Received: 4 October 2020 | Revised: 10 May 2021 | Accepted: 29 July 2021

DOI: 10.1002/vetr.837

Vet Record

ORIGINAL RESEARCH

# Microbial content of non-fermented liquid feed consumed by sows affects the occurrence of neonatal diarrhoea in their piglets: A case–control study

**Gwenaël Boulbria**[1,2] | **Charlotte Teixeira-Costa**[1] | **Céline Chevance**[1,2] | **Romane Grandin**[3] | **Justine Jeusselin**[1,2] | **Pauline Berton**[1] | **Jean Le Guennec**[4] | **Valérie Normand**[1,2] | **Franck Bouchet**[1,2] | **Mathieu Brissonnier**[1] | **Arnaud Lebret**[1,2]

[1] Porc.Spective Swine Vet Practice, ZA de Gohélève, Noyal-Pontivy, France

[2] rezoolution Pig Consulting Services, ZA de Gohélève, Noyal-Pontivy, France

[3] École Supérieure d'Agriculture Angers Loire, Angers, France

[4] Labofarm-Finalab Veterinary Laboratories Group, Loudéac, France

**Correspondence**
Gwenaël Boulbria, Porc.Spective Swine Vet Practice, ZA de Gohélève, rue Joseph et Étienne Montgolfier, Noyal-Pontivy 56920, France.
Email: g.boulbria@porc.spective.fr

## Abstract

**Background:** Microbial colonisation of piglets' intestines starts at birth, especially from contact with sow's faeces. Piglet microbiota could therefore be influenced by the sow's diet. The objective of this study was to evaluate whether the microbiological flora of liquid feed for sows can be associated with the development of neonatal diarrhoea.

**Methods:** This study was carried out on 10 case farms with neonatal diarrhoea and 10 control farms without neonatal diarrhoea. On each farm, a microbiological analysis of gestating and lactating liquid feed was performed. A generalised linear model was used to study the impact of the liquid feed microbiological counts and pH on the probability of neonatal diarrhoea developing.

**Results:** For thermotolerant coliforms, sulphite-reducing bacteria, heterotrophic bacteria and lactic-acid bacteria counts, there was no significant difference between case and control farms. The higher the count of total coliforms, enterococci and yeasts in sow non-fermented liquid feed, the greater the probability of observing neonatal diarrhoea. Moreover, taking into account total coliforms and yeasts counts together is highly predictive of neonatal diarrhoea risk.

**Conclusion:** This study offers new perspectives of investigation and understanding of neonatal diarrhoea in breeding farms feeding sows with a non-fermented liquid feed.

## BACKGROUND

Microbiota play a significant role in host health and metabolism and the swine digestive tract provides the appropriate habitat for a huge number of microbial species. The newborn piglet gut rapidly acquires its microflora by contact with its dam and surrounding environment but a transfer might also occur during gestation.[1] Sow's gastrointestinal tract health can be affected by nutritional regimes,[2,3] particularly the microbiological criteria of liquid feed distributed to sows can affect it.[4] The most significant factor influencing the microflora of the farrowing room environment is the sow in the absence of bedding materials. Therefore, if the microflora introduced by the sow into the environment via faeces can be modified by the microbiological quality of non-fermented liquid feed, this can have an effect on the colonisation of the piglet's gut, and on the emergence of neonatal diarrhoea. Neonatal diarrhoea can be described as faeces with an excess of water in relation to the faecal dry matter content.[5] It affects piglets in the first week of life and significantly increases pre-weaning mortality or growth retardation. The most common causes of this enteric disorder are infections by one or more pathogens or changes in the gut microbiota.[5] Neonatal diarrhoea is associated with common pathogens such as enterotoxigenic *Escherichia coli* (ETEC), *Clostridium perfringens* type C and type A, *Clostridium difficile*, rotavirus and coronavirus. In the last 10 years, *Enterococcus hirae* has also been suspected of being responsible for neonatal diarrhoea.[6–8] Reports

on the impact of non-fermented liquid feed microbial aspects on pig health are scarce, most studies described the benefit of fermented compared to non-fermented liquid feed.[9–11] So, practical recommendations for non-fermented liquid feed management are needed. The aim of this study was to describe some microbiological parameters in non-fermented liquid feed given to sows on ten farms affected with neonatal diarrhoea and on ten control farms and to determine if the microbiological quality of liquid feed can have an impact on the emergence of neonatal diarrhoea.

## MATERIALS AND METHODS

### Farm selection

Twenty conventional herds were enrolled in this study, located in Brittany (France), within 2-h drive of the diagnostic laboratory Labofarm (Finalab, Loudéac, France). Among these 20 herds, 10 exhibited neonatal diarrhoea in more than 20% of litters within a batch for at least two consecutive batches. These herds were defined as 'case' farms. The other 10 herds did not have neonatal diarrhoea and were defined as 'control' farms.

The herds employed various production systems and varied in size from 250 to 1000 sows. Gestating and lactating sows were fed with non-fermented liquid feed, defined as a mixture of feed and water made immediately before feeding. Batchwise farrowing with an all-in/all-out system and cleaning and disinfection of the farrowing rooms between batches was practised in all herds. Neither sows nor piglets received any medication before or during the study. The antibiotic treatment, in particular, was prohibited. For each farm, breed, status regarding porcine reproductive and respiratory disease virus[12] and vaccinations against neonatal diarrhoea for both herds are presented in Table 1. One herd was vaccinated only against ETEC, 16 herds were vaccinated against ETEC and *C. perfringens* type C simultaneously, one herd was vaccinated against *E. coli*, *C. perfringens* type A and *C. perfringens* type C and two herds were not vaccinated at all against neonatal diarrhoea.

### Liquid feed sampling and analyses

At each farm, two samples of non-fermented liquid feed were collected: one approximately 2 weeks prior to farrowing and one in the week after parturition. Both were collected at the end of the liquid feed system line at a feeder level. Each sample of about 200 ml was immediately stored at 4°C and transported to Labofarm within 2 h. The feed samples were cultured within 4 h of collection.

