

# National baseline surveys to characterise processing hygiene and microbial hazards of Australian culled sow meat, retail pork sausages and retail pork mince

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## Abstract

Pork products were sampled at retail to determine the impact of further processing on hazard levels to which consumers may be exposed, compared to carcasses. Surveys of 116 fresh pork sausages and 148 fresh pork mince samples were purchased from supermarkets (n=87, n=105) and butcher shops (n=29, n=43), respectively. For sausages, concentrations of TVC averaged 4.6 log<sub>10</sub> cfu/g. The E. coli prevalence was 16.4% (95% CI: 10.2-24.4%) with average count of 0.65 log<sub>10</sub> cfu/g. The prevalence of coagulase positive Staphylococci was 3.4% (95% CI: 0.9-8.6%) and that for *Listeria monocytogenes* was 16.4% (95% CI: 10.2-24.4%), although none of the samples exceeded levels of 100 cfu/g. The prevalence of *Salmonella* was 8.6% (95% CI: 4.2-15.3%). For pork mince mean TVC were high (6.2 log<sub>10</sub> cfu/g) and a 4 log variation in TVC was observed from product with the same number of days until the use-by-date. In addition commercial retail criteria, which specify a maximum TVC of 6 log<sub>10</sub> cfu/g up to the end of shelf life, were not met by 54.1% of samples (including 50.5% of supermarket samples), an indication of possible cool chain issues. E. coli (6%), coagulase positive *Staphylococcus* (1.3%), *Campylobacter* spp. (2.7%) and *Salmonella* spp. (1.5%), were detected. No E. coli O157:H7, MRSA or *Yersinia enterocolitica* were detected (i.e. prevalence <2.5%).

## Introduction

Based on consumer surveys, it is estimated that in Australia there are around 730 million annual servings of sausages alone, with a beef:pork ratio of 53:47. In addition, in recent years there has been a marketing push by the pork industry to encourage the use of minced meat to increase per capita consumption. Food borne outbreaks associated with pork in Australia are mostly due to Salmonellosis from the food service sector (Pointon and Horchner 2010).

Surprisingly, given the level of consumption, there have been few studies of the microbiology of comminuted meats in the retail chain, which could help indicate if improvements achieved in abattoir processing hygiene have translated to similar low levels in pork retail products or whether further improvements are required. Changes in marketing strategy to increase per capita consumption should take into consideration hazard levels and whether further processing steps will act as contributing factors to consumer risk. Consequently, between 2008 and 2010 national microbiological benchmarking studies were conducted on pork sausages and pork mince at retail.

## Materials and Methods

### Sampling Methods

A total of 116 pork sausages and 148 samples of pork mince were purchased nationally (x 0.5 Kg) from butcher shops (n=29 and n=43, respectively) and supermarkets (n=87 and n=105, respectively). Sample numbers were allocated approximately proportionally on a population basis to the five largest capital cities in Australia as determined by the Australian Bureau of Statistics 2006 Census (Anon., 2006). Sampling of butcher shops and supermarkets, in each city, was in the approximate ratio 1:3 to reflect estimated retail volumes and potential consumer exposure.

Sausage samples were tested for TVC, E. coli, *Salmonella* spp., *Staphylococcus aureus*, *Listeria* spp.

Mince samples were tested for TVC, E. coli, E. coli O157:H7, *Salmonella* spp., *Staphylococcus aureus*, Methicillin Resistant *Staph. aureus* (MRSA), *Campylobacter* spp. and *Yersinia enterocolitica*.

## Laboratory Methods

Samples were placed in insulated containers with ice bricks and transported overnight to the laboratory for testing within 24 hours of collection.

Total Viable Count (TVC) and E. coli Count: 25g of sow meat/mince, pork sausage or pork mince was diluted 1:10 (w/v) with buffered peptone water, stomached for 60 seconds and 10-fold serial dilutions prepared in Peptone Saline Solution (Media Production Unit, The University of Melbourne). Counts were performed using either 3MTM Petrifilm™ Aerobic Plate Count Plates (25°C/96hr) or 3MTM Petrifilm™ E. coli/Coliform Count Plates (37°C/24 hr) (3M Corporation, St Paul, Minnesota). Colonies were identified and counted as per manufacturer's instructions.

E. coli O157:H7: Samples were tested for the presence of E coli O157:H7 using the BIOCONTROL VIP® Gold for EHEC single step immunoassay (BIOCONTROL, Bellevue, Washington). Mince (25g) was added to 225 mL of pre-warmed modified Tryptone Soya Broth (BIOCONTROL, Bellevue, Washington) homogenised for 120 seconds using a stomacher. This broth was incubated for 18 to 28 h at 35 - 37°C. A 100 µL aliquot was then examined as per the manufacturer's instruction.

