

A study of the foodborne pathogens: *Campylobacter*, *Listeria* and *Yersinia*, in faeces from slaughter-age cattle and sheep in Australia

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Abstract

In a study of faeces from 475 slaughter-age cattle and sheep from 19 herds or flocks, *Campylobacter* species (*C. jejuni* and *C. coli*) were cultured from all production systems studied and from 73.7 per cent (14/19) of herds or flocks. Within individual properties there was a higher prevalence in cattle than in sheep, with *Campylobacter* being most commonly isolated from feedlot cattle. The median prevalences and ranges were: for dairy cattle, six per cent (0–24%), feedlot beef cattle, 58 per cent (12–92%) pasture beef cattle, two per cent (0–52%), mutton sheep, 0 per cent (0–4%) and prime lambs eight per cent. *Listeria ivanovii* was cultured from one dairy cow but *Yersinia enterocolitica* was not cultured from any animal. *Campylobacter* is the leading bacterial causative agent of acute diarrhoea in humans in many industrialised countries. While the role of cattle and sheep in producing human campylobacteriosis either directly or via contaminated food, remains to be epidemiologically clarified, this study suggests that the production system, particularly for cattle, may be an important consideration. *Commun Dis Intell* 2003;27:249–257.

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Introduction

Bacterial pathogens associated with human food poisoning may be present in the production animal and therefore be potential sources of contamination. The chain of events from slaughter, through processing, storage and food preparation can allow multiplication of these contaminating organisms.

As part of a larger project investigating Shiga-like toxin producing *E. coli* and *Salmonella* in cattle and sheep, a 'snapshot' study of 19 properties was undertaken to ascertain the prevalence of *Campylobacter* (*C. jejuni* and *C. coli*), *Listeria* (*L. monocytogenes* and *L. ivanovii*) and *Yersinia* (*Y. enterocolitica*) in faeces from slaughter-age animals in New South Wales and Queensland. The aim was to test animals that were about to be slaughtered but had not yet left the farm.

Campylobacter is the leading bacterial cause of acute diarrhoea in man in many industrialised countries including Australia.^{1,2,3} *C. jejuni* and *C. coli*, are the causative agents, with *C. jejuni* most commonly isolated. *C. jejuni* and *C. coli* are almost identical in behaviour and epidemiology⁴ and therefore discussions in this report relating to *C. jejuni*, apply to both organisms. *C. jejuni* is part of the natural intestinal flora of a wide range of birds and animals³ and can be pathogenic in these species. Transmission to humans is usually via faecal contamination of food and water,⁵ and common sources are poultry, unpasteurised milk, untreated water and contact with domestic pets. Campylobacteriosis is more frequently associated with the consumption of poultry than red meat.^{5,6} At present the roles of red meat and production animals in producing this human illness, remain to be epidemiologically clarified.^{2,6}

Listeria is widely distributed in the environment (particularly in soil and vegetation) and in many animal species, with *L. monocytogenes* being pathogenic for humans, animals and birds.⁷ Listeriosis in humans is primarily via contamination of food during production and processing, particularly dairy products.^{8,9} The organism also has been frequently isolated from both red and white meat.¹⁰ Intestinal contents from cattle¹¹ and dirty hides¹² are considered major contributors to carcass contamination. On-farm sources of *Listeria* include poorly made silage,¹³ soil, straw, faeces, and sewage, both

raw and treated.⁸ The level and impact of listerial carriage in the intestine and faeces of cattle and sheep as a contributor to the contamination of meat in Australia is not currently known.