### Liquid feed pH, bacteriology and mycology

For each sample, the pH of the liquid feed was measured using an electronic pH meter (Hanna Instruments HI-98128). Next, liquid feed samples were serially diluted 10-fold in buffered sodium chloride peptone solution (pH 7). Relevant dilutions were plated onto selective media and plates were incubated at the recommended temperature and conditions. All selective media used were obtained from Thermo Fisher Scientific Inc. (Oxoid Microbiology Products). Total coliforms (TColi) and thermotolerant coliforms (ThColi) were cultured aerobically on violet red bile lactose agar plates at 37 and 44°C, respectively. Sulphite-reducing bacteria (SRB, including clostridia) were cultured anaerobically on tryptose sulphite cycloserine agar plates at 37°C. Enterococci (ETC) were cultured aerobically on Slanetz Bartley agar plates at 37°C. Bacterial growths were counted after 12–24 h incubation. Heterotrophic bacteria (HET) were aerobically cultured at 30°C and counted using plate count agar medium and counted after 48 h incubation. Lactic acid bacteria (LAB) were cultured anaerobically on De Man, Rogosa and Sharpe agar plates at 37°C and counted after 72 h incubation. Yeasts (YEA) were cultured aerobically on Sabouraud dextrose agar plates at 37°C and counted after 48 h incubation.

### Diarrhoeic piglet in case herds: selection, necropsy and sampling

In order to clarify the apparent cause of neonatal diarrhoea on the case farms, two diarrhoeic piglets from each case herd under 1 week of age were selected from two different litters. They had shown watery to creamy diarrhoea starting in the previous 24 h, but no other clinical signs (e.g. weight loss, arthritis or omphalitis). No piglets within the litters were treated with antibiotics at selection time.

The piglets were transported alive to the pathology unit of Labofarm for euthanasia and post-mortem examination. The piglets were first sedated with an intramuscular injection of tiletamine and zolazepam, 6 mg/kg (Zoletil 50; Virbac Animal Health, ) and euthanised with an intracardiac injection of tetracaïne 1.3 mg/kg, embutramide 60 mg/kg and mebezonium 8.1 mg/kg (T61; MSD Animal Health). Bacteriological and virological samples were collected by swabs from the intestinal mucosa of the proximal duodenum, the distal jejunum and the distal ileum for *E. coli*, *C. perfringens*, *C. difficile* and enterococci and of the proximal colon for rotavirus. Two swabs per intestinal section were collected (eight swabs per piglet): one for bacteriological cultures and one for genomic and further analyses if required.

### Bacteriological and virological investigation

Swabs were cultured within 30 min of sampling. For *E. coli*, swabs were cultured aerobically on 5% sheep blood agar plates and Drigalski lactose agar plates at 37°C. The plates were examined for bacterial growth after 24 h incubation. For *C. perfringens*, swabs were

**TABLE 1** Characteristics of case herds and piglets: porcine reproductive and respiratory disease virus (PRRSV) status of the herd, sows vaccination, breed, age and weight of necropsied piglets, results of bacteriology, virology and histology for each piglet and aetiology of neonatal diarrhoea

| | | | | | | | | | Bacteriology | | | | Histology | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Herds** | **Breed** | **PRRSV-1 status** | **Neonatal diarrhoea vaccination** | **Pig** | **Gender** | **Primiparous** | **Age (day)** | **Weight (g)** | **hemolytic E. coli** | **Cp** | **Eh** | **Virology R** | **E. coli** | **Cp** | **Eh** | **R** | **Aetiology** |
| A | {LWxLDxT}xPi | Negative | *E. coli* and *Clostridium perfringens* type C | 1 | M | Yes | 3 | 1270 | – | + | + | – | – | – | + | – | *Enterococcus hirae* and *Clostridium perfringens* |
| | | | | 2 | M | No | 3 | 1450 | – | + | + | – | – | + | + | – | |
| B | {LWxLDxT}xPi | Negative | *E. coli* and *Clostridium perfringens* type C | 1 | M | Yes | 4 | / | – | – | + | – | – | – | + | – | *Enterococcus hirae* |
| | | | | 2 | M | No | 5 | / | – | + | + | – | – | – | + | – | |
| C | {LWxLD}xPi | Positive stable | *E. coli* and *Clostridium perfringens* type C | 1 | F | No | 2 | 990 | – | + | + | + | – | + | – | – | *Enterococcus hirae* |
| | | | | 2 | M | No | 2 | 1430 | – | + | + | – | – | + | – | – | |
| D | {LWxLD}xPi | Positive stable | *E. coli* and *Clostridium perfringens* type C | 1 | F | Yes | 2 | / | – | + | – | + | – | – | – | + | Rotavirus |
| | | | | 2 | F | No | 2 | / | – | + | – | + | – | – | – | + | |
| E | {LWxLD}xPi | Positive stable | *E. coli* and *Clostridium perfringens* type C | 1 | F | No | 2 | 1550 | – | – | – | – | – | – | – | – | *Enterococcus hirae* |
| | | | | 2 | M | No | 6 | 3300 | – | + | + | – | – | – | + | – | |
| F | {LWxLD}xPi | Positive stable | *E. coli* and *Clostridium perfringens* type C | 1 | / | / | 5 | / | + | + | + | – | – | – | + | – | *Enterococcus hirae* |
| | | | | 2 | / | / | 5 | / | + | – | + | – | – | – | + | – | |
| G | {LWxLD}xPi | Negative | *E. coli* and *Clostridium perfringens* type C | 1 | M | / | 2 | 1490 | – | – | + | + | – | – | + | + | *Enterococcus hirae* and Rotavirus |
| | | | | 2 | F | / | 2 | 1520 | – | – | + | – | – | – | + | – | |
| H | LWxLW | Negative | *E. coli* and *Clostridium perfringens* type C | 1 | F | Yes | 6 | / | – | + | + | – | – | + | – | – | *Enterococcus hirae* and *Clostridium perfringens* |
| | | | | 2 | F | No | 6 | / | – | + | + | – | – | – | + | – | |
| I | {LWxLD}xPi | Positive stable | No vaccination | 1 | F | Yes | 2 | 1570 | – | + | + | – | – | + | – | – | *Clostridium perfringens* |
| | | | | 2 | M | No | 2 | 1700 | – | + | – | – | – | + | – | – | |
| J | {LWxLD}xPi | Negative | No vaccination | 1 | F | No | 1 | 1330 | – | – | – | – | – | + | – | + | Rotavirus and *Clostridium perfringens* |
| | | | | 2 | M | No | 3 | 1560 | – | – | – | + | – | + | – | + | |

