final report

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Pathogen and antimicrobial resistance in ovine faeces at slaughter

Executive summary of milestones 5 and 6

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Executive summary

Australia is the largest global exporter of sheepmeat, with increased demand from customers in China and the US helping to bolster export volumes in recent years. Australia's strong food safety regulations and reputation for producing clean, green and safe sheepmeat are major factors underpinning the growth in demand for Australian sheepmeat products. Food safety continues to be a major consideration for international customers and the supply of exports into world markets depends on industries capacity to maintain its reputation as a supplier of safe products. The greatest risk to the safe production and processing of sheepmeat comes from bacteria. Thus, assessing sheep production systems for bacteria that pose a risk to humans, such as pathogenic Shiga toxin producing *E. coli* (pathogenic STEC) and antimicrobial resistant bacteria, is one way in which Australia can understand potential risks to human health. This study is designed to assist the Australian sheepmeat industry to improve the value of its commodities by objectively defining food safety risks present in the processing stage of sheepmeat production, with a specific focus on pathogenic STEC and antimicrobial resistant bacteria.

A large national survey was conducted to deliver data on the prevalence and concentration of key foodborne pathogens and the prevalence of antimicrobial resistance in bacteria from sheep faeces at slaughter. A total of 14 Australian sheepmeat processors agreed to participate in this survey, collectively representing 65% of total Australian lamb production and 83% of total Australian mutton production. The number of samples collected from each establishment was adjusted using a probability proportional to size sampling approach to ensure that survey samples were largely representative of Australian sheepmeat production. The survey comprised 800 faecal samples, collected from across three animal groups: pasture-fed lamb (n=414), feedlot lamb (n=163) and sheep (n=223). Lamb were defined as animals with zero permanent incisor teeth that are raised on pasture or on a supplemented pasture diet (pasture-fed lamb) or that were derived from a dedicated/intensive feeding operation (feedlot lamb). Sheep were defined as female or castrate male with at least 1 permanent incisor and males that don't have any secondary sexual characteristics.

Faecal samples were collected weekly in small batches throughout two sampling windows (400 samples per window), with the first sampling window occurring over an 11-week period between September and November 2017 and the second over a 19-week period between February and July 2018. The 800 sampled animals were sourced from five states; NSW (34%), VIC (26%), WA (15%), SA (20%), QLD (0.75%) and unknown origin (4.5%), representing more than 200 different postcodes.

Faecal samples were collected by the processors and shipped to CSIRO under refrigeration conditions. On arrival at CSIRO, samples were tested for the presence of a range of bacteria including Shiga toxin-producing *E. coli* serogroups O26, O45, O103, O111, O121, O145 and O157 (Top 7 STEC), *Salmonella*, *Enterococcus* and generic *E. coli*. Top 7 STEC and *Salmonella* were isolated and enumerated using immunomagnetic separation and most probable number techniques, respectively. Top 7 STEC were sequenced, molecularly characterised and compared to previous cattle isolates. *E. coli* and enterococci were isolated using Petrifilm *E. coli*/coliform count plates and Enterococcosel broth and agar, respectively.

By culture confirmation, Top 7 STEC were recovered from 28 of the 800 samples processed (3.5%); 27 samples contained STEC O157 (3.4%), two samples contained STEC O26 (0.3%), with one sample containing both O157 and O26. Analysis of animal groups revealed that Top 7 STEC were present in 4.9% of sheep, 4.3% of feedlot lamb and 2.4% of pasture-fed lamb. Despite considerable effort to isolate Top 7 STEC, serogroups O45, O103, O111, O121, and O145 were not isolated from any sample. Counts of STEC O157 were generally low with 17 of the 27 samples (63%) containing O157 at concentrations less than 1 $\log_{10}$ MPN/g faeces. The remaining samples contained O157 at 1 (n=1), 1.7 (n=1), 1.8 (n=2), 2.3 (n=1), 3.3 (n=2), 3.7 (n=2) and 6.3 (n=1) $\log_{10}$ MPN/g of faeces. The two STEC O26 isolates were present at 0.15 and 3.0 $\log_{10}$ MPN/g faeces. Characterisation data showed that STEC O157 isolates most often possess stx_{1a} and stx_{2c} toxin subtypes (72%), which places them into level 3 of the risk classification scheme proposed by JEMRA and is consistent with the predominant *stx* subtypes observed in Australian cattle populations. The remaining O157 isolates were shown to possess stx_{1a} alone (16%; JEMRA level 4) and stx_{2c} alone (12%; JEMRA level 3). The two O26 isolates possessed a single toxin type, stx_{1a} (JEMRA level 4), which is also consistent with the predominant profile observed in Australian cattle isolates.

Salmonella were isolated from 81 of 800 samples (10.1%) tested. The prevalence of *Salmonella* was higher in sheep (19.3%) than pasture-fed (7.5%) or feedlot (4.3%) lamb samples. Counts of *Salmonella* were generally very low with mean counts of 0.9, 0.6 and 0.4 $\log_{10}$ MPN/g faeces recorded for feedlot lamb, sheep and pasture-fed lamb, respectively. *E. coli* (96%) and *Enterococcus* (98%) were isolated from most samples, with clinically relevant *Enterococcus faecium* and *faecalis* species isolated from 42 (5.3%) and 34 (4.3%) of the 800 samples, respectively. A subsample of 100 *E. coli* (randomly selected), along with 76 *Enterococcus* (preferencing clinically significant species - *E. faecalis* and *E. faecium*) and all 81 *Salmonella* isolates were tested for antimicrobial susceptibility. The resistance to clinically significant antimicrobials was generally low across all isolate groups. Of

the 100 *E. coli* tested, 97% were considered pan-susceptible regardless of whether epidemiological (ECOFF) or clinical (CLSI) breakpoints were used, 2% were non-wild for tetracycline and 1% were considered clinically resistant to sulfisoxazole. When ECOFF breakpoints were considered, 100% of *E. faecalis* (n=34) and 83% of *E. faecium* (35 of 42 isolates) were considered wild type. Of the remaining *E. faecium*, 6 (14.2%) were considered non-wild for ciprofloxacin and 1 (2.4%) for streptomycin. Of the 81 *Salmonella* tested, 80 (99%) were considered pan-susceptible, with just a single isolate confirmed as non-wild type for ampicillin, streptomycin and tetracycline. Genetic markers were determined for tetracycline, beta-lactams and sulphonamides.

This is the first national survey of STEC in sheep undertaken in Australia. The results indicate that Australian sheep are a potential reservoir for STEC O157 and O26; however, the very low prevalence of STEC O26 and lack of isolation of other Top 7 STEC suggests that these serogroups are uncommon, or not present in Australian sheep populations. The rate of detection of AMR in isolates from sheep was low, suggesting that sheep production practices are likely to have minimal impact on the development of resistance to antimicrobials considered highest priority critically important to human medicine. The prevalence of Top 7 STEC, *Salmonella* and antimicrobial resistance in bacteria from sheep are consistent with previous Australian surveys of beef cattle. The information provides a platform through which industry can engage in evidence-based discussions about the risks associated with Top 7 STEC and AMR bacteria in sheepmeat. The information may be used to educate industry on potential risks, address trade partner concerns and inform future research direction into the use of intervention strategies for reducing bacterial loads on carcases during processing. Scientific outcomes may also be used to inform domestic and international markets of our monitoring programs and ongoing commitment to ensuring Australian sheep exports remain safe for consumers.