Yersinia enterocolitica is recognised worldwide as a foodborne pathogen. From human disease studies, pig meat features more prominently than red meat as the likely source.^{14,15} Pigs, dogs and cats appear to be the main animal reservoirs for the strains of *Y. enterocolitica* associated with human disease. In contrast, the reported bovine and ovine isolation rates are lower.^{15,16}

Materials and methods

Property selection

Nineteen commercial cattle and sheep properties in New South Wales and Queensland were selected to cover all production systems producing red meat: six dairy cattle properties, four feedlot beef cattle properties, four pasture beef cattle properties, two prime-lamb properties and three mutton-sheep properties (Table). These properties were a subset of 215 properties in a larger research study, in which properties were selected with and without a history of *Salmonella* in the preceding two years. Of the 19 properties in this study, nine had a history of *Salmonella*. There was no selection in relation to a history of the three pathogens in this study.

Animal selection and sampling

Animal Care and Ethics approval was given in New South Wales and Queensland. From each property, 25 animals were selected at random from those meeting the following criteria: animals were to be within one month of the expected slaughter date or equivalent age; grazing animals were to be fresh off pasture and to be sampled within four hours of yarding and not yarded overnight; feedlot cattle were to have been on feed for a minimum of 60 days; dairy cattle were to be greater than four years old and in the last 100 days of a lactation cycle. Twenty-five animals were selected because this represented the available number of animals that were ready for slaughter from the properties and was considered a large enough sample to demonstrate potential significant differences. At least 2 g of faeces was collected per rectum

(using a new sterile glove for each animal), and placed in an individually numbered sterile specimen container. Specimens were transported chilled to arrive at the laboratory within 24 hours of collection.

Data collection

Statistical analysis was done using MS Excel 97. Questionnaires for each production system were prepared and analysed in Epi Info 6.04 (Centers for Disease Control and Prevention, Atlanta, Georgia, USA). They were designed to identify possible risk factors associated with the excretion of the bacterial pathogens and included approximately 100 questions under the following headings: property management; environment; management of sampled animals; access to manures; nutrition and feed; water; health status—animal and human. The questionnaire was completed by the producer and veterinarian.

Laboratory testing

Campylobacter culture

Faeces (1.0 g) were inoculated into 10 mL Preston Selective Enrichment Broth (Oxoid), incubated at 42°C for 48 hours in a microaerophilic atmosphere (CampyGen, Oxoid or a gas mixture consisting of 5% O₂, 10% CO₂, 85% N₂), then subcultured (10 µL) onto Preston *Campylobacter* Selective Agar (Oxoid) plates which were incubated as described above.

Identification of Campylobacter spp.

Up to three suspect colonies (based on characteristic morphological appearance) were subcultured. Isolates were considered to be *Campylobacter* spp. if they were oxidase positive, motile and Gram stained smears of suspect colonies revealed small tightly coiled spiral organisms. Isolates were identified as *C. jejuni* or *C. coli* as described by Barrow *et al.*¹⁷

Listeria culture

Faeces (0.1 g) were inoculated into 10 mL *Listeria* Primary Selective Enrichment Medium (UVM I, Oxoid), and incubated at 30°C for 24 hours, then 0.1 mL of the UVM I broth was inoculated into *Listeria* Secondary Selective Enrichment Medium (UVM II), incubated at 30°C for 24 hours. This was then subcultured (10 µL) onto an Oxford (Oxoid) plate and a Palcam (Oxoid) plate which were incubated aerobically at 37°C for 48 hours.

Identification of Listeria spp.

Up to five colonies were subcultured onto sheep blood agar. β-haemolytic colonies that were Gram positive short rods with rounded ends were further tested and identified as *L. monocytogenes* or *L. ivanovii* according to Barrow *et al.*¹⁷

Yersinia culture

Faeces (1.0 g) were added to 10 mL phosphate buffered saline (pH 7.2), incubated at 4°C for seven days, vortexed to allow large particles to settle, subcultured (10 µL) onto a Cefsulodin-Irgasan-Novobiocin (CIN) (Oxoid) plate and incubated at 32°C for 48 hours.

Identification of Yersinia spp.