Abbreviations: LW, Large White; LD, Landrace; Pi, Pietrain; T, Tai Zumu; M, male; F, female; *E. coli*, *Escherichia coli*; Cp, *Clostridium perfringens*; Eh, *Enterococcus hirae*; R, rotavirus.

cultured anaerobically on 5% sheep blood agar plates and nalidixic acid agar plates at 37°C and incubated for 48 h. In this study, since no toxigenic typing was performed, *C. perfringens* serotype was not determined. Enterococci were also cultured aerobically at 37°C on nalidixic acid agar plates. All bacterial species were identified using matrix assisted laser desorption ionization - time of flight (MALDI-TOF) mass spectrometry (Brucker Daltronics), the identification of *E. hirae* was confirmed specifically using this method. For all piglets, the presence of rotavirus in faeces was tested using a rapid enzyme immunoassay (DipFit Rotavirus Porcin; Bio-X Diagnostics, Finalab) according to the manufacturer's instructions.

## Histopathological examinations

Tissue samples were collected from the proximal duodenum, proximal jejunum, distal jejunum, ileum and colon. Specimens were flushed and then fixed with 10% neutral-buffered formalin and transported to the Orbio diagnostic laboratory (Finalab) for microscopic examination.

## Decision process to determine the aetiology of neonatal diarrhoea in case farms

The aetiology of neonatal diarrhoea in case farms was determined when one or both sampled piglets displayed bacterial growth or positive rotavirus ELISA and simultaneous histological lesions consistent with.

## Statistical analyses

Only the aetiology of neonatal diarrhoea in case farms were described in this study. The bacterial counts per mL of liquid feed were log-transformed and tabulated. The LAB:TColi ratio was calculated to describe the balance between these two bacterial populations. The relationships between each microbiological parameter (TColi, ThColi, SRB, ETC, HET, LAB and YEA) and pH were described using a principal component analysis (PCA). The significance of the relationship between the two parameters was tested using regression analysis. The impact of farm status regarding neonatal diarrhoea and the source of liquid feed (gestating or farrowing room) on microbiological counts per mL in sow liquid feed were compared by an analysis of variance (ANOVA). Statistical analyses were performed in R Studio version 4.0.2 (R Core Team, 2020). Finally, a generalised linear model (GLM) was adjusted to test if the bacteriological and mycological counts in liquid feed distributed to sows were predictive of the occurrence of neonatal diarrhoea, taking into account the source of the liquid feed. The final model was selected using the R stepwise function. Statistical significance was set at $p < 0.05$.

# RESULTS

## Detection of neonatal enteropathogens in case herds

Characteristics of case herds and piglets are presented in Table 1. *Enterococcus hirae*, *C. perfringens* and rotavirus were identified as enteropathogens involved in clinical neonatal diarrhoea in 7, 4 and 3 herds respectively, sometimes in coinfection. *Clostridium perfringens* evocative lesions included superficial villus tip necrosis and the accumulation of fibrin. Jejunal and ileal lesions may be heavily colonised with large, rectangular Gram-positive bacilli with rounded or truncated ends, although it was common to find masses of organisms in the lumen. Capillaries were dilated, but there was no haemorrhage. Lesions compatible with *E. hirae* were small intestinal colonisation by entero-adherent Gram-positive cocci accompanied by villous atrophy and mild epithelial lesions, including increased apoptosis of enterocytes. Finally, typical rotavirus lesions were observed in the small intestine with massive destruction of the villi and subsequent adaptive and regenerative responses. The tips of atrophic villi were eroded or were covered by swollen or attenuated, nearly squamous epithelial cells.

## Microbiological quality and pH of sow liquid feed in case and control herds

The PCA (Figure 1) was useful to represent all correlations between the microbiological criteria. For example, it revealed a negative correlation between pH and lactic acid bacterial count ($R^2 = 0.44$, $p < 0.05$), between pH and the LAB:TColi ratio ($R^2 = 0.27$, $p < 0.05$) and between pH and heterotrophic bacteria counts ($R^2 = 0.28$, $p < 0.05$). Other correlations were not statistically significant, particularly there was no relationship between yeast, enterococci and total coliforms counts.

Average results for each criterion (log CFU/ml) are presented in Table 2. All dispersions of analysed data for each microbiological criterion and pH are represented in Figure 2. In our study, there was no statistically significant difference between affected and unaffected farms for lactic acid bacteria counts. However, we observed a higher dispersion of LAB counts for the case farms. It was the only criterion where the source of liquid feed had an impact on counts ($p < 0.05$; Figure 3). There were more lactic acid bacteria in sow liquid feed sampled in farrowing than in gestating facilities. The heterotrophic bacteria counts were also closed in both groups, and no statistically significant difference between the two groups was observed. Total coliforms counts were significantly higher on case farms ($p < 0.05$). However, this difference was not observed for thermotolerant coliforms, although the dispersion of thermotolerant coliform counts is higher in case farms. For sulphite-reducing bacteria, the difference between the two groups was not

**TABLE 2** Comparison of the results of microbiological counts (in log CFU/ml), LAB:TColi ratio and pH of liquid feed in case and control farms

| | | Microbiological criteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | pH | LAB | HET | TColi | ThColi | SRB | ETC | YEA | LAB :TColi |
| **Control herds** | | | | | | | | | |
| Mean | 5.08 | 8.18 | 8.38 | 5.32 | 2.57 | 2.83 | 4.99 | 3.99 | 2.85 |
| Median | 4.96 | 7.86 | 9.11 | 4.71 | 1.48 | 1.74 | 2.81 | 3.70 | 3.15 |
| SD | 0.75 | 8.45 | 8.57 | 5.61 | 3.04 | 3.11 | 5.62 | 4.08 | 2.83 |
| **Case herds** | | | | | | | | | |
| Mean | 5.18 | 7.98 | 8.28 | 5.46 | 4.11 | 3.54 | 4.30 | 4.89 | 2.52 |
| Median | 5.02 | 7.60 | 8.15 | 5.11 | 1.70 | 2.34 | 3.74 | 4.26 | 2.49 |
| SD | 0.68 | 8.18 | 8.28 | 5.62 | 4.73 | 4.00 | 4.52 | 5.20 | 2.55 |

Abbreviations: ETC, enterococci; HET, heterotrophic bacteria; LAB, lactic acid bacteria; LAB:Tcoli, lactic acid bacteria:total coliforms ratio
SD, standard deviation; SRB, sulphite-reducing bacteria; TColi, total coliforms; ThColi, thermotolerant coliforms; YEA, yeasts.

