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Salmonella occurrence and *Enterobacteriaceae* counts in pig feed ingredients and compound feed from feed mills in Ireland

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ABSTRACT

The purpose of this study was to assess the occurrence of non-typhoidal *Salmonellae* and Enterobacteriaceae counts in raw ingredients and compound feeds sampled from feed mills manufacturing pig diets. Between November 2012 and September 2013, feed ingredients ($n = 340$) and compound pig feed ($n = 313$) samples were collected from five commercial feed mills and one home compounder at various locations throughout Ireland. Feed ingredients included cereals, vegetable protein sources and by-products of oil extraction and ethanol production. The compound feeds included meal and pelleted feed for all stages of pig production. Samples were analysed for *Salmonella* using standard enrichment procedures. Recovered isolates were serotyped, characterised for antibiotic resistance and subtyped by multi locus variance analysis (MLVA). Total *Enterobacteriaceae* counts were also performed. *Salmonella* was recovered from 2/338 (0.6%) ingredients (wheat and soybean meal), at two of the six mills. *Salmonella* was also detected in 3/317 (0.95%) compound feeds including pelleted feed which undergoes heat treatment. All isolates recovered from feed ingredient and compound feed samples were verified as *Salmonella enterica* subsp. *enterica* serotype (4,[5],12:i:-) that lack the expression of flagellar Phase 2 antigens representing monophasic variants of *Salmonella* Typhimurium (4,[5],12:i:-). Isolates exhibited resistance to between two and seven antimicrobials. Two distinct MLVA profiles were observed, with the same profile recovered from both feed and ingredients, although these did not originate at the same mill. There was no relationship between the occurrence of *Salmonella* and a high *Enterobacteriaceae* counts but it was shown that *Enterobacteriaceae* counts were significantly lower in pelleted feed (heat treated) than in meal (no heat treatment) and that *Enterobacteriaceae* counts would be very useful indicator in HACPP programme. Overall, although the prevalence of *Salmonella* in pig feed and feed ingredients in the present study was low, even minor *Salmonella* contamination in feed has the potential to affect many herds and may subsequently cause human infection. Furthermore, the recovery of a recently emerged serovar with multi-antibiotic resistance is a potential cause for concern.

1. Introduction

Non-typhoidal *Salmonellae* can colonise a wide range of hosts including all the major livestock species (poultry, cattle, and pigs), often asymptomatically, potentially leading to contamination of meat and other food products (Stephens et al., 2009). Following a "farm-to-fork" model, animal feed is at the beginning of the food safety chain. Therefore, the presence of *Salmonella* in animal feed or feed ingredients at the feed mill or on-farm is a cause for public health concern. This is evidenced by a number of incidences where animal infection has been traced back to contaminated animal feed. For example, Österberg et al. (2006) established that contaminated feed was the cause of an outbreak of *Salmonella* Cubana on a number of Swedish pig farms. Furthermore, Molla et al. (2010) found genotypically related and in some cases clonal *Salmonella* strains in commercially processed pig feed and pig faecal samples.

Abbreviations: MLVA, multi locus variance analysis; EFSA, European Food Safety Authority; NSRL, National Salmonella Reference Laboratory; MPN, most probable number; BPW, buffered peptone water; PCR, polymerase chain reaction; VNTR, variable number tandem repeat.

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A number of different feed ingredients may potentially harbour pathogenic micro-organisms including non-typhoidal *Salmonellae*. Historically, a number of studies have shown the presence of *Salmonella* in feed ingredients of animal origin (e.g. rendered animal by-products) (Clise and Swecker, 1965; Franco, 2005); however, such ingredients are no longer an issue following their ban in animal feed in the European Union (EU) in 2001 in the aftermath of the Bovine Spongiform Encephalopathy (BSE) crisis (Commission Regulation (EC) No 163, 2009). Exceptions have been made for the use of certain animal protein sources including fish meal, milk powders, certain blood products and dicalcium phosphate by-products (e.g. from the production of gelatin) as feed for monogastric animals (Commission Regulation (EC) No 1292, 2005). However, these ingredients are not without risk, as evidenced by the reported introduction of *S.* Agona to the United States (US) food chain via imported Peruvian fish meal, as quoted by Clark et al., 1973.

However, the risk of *Salmonella* contamination of pig feed from ingredients of animal origin may not be an issue, as the protein-rich ingredients currently used to formulate pig diets are principally of vegetable origin. Any ingredient of vegetable origin may become contaminated with *Salmonella* from contact with infected or carrier wildlife or production animals during storage or transit and/or from the use of manure or sludge as fertilizers on the growing crop. However, the risk is greater with imported ingredients as they may originate in countries with different regulations and there is an opportunity for contamination during transit. The Republic of Ireland relies on importing a much higher proportion of its animal feed requirement compared to other EU countries. In 2014, Ireland was importing 65% of its requirements, with ~3 million tonnes of cereals being imported annually, ~55% of which comes from countries outside the EU (DAFM, 2015). The EU in 2014 was 35% deficient in its requirement for protein for animal feed, so third-country imports are unavoidable (Popp et al., 2013; DAFM, 2015). In the EU, these are largely imported in the form of soybean from North and South America (de Visser et al., 2014). The contamination of cereals with *Salmonella* was estimated to range between 0.2 and 0.6% in 2012 in a study by the European Food Safety Authority (EFSA, 2014). This is much lower than for feed ingredients such as soybean meal (3.2–6.7%) and rapeseed (6.8%) which are by-products from other processing operations (EFSA, 2008). In one surveillance study, *Salmonella* was isolated from 14.6% of soybean meal consignments and 10% of rapeseed meal samples (Wierup and Haggblom, 2010).