Up to three typical colonies were subcultured onto sheep blood agar. Colonies were considered typical of *Yersinia enterocolitica* if they appeared as 'bull's eye' colonies with deep red centres surrounded by a transparent periphery on CIN agar. Isolates were identified as *Yersinia enterocolitica* according to Barrow *et al.*¹⁷

Results

The results and prevalence rates of *Campylobacter*, *Listeria* and *Yersinia* in each of the 19 herds and flocks sampled are summarised in the Table.

Table. Prevalence of *Campylobacter*, *Listeria*, and *Yersinia* in cattle and sheep from 19 herds or flocks, based on testing 25 faecal samples per property

Product type	Property number	Date	District	Approximate stocking rate (DSE/ hectare)	Number of head/m ²	Diarrhoea family/ workers	Campylobacter isolations (%)	Listeria isolations (%)	Yersinia isolations (%)
Dairy cattle	1	1/06/98	Northern NSW	24		Y	24	0	0
	2	26/08/98	Southern NSW	20		Y	24	0	0
	3	2/06/98	Northern NSW	23		N	8	0	0
	4	12/05/98	Central NSW	7		N	4	0	0
	5	26/05/98	Southern NSW	10		Y	4*	4 [†]	0
	6	13/05/98	Southern NSW	20		N	0	0	0
							Median 6 Mean 10.7		
Feedlot beef	1	5/08/98	Southern Qld		0.09	N	92	0	0
	2	16/07/98	Central NSW		0.09	N	76	0	0
	3	21/07/98	South-East Qld		0.10	N	40	0	0
	4	22/04/98	Southern NSW		0.06	N	12	0	0
						Median 58 Mean 55			

Table continued. Prevalence of *Campylobacter*, *Listeria*, and *Yersinia* in cattle and sheep from 19 herds or flocks, based on testing 25 faecal samples per property

Product type	Property number	Date	District	Approximate stocking rate (DSE/ hectare)	Number of head/m ²	Diarrhoea family/ workers	Campylobacter isolations (%)	Listeria isolations (%)	Yersinia isolations (%)
Pasture beef	1	28/10/98	Southern NSW	2		N	56	0	0
	2	4/08/98	Western NSW	1		N	4	0	0
	3	5/08/98	Western NSW	1		Y	0	0	0
	4	28/10/98	Southern NSW	3		N	0	0	0
							Median 2 Mean 15		
Mutton sheep	1	7/05/98	South-west NSW	1		N	4	0	0
	2	13/05/98	South-west NSW	1		N	0	0	0
	3	10/06/98	Central NSW	3		N	0	0	0
							Median 0 Mean 1.3		
Prime lambs	1	11/08/98	Southern NSW	3		N	8	0	0
	2	30/06/98	Central NSW	4		N	8	0	0
							Median 8 Mean 8		

DSE Dry sheep equivalent.

Y Yes.

N No.

* *Campylobacter coli*; all other *Campylobacter* isolates were *Campylobacter jejuni*.

† *Listeria ivanovii*.

Campylobacter

From the 19 herds or flocks, *Campylobacter* spp. were found in all production systems and 73.7 per cent (14/19) of all herds and flocks tested. *Campylobacter jejuni* was isolated from all production systems and one isolation of *C. coli* was made from one dairy cow. Within individual properties there was an apparent higher prevalence in cattle than in sheep, with *Campylobacter* being most commonly isolated from feedlot cattle. The median prevalences and ranges were: for dairy cattle, six per cent (0%–24%), feedlot beef cattle, 58 per cent (12%–92%) and pasture beef cattle, two per cent (0%–52%), mutton sheep, 0 per cent (0%–4%) and prime lambs eight per cent. No significant differences ($p < 0.05$) were detected between pairs of means when the means for all cattle properties and all sheep properties were compared or when different production systems were compared using a 2 tailed t-test for two samples assuming equal variances