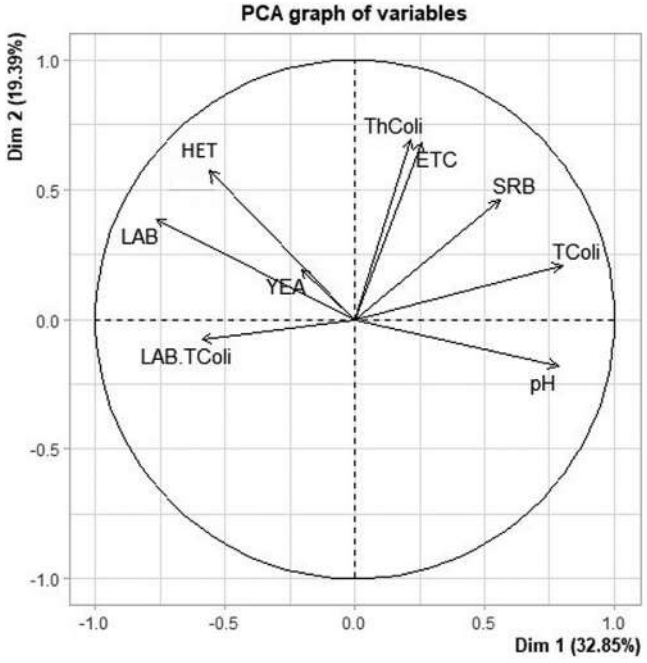


**FIGURE 1** Loading plots of the principal component analysis (PCA) reported the results of the elaborations carried out with datasets. In the axis title of each component, the explained variance is reported. LAB: lactic acid bacteria; HET: heterotrophic bacteria; YEA: yeast; ThColi: thermotolerant coliforms; ETC: *Enterococci*; SRB: sulphite-reducing bacteria; TColi: total coliforms; LAB:Tcoli: lactic acid bacteria: total coliforms ratio

significant. However, more than half of the sow liquid feeds collected from case farms had a count above 2 log CFU/ml, whereas more than half of the liquid feeds collected from control farms had a count below 2 log CFU/ml. Enterococci counts were significantly higher on case farms ($p < 0.05$). Yeast counts were significantly higher on case farms ($p < 0.05$). Finally, no statistical difference was noticed between both groups in the pH of liquid feed distributed to sows (Figure 3).

## Risk criteria for neonatal diarrhoea occurrence

The GLM emphasised three criteria for the bacteriological and mycological analysis of liquid feed distributed to sows that are predictive of the occurrence of neonatal diarrhoea: total coliforms, enterococci and yeast counts. It appears that the higher their counts were, the higher the risk of neonatal diarrhoea emergence. In the GLM, the source of liquid feed had no impact on the counts of total coliforms ($p = 0.68$), enterococci ($p = 0.8$) and yeasts ($p = 0.78$), which confirmed the results of ANOVA. Moreover, their counts in liquid feed were independent of each other as previously described using the PCA. When all criteria were considered, the stepwise model selection gave a final model including the total coliforms and yeast counts, both simultaneously significant ($p < 0.05$), meaning that in this study these both criteria, taken into account together, are the most predictive of neonatal diarrhoea emergence. When the enterococci counts were added to this model, all three criteria appeared to have a slight effect simultaneously ($p < 0.15$). In our study, for results above 5 log CFU/ml of total coliforms (Figure 4a), 4 log CFU/ml of enterococci (Figure 4b) and 4 log CFU/ml of yeasts (Figure 4c), the probability of neonatal diarrhoea occurring is greater than 50%.

## DISCUSSION

Neonatal porcine diarrhoea is a common problem in commercial swine herds and is of significant importance for the pig industry worldwide because it causes major economic losses due to increased morbidity and mortality, decreased weight gain and the need for medications.[13] Over the last 10 years, increasing attention has been focused on neonatal diarrhoea in piglets in which routine diagnostic protocols fail to isolate known enteric pathogens such as haemolytic *E. coli*, *C. difficile*, *C. perfringens* type A or type C or parasites.[13] In our study, ETEC were not involved in neonatal diarrhoea in the case farm, suggesting the effectiveness of marketed vaccines. We identified *C. perfringens* as the pathogen involved in two case farms despite vaccination against *C. perfringens* type C. We can hypothesise a failure in vaccination but maybe the most credible hypothesis is the implication of *C. perfringens* type A, as suggested by slight microscopic lesions observed during our study. Indeed, *C. perfringens* type A was