The reported incidence of *Salmonella* in compound animal feed is generally low and when present, prevalence ranges on average from 0.6 to 1.7% (EFSA, 2008). It is also considered that the reported incidence in both feed ingredients and compound feed is probably lower than the true incidence due to under-reporting, sub optimal sampling procedures and for other reasons such as *Salmonella* detection methods may not offer all *Salmonella* serotypes an equal chance of isolation (Jones, 2011), especially in samples where multiple serotypes are present (De Busser et al., 2013a). A comprehensive sampling plan is therefore required for the monitoring of *Salmonella* in animal feed, as *Salmonella,* when present, is usually in low numbers and unevenly distributed. However, even low numbers of *Salmonella* may be sufficient to cause infection (Finn et al., 2013). This is particularly true for feeds of high fat content in which *Salmonella* can be protected from host gastric defence mechanisms (Jones et al., 1982). *Salmonella,* if present in the feed, also has the potential to multiply in warm, moist conditions, either at the feed mill or on the farm (Davies and Hinton, 2000; Hilbert et al., 2012).

As food-producing animals are the primary source of *Salmonella* infections in humans (Forshell and Wierup, 2006), it follows that contamination of animal feed with this pathogen should not be overlooked as an important origin of foodborne illness and outbreaks. The same *Salmonella* serotypes have been recovered from commercial pig feed and pigs sampled on the same farm (Burns et al., 2013). However, it remains unclear whether the feed contamination arose on-farm or whether the commercial feed introduced onto the farm was already contaminated.

The total number of *Enterobacteriaceae* can serve as a hygiene indicator in food and feed. *Enterobacteriaceae* have the advantage of being enumerated inexpensively and easily and are useful for quantifying the hygienic performance of a production process, when particular pathogens or spoilage organisms might be difficult to detect (Jordan et al., 2007). In the EU there is legislation (Commission Regulation (EC) No 2073, 2005) setting microbial process hygiene criteria for *Enterobacteriaceae* counts on foods including carcasses, milk and dairy products, and eggs. Equally, the determination of *Enterobacteriaceae* counts could be used to assess and subsequently improve mill hygiene and the quality of animal feeds (Jones and Richardson, 2004, Veldman et al., 1995). However the relevance of *Enterobacteriaceae* in feed should, however, be assessed and interpreted carefully and recognition given that there is conflicting studies on the correlation between *Enterobacteriaceae* count and the presence of *Salmonella* in feed. Jones and Richardson (2004) reported that poultry feed samples, meal and pellets, contaminated with *Salmonella* contained significantly higher *Enterobacteriaceae* counts. A study by Veldman et al. (1995), isolated predominantly thermotrophic *Enterobacteriaceae* from feedstuffs and found them to be useful markers of the rate of contamination with *salmonellae* and of the efficiency of decontamination of the feedstuffs by pelletisation. Whereas a study by Cox et al. (1983) showed no correlation between *Enterobacteriaceae* and *Salmonella*. Further studies showing the benefit of using as a hygiene indicator in feed therefore would be of benefit.

Therefore, the objective of this study was to assess the occurrence and characteristics of *Salmonella* in a range of feed ingredients and compound feeds sampled from feed mills supplying high *Salmonella* sero-prevalent pig farms in the Republic of Ireland, where on-farm bacteriology had confirmed *Salmonella* presence in both pigs and feed (Burns et al., 2013). *Enterobacteriaceae* counts were also performed and these may provide valuable data that could be used as a baseline for assessment of the hygienic standard of feed, which is currently rare in other studies.

2. Material and methods

2.1. Sample collection

Samples of feed ingredients and compound pig feed were collected monthly from five commercial feed mills (Mills A–D and F) and one home compounder (Mill E). All mills were operating under hazard analysis and critical control points (HACCP) quality assurance schemes and were all producing both meal and pelleted feed from a wide variety of ingredients. In all mills, pelleting was preceded by a steam conditioning step, whereas no heat treatment was applied to meal feed. Samples from each feed mill were taken over a 6 month period between November 2012 and September 2013. A total of 338 raw ingredients and 317 compound feed samples were obtained. The feed ingredients included cereals, vegetable protein ingredients and by-products of oil extraction and ethanol production and were the ingredients used in pig diet formulation at the time of the study. Compound feeds included meal and pelleted feed for all stages of pig production. For pelleted feed, pelleting was preceded by a steam conditioning step, whereas no heat treatment whatsoever was applied to meal feed. Feed ingredients were sampled at mill intakes from every ingredient load and finished feeds were sampled from every batch (from storage bins at the feed mills). All samples were composite samples taken by mill personnel in accordance with Commission Regulation (EC) No 152 (2009). Sub

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