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Clinical utility and performance of sock sampling in weaner pig diarrhoea

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ABSTRACT

Low pathogen diarrhoea is a group-level diagnosis, characterised by non-haemorrhagic diarrhoea. In the current study, the apparent prevalence of low pathogen diarrhoea outbreaks in Danish herds was investigated along with the clinical utility of a laboratory examination for intestinal disease, agreement between three consecutive herd examinations from the same herd and agreement between quantitative PCR results from pooled faecal samples and sock samples.

Twenty-four veterinarians submitted faecal and sock samples for quantitative PCR testing from outbreaks of diarrhoea in nursery pigs ($n = 38$ herds) where the farmer or veterinarian had decided that antimicrobial treatment was necessary. The veterinarians were asked to fill in a questionnaire and participate in telephone interviews.

The apparent prevalence of low pathogen diarrhoea was 0.18 (95% CL: 0.08–0.34). Agreement between the veterinarians' clinical aetiological diagnosis and the pooled faecal sample was 0.18 (95% CL: 0.08–0.34), and Cohen's Kappa was 0.03 (95% CL: −0.08 to 0.14). Antibiotic treatment or prevention strategies were changed in 0.63 (95% CL: 0.46–0.78) of the herds, and the veterinarians indicated that, for 0.32 (95% CL: 0.18–0.50) of the herds, changes were related to the diagnostic results from the laboratory examination performed in the study.

In 0.16 (95% CL: 0.05–0.36) of the herds, the same infections were demonstrated at all three consecutive examinations. No herds had three consecutive diarrhoea outbreaks classified as low pathogen diarrhoea. For the quantitative results ($\log_{10}$ of the summed amounts of *Lawsonia intracellularis*, *Brachyspira pilosicoli*, *Escherichia coli* F4 and F18) agreement between pooled faecal samples and sock samples was evaluated. Lin's concordance correlation coefficient was 0.69 (95% CL: 0.48–0.82), and the mean difference between the two types of samples was −0.38 $\log_{10}$ bacteria/g faeces (SD = 1.59 $\log_{10}$ bacteria/g faeces; 95% CI: −0.90 to 0.14 $\log_{10}$ bacteria/g faeces). Agreement for the dichotomised results was 0.89 (95% CI: 0.75–0.97) when test results were classified as low pathogen diarrhoea or not, and Cohen's Kappa was 0.61 (95% CI: 0.26–0.95). In relation to detection of the individual infections, agreement was 0.63 (95% CI: 0.46–0.78), and Cohen's Kappa was 0.53 (95% CI: 0.34–0.71).

In conclusion, low pathogen diarrhoea is a common finding amongst diarrhoea outbreaks that are subjected to antibiotic batch treatment in Danish nursery pigs. Sock samples seem to offer a reliable diagnostic method with impact on clinical decisions for treatment and prevention. However, both the diarrhoea type and the aetiology change with time in the majority of herds, indicating a potential need for frequent diagnostic examinations.

1. Introduction

Correct application of antibiotics in both humans and animals has received increased attention because of the risk of antibiotic resistance development (Chan, 2012). The consumption of antibiotics in production animals has raised concern due to the

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potential spread of resistant bacteria or genes to humans by direct contact with infected animals or spread by animal products (Aarestrup et al., 2008; Kumar and Singh, 2013; Wegener, 2003). Examples of such spread include methicillin-resistant *Staphylococcus aureus* and extended-spectrum beta-lactamase-producing *Escherichia coli* (Kumar and Singh, 2013).

In some European countries, initiatives have been implemented in order to monitor, optimise and eventually reduce antimicrobial use. In Denmark, these include: (1) antimicrobial resistance in bacteria from animals, humans and food is reported yearly in the DANMAP report (DANMAP, 2013), (2) all use of antimicrobials in pigs is therapeutic and prescribed by a veterinarian, (3) prescription data from antimicrobials used in pigs are collected by herd, age, and indication in a public national database named VetStat (Stege et al., 2003), (4) in 2010, the Danish authorities established acceptable levels for antimicrobials used on farms called the "Yellow Card initiative" (Alban et al., 2013), and (5) legislation requiring laboratory diagnostic documentation in relation to group medications for enteric and respiratory diseases has recently been implemented in 2014.