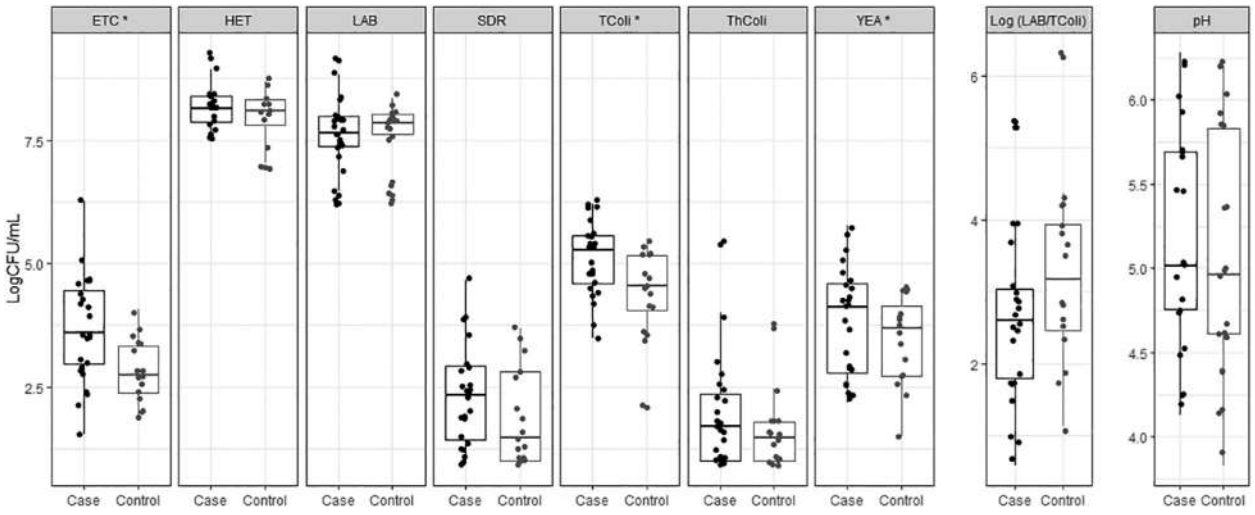


**FIGURE 2** Dispersion of analysed criteria in sow non-fermented liquid feed between case and control farms (* = significant level of a mixed regression model, $p$-value < 0.05). ETC: *Enterococci*; HET: heterotrophic bacteria; LAB: Lactic acid bacteria; SRB: sulphite-reducing bacteria; TColi: total coliforms; ThColi: thermotolerant coliforms; YEA: yeast; LAB/Tcoli: lactic acid bacteria:total coliforms ratio

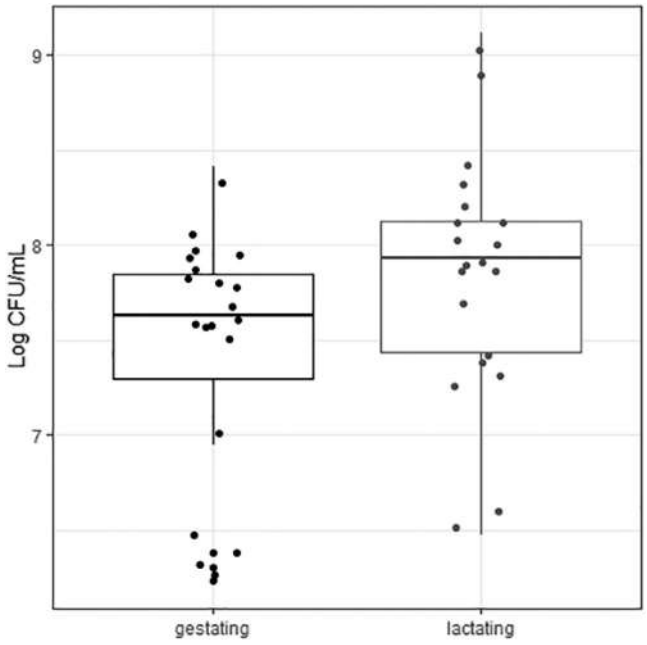


**FIGURE 3** Dispersion of lactic acid bacteria (LAB) depending on the source of sow liquid feed (gestating or farrowing facilities) (log CFU/ml)

reported to be frequently detected in a survey on the aetiology of recurrent neonatal diarrhoea in Spain.[14] Unfortunately we did not perform toxigenic typing for this study. *Enterococcus hirae* is part of the normal intestinal flora of pigs but Swedish and Danish publications suggested a possible pathogenic role in neonatal diarrhoea.[6,8] It has been reported that the genus *Enterococcus* was 24 times more abundant in diarrhoeic piglets compared to healthy piglets.[13] In our study, *E. hirae* was suspected to be the pathogen involved in neonatal diarrhoea in seven out of 10 case farms. This is consistent with our previous experience. Using the same decisional process that the one described in this study to determine the aetiology of neonatal diarrhoea, we observed during our routine diagnosis a high detection of *E. hirae* in diarrhoeic neonatal piglets, in 72 out of 165 piglets submitted alive to laboratories for neonatal diarrhoea diagnosis between 2017 and 2019. Finally, rotavirus diarrhoea was evidenced in three cases, alone or in association with other pathogens. This was in accordance with a recent report on the aetiology of recurrent neonatal diarrhoea in Spain.[14]

During recurrent neonatal diarrhoea cases investigation, all risk factors must be reviewed (including vaccination,[15] maternal immunity transfer, sow parity,[16] disease status of the farm, washing and disinfection protocols of the farrowing unit between batch[15] and environmental conditions). Among these critical points, the microbiological quality of liquid feed for sows is investigated in this study.

Gut colonization has a major impact on host health and disease. The microbiota helps in the maturation of the gastrointestinal immune system and protects against pathogen colonisation through competitive exclusion.[17] The development of many diseases can be triggered by the microbiota composition. The bacterial colonisation of the piglet intestine is believed to begin immediately after birth and is mainly influenced by their mothers as previously reported.[18] The sow's faeces, skin and the surrounding environment contain a large number of bacteria, which are likely to be the source of the intestinal microbiota of piglets.[19] Indeed, it has been shown that piglets ingest large quantities of the sow's faeces.[20]

The sow's gastrointestinal tract health may be affected by nutrition and feeding.[21] It can therefore be assumed that the bacteriological and mycological quality of liquid feed distributed to sows may affect the gut composition of the dam and its offspring. A previous report demonstrated that nutritional regimes that influence the microbiology of the sow's faeces are also likely to influence that of neonates.[4] Remarkable alterations in faecal microbiota were reported in neonatal piglets with diarrhoea of unknown aetiology.[6,13] We, therefore, hypothesised that the quality of liquid feed