Salmonella spp. The remaining 1:10 (w/v) dilution, as described under the TVC/ E. coli section above, was examined as per Australian Standard (AS) 5013.10-2004. All colonies identified as Salmonella spp. were forwarded to The Salmonella Reference Centre at The Institute of Medical and Veterinary Science (IMVS), Adelaide, South Australia for serotyping.

Staphylococcus aureus: Aliquots (1 mL) from each serial dilution were inoculated onto either 3M™ Petrifilm™ Staph Express Count Plates (3M Corporation, St Paul, Minnesota) and incubated at 37°C for 24 h Colonies were identified and counted as per manufacturer's instructions.

MRSA: A loopful of the overnight buffered peptone enrichment was streaked onto a Brilliance MRSA agar plate (Oxoid, Thebarton, South Australia) and incubated for 18-20 hours at 37°C. The plate was then observed for typical colonies as per the manufacturer's instructions. Suspect colonies were streaked for purity onto Nutrient Agar and screened by polymerase chain reaction (PCR) as per Jonas et al. (2002).

Listeria spp.: A second 25 g sample of sausage meat was examined for the presence or absence of Listeria species following FSIS MLG 8.05 – Isolation and identification of Listeria monocytogenes from red meat, poultry, egg and environmental samples. Levels of Listeria organisms were determined by spread inoculating 0.1 mL aliquots of the 1:10 dilution prepare above onto plates of Modified Oxford (MOX) Agar (Oxoid Ltd., Basingstoke Hampshire England) with the limit of detection 100 cfu/g.

Campylobacter spp.: Mince (25g) was weighed aseptically into a stomacher bag and examined as per AS 5013.6-2004: Examination for specific organisms – Campylobacter.

Yersinia enterocolitica: Mince (25 g) was diluted 1:10 (w/v) in tris-buffered peptone water and homogenized in a stomacher for 1 min with the examination carried out following Roberts D and Greenwood M. 2003. The identity of suspect colonies was confirmed using Microbact 24E incubated at 25°C for 48 h. (J Holds, SA Pathology, Adelaide, South Australia pers. comm.). Those cultures presumptively identified as Yersinia enterocolitica were sent for confirmation, biotyping and serotyping by the Salmonella Reference Centre at the IMVS, Adelaide, South Australia.

## Results

A summary of the results of the surveys are presented in Table 1. Mean log<sub>10</sub> TVC(cfu/g) in sausages and retail mince was 4.6 and 6.2, respectively. Mean log<sub>10</sub> E. coli concentration was 1.63 and 1.33, being detected in 16.4 and 10.1% of samples. Salmonella spp. were isolated from 8.6% and 1.5% of sausages and mince samples, respectively. Serovars included S. typhimurium (3: pt 8, 108, 197), S. rissen (1), S. infantis (2), S. sofia (2), S. agona (1), S. derby (1), S. ohio (1). L. monocytogenes was isolated from 16.4 % of retail pork sausages. E. coli O157:H7, MRSA and Yersinia enterocolitica were not detected in the products tested (Table 1).

Table 1. Summary of overall microbiological results for pork sausages and retail pork mince.

	Pork Sausages # (%)	Pork Mince # (%)
N	116	148
Log <sub>10</sub> TVC(cfu/g)	4.6	6.2
<i>E. coli</i> prevalence	19 (16.4)	15 (10.1)
Log <sub>10</sub> <i>E. coli</i> (cfu/g)	1.63	1.33
<i>E coli O157:H7</i>	Nt	0 (<2.5)
<i>Salmonella</i> <sup>1</sup>	10 (8.6)	2 (1.5)
<i>L. monocytogenes</i> <sup>2</sup>	19 (16.4)	Nt
<i>Listeria</i> (other)	42 (36.2)	Nt
<i>Staph</i> (Coag. Pos)	4 (3.4)	2 (1.3)
MRSA	Nt	0 (<2.5)
<i>Campylobacter</i> spp.	Nt	4 (2.7)
<i>Yersinia enterocolitica</i>	Nt	0 (<2.5)

<sup>1</sup> Includes *S. typhimurium* (3: pt 8, 108, 197), *S. infantis* (2), *S. rissen* (1), *S. sofia* (2), *S. agona* (1), *S. derby* (1), *S. ohio* (1).

