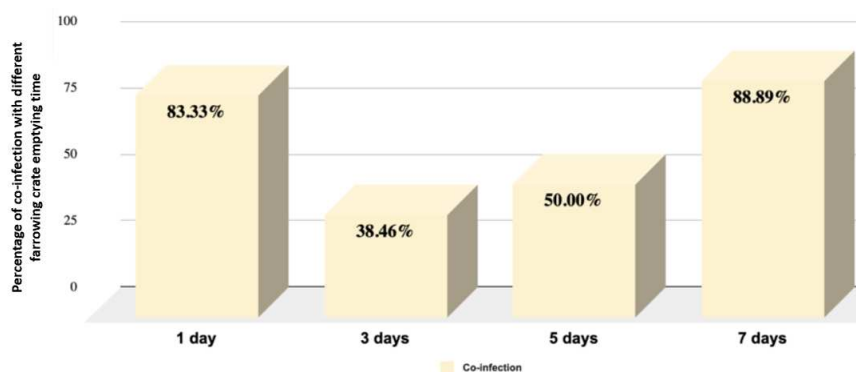


Figure 4: Percentage of co-infection with different farrowing crate emptying time

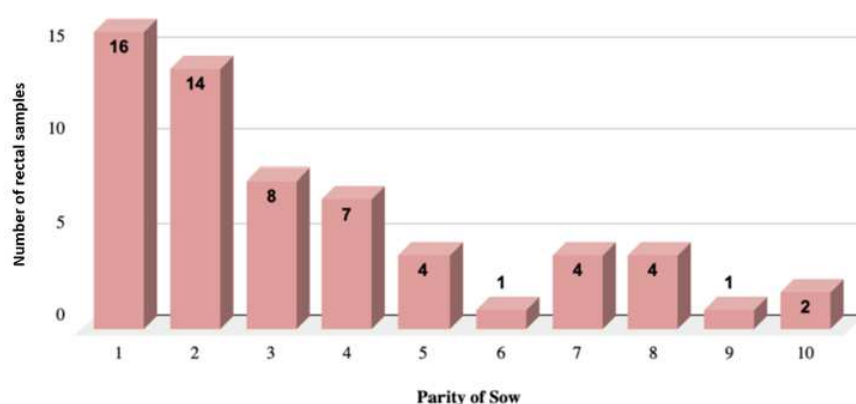


Figure 5: Number of rectal samples from different parity of sow

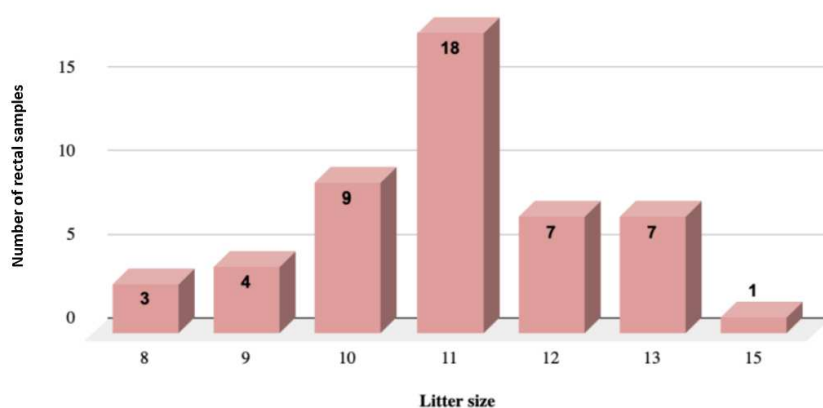


Figure 6: Number of rectal samples from different litter size

## CONCLUSION

In conclusion, all four common pathogens examined were all detected in rectal samples from pre-weaned piglets with diarrhoea. The two most commonly detected pathogens are Coronaviridae and *C. perfringens* which potentially cause viral infection and bacterial infection in piglets respectively. Furthermore, these pathogens often co-exist in pre-weaned piglet diarrhoea which causes significant economic losses to farmers. Besides the pathogens themselves causing the losses, farm husbandry factors such as farrowing crate emptying time also

contribute to pre-weaned piglet diarrhoea.

## CONFLICT OF INTEREST

None of the authors of this paper has financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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