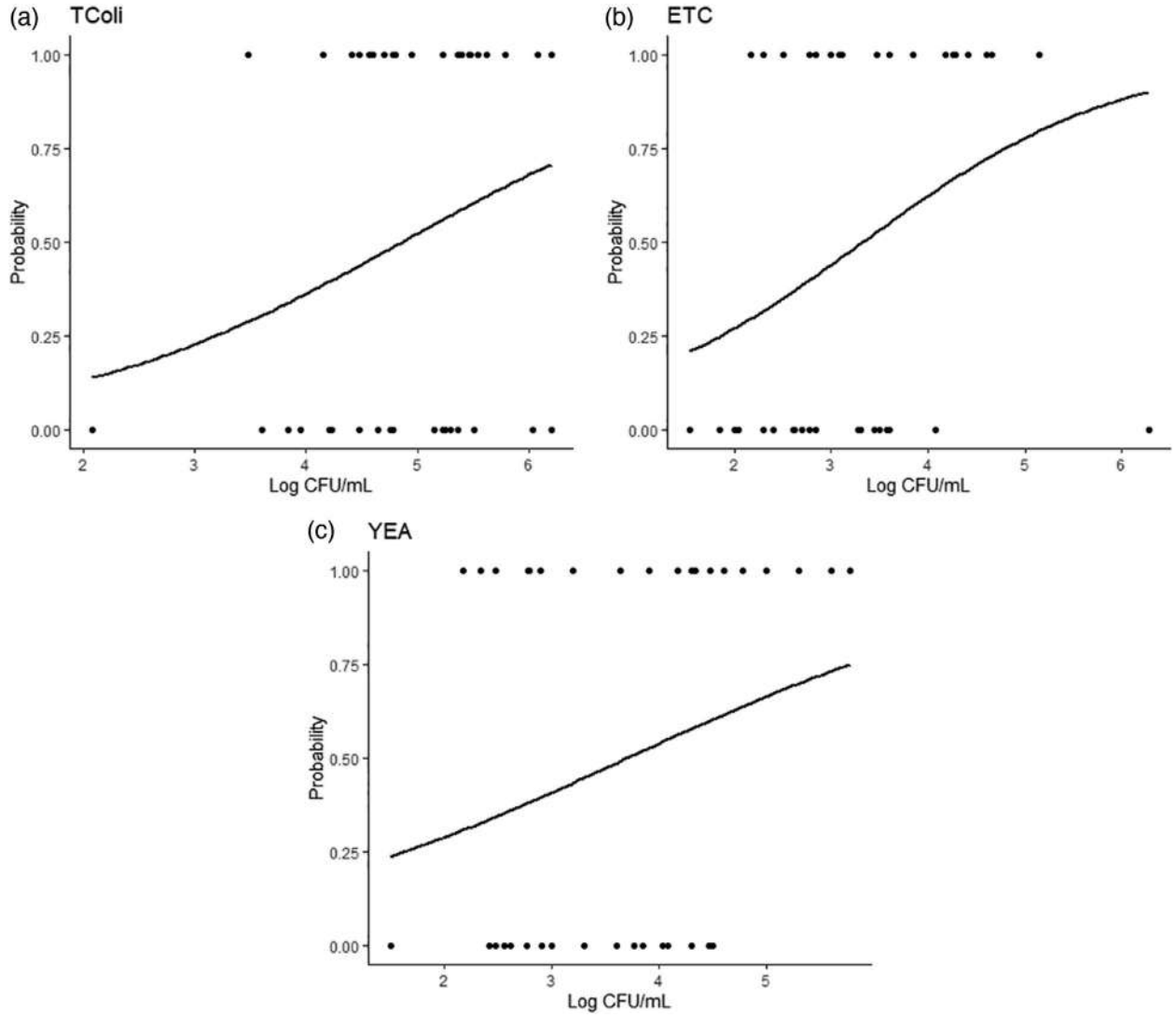


**FIGURE 4** Probability of observing neonatal diarrhoea depending on total coliforms (a), enterococci (b) and yeasts (c) count in liquid feed distributed to sows (log CFU/ml)

distributed to sows can affect the gut health of piglets by causing dysbiosis with the overgrowth of one or more pathogenic bacteria or viruses.

In this study, samples were collected at the end of liquid feed system lines at the feeder level, which is the segment of the liquid feed distribution system with the highest level of microbiological flora.[22] Some previously published studies characterised the microbiological flora of liquid feed distributed to growing pigs in the feeder,[23–26] but to our knowledge, there are few studies on the microbiological quality of liquid feed distributed to sows.

In a field, the liquid feed could be contaminated in four ways that must be considered when practices are explored: contamination because of a bad water microbial quality, a bad dry feed microbial quality, inappropriate fermentations during the feed process and improper hygiene of the feeding system. Analysis of water quality used to prepare the liquid feed is sometimes challenging in commercial farms. An attentive examination of each stage of water supply, from the water source to the end of the water line at the mixing tank level is recommended. A dry feed could also be a source of microbial contamination and must be tested. During our study, these two components of liquid feed were not investigated. Improper fermentation could happen on farms depending on the residence time of the liquid feed in feeding pipelines or the presence of a rest tank for example. We did not record residence time during our trial. Finally, the hygiene of the liquid feeding system is an important consideration. Two microbiological criteria have been previously described to evaluate global pathogen microbiological contamination: the bacterial load of total coliforms (also described as *Enterobacteriaceae*) and heterotrophic microorganisms.[27] The pathogenic role of total coliforms contamination in growing pigs liquid feed has been previously reported as increasing the risk of haemorrhagic bowel syndrome.[25] One reference value for total coliforms has been proposed for animals liquid feed, the total coliform load should be below 4 log CFU/ml.[28] In our study, the total coliform load was around 4 log CFU/ml. This is in accordance with previous studies which measured total coliform load between 2 log and 5 log CFU/ml[22] and around 4 log CFU/ml in liquid feed distributed to fattening

pigs.[24] In our study, the total coliform load in sow liquid feed was significantly higher in affected farms. This result confirmed the importance of feeding system hygiene on sow farms. Furthermore, the mean heterotrophic microorganism load was 8 log CFU/ml, this is in accordance with a previous study.[22] For this count, we observed no statistical difference between farms whatever their status regarding neonatal diarrhoea.

*Enterococcus hirae* was frequently involved in neonatal diarrhoea cases in our practice, so, in this study, we decided to count enterococci in liquid feed distributed to sows. The amount of genus *Enterococcus* was on average 4 log CFU/ml in this study and we observed a statistically significant difference between affected and non-affected farms. This finding is of interest but needs more investigations to be confirmed.