<sup>2</sup> All positive *L. monocytogenes* < 100cfu/gm. Nt= not tested

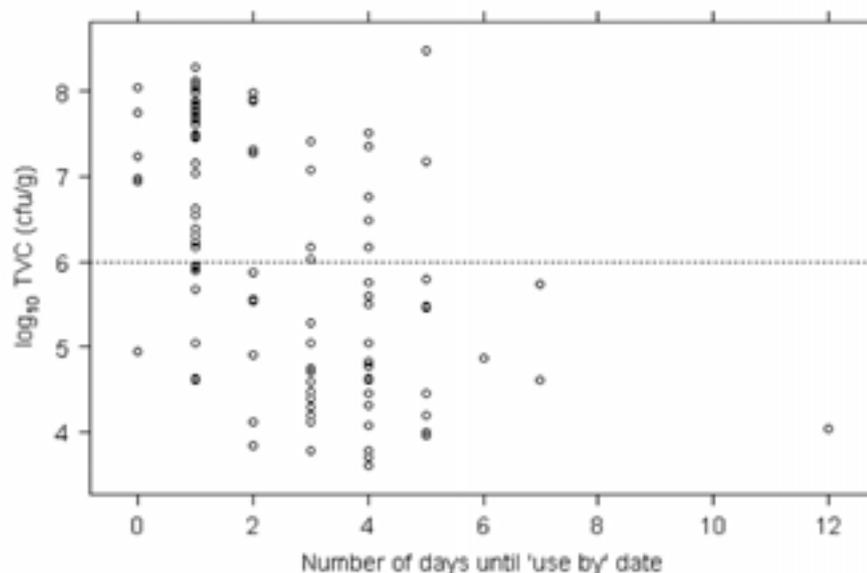


Figure 1. Scatter plot of pork mince TVC versus number of days until 'use by' date

## Discussion

A recent National Finisher Baseline Survey (Hamilton et al 2010) indicated Australian pig carcasses hygiene was good, with a mean *E. coli* prevalence of 20.8% and a -0.79 mean log<sub>10</sub> cfu/cm<sup>2</sup>. Mean *Salmonella* prevalence was 0.4% (Hamilton et al 2011). However, comminuted meats such as sausages and mince by their very nature are problematic from a food safety point of view. Their production entails considerable mixing of lots, potentially leading to wider distribution of contamination and hence increased potential for consumer exposure.

The survey results show that for sausages there is considerable contamination, probably associated with the use of carcass trims, and in the case of retail butcher shops the potential for cross contamination. This point is demonstrated by the isolation of *S. sofia*, a chicken associated serovar (Pointon et al 2008), from pork sausages and mince in this study. The sausage *Salmonella* prevalence of 8.6% is considerably lower than the 24% reported in a similar Brazilian study (Mürmann et al 2011) and higher than 2.9% reported from Ireland (Broughton et al 2004). It remains a potential food safety issue if sausages are improperly cooked, when process control is absent at major community events.

For mince the *Salmonella* levels are relatively low (1.5%) despite the processing hygiene indicators (TVC and *E. coli* prevalence and counts) being similar to sausages (Table 1). An explanation may be the source of the raw material.

Supermarket mince constitutes the predominant source consumed, and is generally processed centrally from shoulder primal cuts. This may explain why mince Salmonella detection rates more closely reflect carcass levels (Hamilton et al 2011). Similar studies have reported prevalence in pork mince ranging from 0.3% (Delhalle et al 2009) to 12.5% (Duffy et al 2001)

Mince, which unlike sausages is not allowed to contain preservatives, had a mean log<sub>10</sub> cfu/g of 6.2. Of particular concern was the fact that for pre-packaged mince with the same number of days to the use-by-date there was a 4 log variation in TVC across samples. In addition commercial retail criteria, which specify a maximum TVC of 6 log<sub>10</sub> cfu/g up to the end of shelf life, were not met by 54.1% of samples, including 50.5% of supermarket samples (Fig 1). This is a very significant result that indicates an underlying problem in either mincing hygiene, cool chain abuse and/or an overly optimistic shelf life claim. While mince is widely promoted for use in Bolognese style dishes marketing promotions should be aware of the potential risk associated with end-uses that have less certain hazard reducing steps such as fresh pork burgers.

## Conclusion

This study is a prudent reminder that even when carcass hygiene is high by international standards, final hazard levels in retail products and potential consumer risk are subject to a number of contributing factors including carcass levels, use of trim or primal cuts, cross contamination, cool chain temperature abuse and added ingredients.

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