Several guidelines for prudent antimicrobial use in veterinary practice have been published, and the main guidelines published in English have recently been reviewed (Teale and Moulin, 2012). A key step is to decide whether antibiotic treatment is necessary or whether the disease should be managed in another way. If antibiotic treatment is needed, the second step is selection of the most appropriate antibiotic (EPRUMA, 2008). Group medications are common in the pig industry and are considered to be either preventive or metaphylactic. The relative importance of these two antibiotic strategies differs amongst the individual European countries (Callens et al., 2012). In Denmark, metaphylactic use is considered to be the predominant strategy, and intestinal diseases are the most important indication in relation to antibiotic consumption in pigs (Hybschmann et al., 2011). Intestinal disease clinically characterised by diarrhoea may have a non-infectious cause (Chase-Topping et al., 2007), and in some diarrhoea outbreaks that are treated with antibiotics only few pigs experience a bacterial intestinal disease (Pedersen et al., 2014). Therefore, low pathogen diarrhoea (LP diarrhoea) has been suggested as a group-level diagnosis, characterised by non-haemorrhagic diarrhoea in more than 20% of the pigs in a group in which known bacterial pathogens can be demonstrated in fewer than 15% of the pigs within the group (Pedersen et al., 2014). Such LP diarrhoea outbreaks probably do not need antibiotic treatment, because no or only few pigs would be suffering from bacterial intestinal disease. Omission of LP diarrhoea treatment would therefore comply with the guidelines for prudent use of antimicrobials and would result in reduced antimicrobial consumption.

We have previously suggested criteria for herd diagnosis of LP diarrhoea using a fast and relatively low-cost diagnostic strategy (Pedersen et al., 2014), that potentially offers evidence-based decision support to the veterinarians in relation to antibiotic treatment of diarrhoea. However, the value of such a diagnostic strategy should be compared with the clinical examinations and other elements of decision- making already in use. Further, it should be demonstrated that a diagnostic test has an impact, i.e. it results in changes of therapeutic interventions (Jarvik et al., 1996). Clinical or therapeutic impact for a diagnostic test may be measured using questionnaires as described for the assessment of "impact on patient management" in the guidelines on clinical evaluation of diagnostic agents (EMEA, 2009). Therefore, measuring and assessing the clinical utility of a diagnostic method for diarrhoea in pigs should include evaluating whether the diagnostic method has a clinical or therapeutic impact resulting in actual changes in the behaviour of the user.

The suggested criteria for LP diarrhoea are based on quantitative PCR testing of a mixture of freshly deposited normal and diarrhoeic faecal samples, which were pooled by weight in the laboratory. This involves the collection of individual faecal samples on the farm, shipment to the laboratory and pooling of the samples at the laboratory. All of these steps are time-consuming and laborious and result in increasing costs.

Sock samples have been used for detection of *Salmonella* spp. in broiler flocks (Skov et al., 1999) and could provide an easier and cheaper alternative to laboratory pooling of samples. However, results from qPCR testing of sock samples may be more difficult to interpret in relation to the current disease status of the pigs.

The study focused on cases of intestinal diseases in nursery pigs that veterinarians and farmers decided to treat with antimicrobials. Four different objectives were investigated. Objective 1 was to estimate the apparent prevalence of LP diarrhoea outbreaks. Objective 2 was to evaluate the clinical utility of a laboratory examination in relation to intestinal disease.

Objective 3 was to determine agreement between three consecutive herd examinations from the same herd in relation to herd diagnosis of LP diarrhoea and intestinal infections. Objective 4 was to evaluate agreement between qPCR results from pooled faecal samples and sock samples.

2. Materials and methods

2.1. Study design

Objectives 1 and 2 were investigated by qPCR examination of diarrhoea outbreaks in a number of Danish nursery herds combined with questionnaires and telephone interviews of the herd veterinarians.

Objective 3 was investigated by performing qPCR examination of three consecutive diarrhoea outbreaks in the same herds.

Objective 4 was investigated by comparing qPCR results from pooled faecal samples and sock samples obtained from the same diarrhoea outbreaks.

2.2. Sample size

Sample size calculations for objectives 1–3 were performed using formulae to estimate a sample proportion (Dohoo et al., 2009a). For the objectives where no previous data were available for sample size calculations, a sample proportion of 0.50, which provided the largest sample size, was used in the calculations.

For objective 1, an expected prevalence of LP diarrhoea of 0.25, 95% confidence and precision of 0.12 provided a sample size of approximately 50 diarrhoea outbreaks. For objective 2, no previous data were available for sample size calculations. A sample size of 50 diarrhoea outbreaks, 95% confidence and sample estimates of 0.50 would provide a precision of 0.15. This was considered acceptable, and it was decided to include a total of 50 diarrhoea outbreaks (one outbreak in each of 50 herds) to investigate objectives 1 and 2.

For objective 3, no previous data were available for sample size calculations. A sample size of 25 herds with examination of three consecutive diarrhoea outbreaks per herd, 95% confidence and a sample estimate of 0.50 would provide a precision of 0.20. This was considered acceptable, because the investigation of objective 3 was considered a pilot study.

For objective 4, sample size calculation was performed using formulae to estimate a sample mean (Dohoo et al., 2009a). No previous data on the expected difference between $\log_{10}$ transformed quantitative qPCR results obtained from testing a pooled faecal sample and a sock sample were available. Using an expected mean