Publications about the role of yeasts in porcine diets are scarce, and most focus on fattening pigs and sometimes with conflicting conclusions. Some studies described yeast overgrowth as an important risk factor for pig intestinal health,[25,29] especially if the amount of yeast is over 6 log UFC/g.[30] One reference value for yeast has been proposed; in liquid feed, the amount of yeast should be below 5 log CFU/ml.[28] Another study did not reveal any difference between pig farms classified at risk or not for intestinal diseases.[23] In our study, 4 log CFU/ml of yeast were counted on average in sow liquid feed. This result is close to the mean described in a previous study,[31] but lower than the mean observed in France in liquid feed distributed to growing pigs which was around 6 log CFU/ml.[24] However, in our case study, a statistically significant difference was found in the amount of yeast in sow liquid feed between farms affected or not with neonatal diarrhoea. This study supports the pathological role of the overgrowth of yeast in sow liquid feed.

Other bacteriological criteria were evaluated in this study, but we could not demonstrate any significant difference between affected and unaffected farms regarding these criteria. Lactic acid bacteria were the dominant flora in liquid feed samples analysed in this study. Enabling conditions for the implantation and development of lactic acid bacteria in liquid feed system lines are recommended.[22] Indeed, these bacteria produce lactic and acetic acids, which have an inhibitory effect on pathogenic flora. The effect of lactic acid bacteria to limit the growth of various Gram-negative bacteria, especially pathogenic *E. coli*[4] and *Salmonella typhimurium*[11] is well documented. It has been demonstrated that the sow is a source of piglets lactic acid flora.[4] In this study, the average lactic acid bacteria count in sow liquid feed was higher in control farms, however, this difference was not significant. Moreover, LAB counts were significantly higher in lactating than in the gestating liquid feed. The reason for this observation is unknown but we can hypothesise that Lactobacilli might be added as probiotics in lactating feeds for example. This data was not recorded during our study. The LAB:TColi ratio is proposed to characterise the balance between these two populations in liquid feed,[24] and to describe if this balance is favourable or not in gut microbiota.[4] A higher LAB:TColi ratio in faeces is usually associated with a bacterial flora that contributes to improved animal performance. In our study, we observed no statistical difference between case and control farms in LAB:TColi ratio in the liquid feed.

Finally, regarding sulphite-reducing bacteria, bacterial loads were around 4 log CFU/ml in our study. These bacterial populations were detected in all liquid feed samples. But the bacterial load was below 1 log CFU/ml in six unaffected farms and two affected farms. However, a previous study described the detection of these bacterial populations in only 8% of samples (results based on 120 liquid feed samples).[22] No statistical difference was observed in this study between affected and unaffected farms. The presence of sulphates in feed can promote the proliferation of certain bacteria, especially *E. coli*, so SRB can be a risk factor for gastrointestinal disturbances in pigs.

## CONCLUSION

This case–control study highlighted three criteria – two bacteriological criteria and one mycological criterion – worthy of consideration in farms feeding sows with a liquid feed system for the prevention or management of neonatal diarrhoea: the level of contamination of the liquid feed by total coliforms, enterococci and yeasts. This study was carried out on a relatively small number of farms and these results should be consolidated by further results over time. For the moment, we could suppose that sow non-fermented liquid feed with total coliforms counts higher than 5 log CFU/ml and/or enterococci counts higher than 4 log CFU/ml and/or yeasts count higher than 4 log CFU/ml is at risk for neonatal diarrhoea. Contamination by both total coliforms and yeasts together is highly predictive of neonatal diarrhoea occurrence. On farms feeding sows with non-fermented liquid feed, an analysis of a sample is recommended, whatever the stage of the reproductive cycle (gestating or lactating feed), and if the advisor identifies abnormalities, the source of contamination (dry feeds, water, fermentation and hygiene) must be investigated. Finally, the role of *E. hirae* in neonatal diarrhoea and the link between the presence of genus *Enterococcus* in liquid feed and the occurrence of neonatal enterococcal diarrhoea should be further investigated.

### ACKNOWLEDGEMENTS

The authors would like to thank the herd owners for their cooperation and Finalab's team for their support in analyses and their help for redaction. Finally, we would like to acknowledge Maela Kloareg

(Kuzulia, Plabennec, France) for her assistance and advice in statistical analyses.

## FUNDING INFORMATION

Porc.Spective Swine Vet Practice; Labofarm Finalab.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## ETHICS APPROVAL

Piglets included in this survey were commercial pigs kept on farms in accordance with French regulations and standards for pig breeding and rearing. According to the French legislation, neonatal diarrhoea diagnostics, including piglet euthanasia, were performed during veterinary herd health monitoring. No specific authorisation was requested for applied research derived from routine veterinary work in France. Consents were obtained from herd owners.

## AUTHOR CONTRIBUTIONS

Study conception and design: GB, AL. Data acquisition: GB, RG, AL, CC, JJ, VN, PB, FB, MB. Laboratory investigations: GB, JLG. Data analysis and interpretation: GB, AL, CTC. Drafting the manuscript: GB, AL, CTC. All authors read, critically revised and approved the final manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ORCID

*Gwenaël Boulbria* https://orcid.org/0000-0001-9169-4420

## REFERENCES

1. Leblois J, Massart S, Li B, Wavreille J, Bindelle J, Everaert N. Modulation of piglets' microbiota: differential effects by a high wheat bran maternal diet during gestation and lactation. Sci Rep. 2017;7(1):7426.
2. Hasan S, Saha S, Junnikkala S, Orro T, Peltoniemi O, Oliviero C. Late gestation diet supplementation of resin acid-enriched composition increases sow colostrum immunoglobulin G content, piglet colostrum intake and improve sow gut microbiota. Animal 2018;1–8.
3. Hasan S, Junnikkala S, Peltoniemi O, Paulin L, Lyyski A, Vuorenmaa J, et al. Dietary supplementation with yeast hydrolysate in pregnancy influences colostrum yield and gut microbiota of sows and piglets after birth. PLoS One. 2018;13(5): e0197586.
4. Demecková V, Kelly D, Coutts AGP, Brooks PH, Campbell A. The effect of fermented liquid feeding on the faecal microbiology and colostrum quality of farrowing sows. Int J Food Microbiol. 2002;79(1–2):85–97.
5. Thomson JR, Friendship RM. Digestive system. In: Zimmermann J, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, editors. *Diseases of swine.* West Sussex, UK: Wiley-Blackwell; 2012. p. 216–8.
6. Kongsted H, Pedersen K, Hjulsager CK, Larsen LE, Pedersen KS, Jorsal SE, et al. Diarrhoea in neonatal piglets: a case control study on microbiological findings. Porcine Health Manag. 2018;4:17.
7. Larsson J, Aspán A, Lindberg R, Grandon R, Båverud V, Fall N, et al. Pathological and bacteriological characterization of neonatal porcine diarrhoea of uncertain aetiology. J Med Microbiol. 2015;64(8):916–26.
8. Larsson J, Lindberg R, Aspán A, Grandon R, Westergren E, Jacobson M. Neonatal piglet diarrhoea associated with enteroadherent *Enterococcus hirae.* J Comp Pathol. 2014;151(2–3):137–47.
9. Canibe N, Jensen BB. Fermented and nonfermented liquid feed to growing pigs: Effect on aspects of gastrointestinal ecology and growth performance. J Anim Sci. 2003;81(8):2019–31.
10. Bunte S, Grone R, Keller B, Keller C, Galvez E, Strowig T, et al. Intestinal microbiota of fattening pigs offered non-fermented and fermented liquid feed with and without the supplementation of non-fermented coarse cereals. Microorganisms 2020;8(5):638.
11. Canibe N, Jensen BB. Fermented liquid feed—Microbial and nutritional aspects and impact on enteric diseases in pigs. Anim Feed Sci Technol. 2012;173(1):17–40.
12. Holtkamp DJ, Polson DD, Torremorell M, Morrison B, Classen DM, Becton L, et al. Terminology for classifying swine herds by porcine reproductive and respiratory syndrome virus status. J Swine Health Prod . 2011;19(1):44–56.
13. Hermann-Bank ML, Skovgaard K, Stockmarr A, Strube ML, Larsen N, Kongsted H, et al. Characterization of the bacterial gut microbiota of piglets suffering from new neonatal porcine diarrhoea. BMC Vet Res. 2015;11(139).
14. Mesonero-Escuredo S, Strutzberg-Minder K, Casanovas C, Segalés J. Viral and bacterial investigations on the aetiology of recurrent pig neonatal diarrhoea cases in Spain. Porcine Health Manag. 2018;4:5.
15. Larsson J, Fall N, Lindberg M, Jacobson M. Farm characteristics and management routines related to neonatal porcine diarrhoea: a survey among Swedish piglet producers. Acta Vet Scand. 2016;58(1):77.
16. Kongsted H, Toft N, Nielsen JP. Risk factors and epidemiological characteristics of new neonatal porcine diarrhoea syndrome in four Danish herds. BMC Vet Res. 2014;10: 151.
17. Bauer E, Williams BA, Smidt H, Verstegen MWA, Mosenthin R. Influence of the gastrointestinal microbiota on development of the immune system in young animals. Curr Issues Intest Microbiol. 2006;7(2):35–51.
18. Katouli M, Lund A, Wallgren P, Kühn I, Söderlind O, Möllby R. Metabolic fingerprinting and fermentative capacity of the intestinal flora of pigs during pre- and post-weaning periods. J Appl Microbiol. 1997;83(2):147–54.
19. Thompson CL, Wang B, Holmes AJ. The immediate environment during postnatal development has long-term impact on gut community structure in pigs. ISME J. 2008;2(7):739–48.
20. Sansom BF, Gleed PT. The ingestion of sow's faeces by suckling piglets. Br J Nutr. 1981;46(3):451–6.
21. Pluske JR, Turpin DL, Kim J-C. Gastrointestinal tract (gut) health in the young pig. Anim Nutr. 2018;4(2):187–96.
22. Royer E, Moundy G, Albar J & Martineau GP. Analyse descriptive du degré d'hygiène microbiologique de la machine à soupe dans neuf élevages porcins : Influence des différents maillons du circuit. Rev Méd Vét. 2004;155:609–18.
23. Royer E, Moundy G, Albar J. Analyse descriptive du degré d'hygiène micro- biologique de la machine à soupe dans neuf élevages porcins. Revue Méd Vét. 2005;7.
24. Brunon M, Trombani C, Ropars M, Le Roux M, Launay S. Déterminants de la flore microbiologique de la soupe et ses conséquences sur les porcs charcutiers en engraissement. Journées Recherche Porcine. 2020;52:147–52
25. Grahofer A, Gurtner C, Nathues H. Haemorrhagic bowel syndrome in fattening pigs. Porcine Health Manag. 2017;3(1): 27.
26. O' Meara FM, Gardiner GE, O' Doherty JV, Lawlor PG. Effect of dietary inclusion of benzoic acid (VevoVitall®) on the

microbial quality of liquid feed and the growth and carcass quality of grow-finisher pigs. Livest Sci. 2020;237:104043.
27. Royer E, Moundy G, Albar J, Martineau GP. Evaluations quantitatives de l'hygiène des différents maillons des machines à soupe. Paper presented at: Symposium ISPAIA – SOGEVAL De la démarche hygiène à la biosécurité, Ploufragan, France. 24 April 2002.
28. Kamphues J, Coenen M, Kienzle E, Pallauf J, Simon O, Zentek J. *Supplemente zu Vorlesungen und übungen in der Tierernährung*. 10th ed. Hannover: M. & H. Schaper Alfeld; 2004.
29. Häni H, Zimmermann W, Huber A, Schmidt J. Das hämorrhagische intestinalsyndrome (HIS) des Schweines: klinische, pathologischanatomische und ätiopathogenetische Aspekte. Schweiz Arch Tierheilkd. 1993;135:117–24.
30. Drochner W, Scholz H, Hertrampf B. Untersuchungen über die Zusammensetzung von Mischfuttermitteln für Ferkel und Mastschweine sowie Einzelfuttermitteln in Problembeständen (ein Erfahrungsbericht). Dtsch tierärtzl Wochenschr. 1983;121–8.
31. Geary TM Improving the performance of weaner pigs through developments in liquid feeding. 1997. Thesis submitted to the University of Plymouth for the degree ofDoctor of Philisophy. Available from: https://pearl.plymouth.ac.uk/handle/10026.1/2793

**How to cite this article:** Boulbria G, Teixeira-Costa C, Chevance C, Grandin R, Jeusselin J, Berton P, et al. Microbial content of non-fermented liquid feed consumed by sows affects the occurrence of neonatal diarrhoea in their piglets: A case–control study. *Vet Rec*. 2021;e837. https://doi.org/10.1002/vetr.837