For the four feedlots sampled, two factors, stocking density and weather conditions, were identified from the questionnaires as possible contributors to the number of animals shedding *Campylobacter*. Feedlot beef property 4, which had the lowest prevalence of *Campylobacter*, also had the lowest cattle density and was the only feedlot where dry weather conditions prevailed. Of the pastured animals: dairy cattle, pasture beef cattle, mutton sheep and prime lambs; the dairy cattle had the highest stocking rates and also the highest prevalence of *Campylobacter*. Of the four pasture beef properties sampled, property 1 had higher levels of *Campylobacter* (56% of samples positive) than the other pasture beef properties (0%–4% of samples positive). From the questionnaire, a possible risk factor was identified: because of drought conditions, the cattle had been grazed near the house septic tank absorption trench. No other property reported that sampled animals had grazed near an absorption trench. Because of the limited nature of this survey, results from the questionnaires were not proven to be statistically significant associations, but have been reported as possible associations.

Listeria

L. monocytogenes was not isolated from any animal. One dairy cow (dairy cattle property 5) was positive for *L. ivanovii*. Of the 19 properties, two feedlots and three dairies included silage in the ration, but dairy cattle property 5 did not.

Yersinia

No *Yersinia* associated with human disease (*Y. enterocolitica*) was isolated from any of the properties.

Three of the six dairies and one of the four pasture beef properties reported diarrhoea in the family or workers at the property in the two months prior to collecting the cattle faecal samples. No causative agent was identified for any of these human cases. The three dairies, but not the pasture beef property were detected to have animals shedding *Campylobacter*, but there was no statistically significant association with the human illness reported.

Discussion

C. jejuni and *C. coli*, *L. monocytogenes* (to a lesser extent *L. ivanovii*) and *Y. enterocolitica* are bacterial pathogens that cause food poisoning in humans. In our study, *C. jejuni* was commonly isolated and there was a higher prevalence in cattle than in sheep. This study demonstrated a difference between cattle from different production systems, with feedlot cattle having a higher prevalence than either dairy cattle or pasture beef cattle. In contrast, *L. ivanovii* was isolated from only one bovine; *L. monocytogenes* and *Y. enterocolitica* were not isolated at all.

Studies from other countries report a wide variation of *campylobacter* carriage rate in domestic food-producing animals. This may reflect the different geographic/climatic conditions, and management practices.² New Zealand abattoir studies in both dairy cattle and sheep demonstrated higher prevalence rates than our study: New Zealand dairy cattle had isolation rates for *C. jejuni* or *C. coli* from rectal swabs of 24 per cent, 31 per cent and 12 per cent during summer, autumn and winter respectively,¹⁸ and New Zealand sheep had prevalence rates of 2.4 per cent for lambs and 14 per cent for adult sheep.¹⁹ In the study of New Zealand dairy cattle, approximately half of the isolates were *C. jejuni* and the other half, *C. coli*. Interestingly, we only isolated *C. coli* from one animal in our study. An abattoir study in Australia by Grau²⁰ found *C. jejuni* in 54 per cent of calf faecal samples and 12.5 per cent of cow faecal samples and also observed that lot-fed cattle were more likely to have *C. jejuni* in their intestinal tracts and on their carcasses than were pasture-fed cattle. The true proportion of animals carrying *Campylobacter* can only be

ascertained by field studies, as abattoir surveys may give false (higher) figures due to transport associated stress, cross-infection during transport and mixing of animals before slaughter.

The higher stocking rate in dairies, compared with other grazing cattle and sheep, was identified as a possible risk factor for *Campylobacter* prevalence. High stocking density and wet weather were identified as possible contributors to the number of feedlot animals shedding *Campylobacter*. Both these factors would increase the level of moisture in the pen and encourage the survival of the organisms. In addition to these possible contributing factors, feedlot rations are high in carbohydrate compared with pasture and therefore may provide a more suitable environment in the gastrointestinal tract for *Campylobacter* to survive and proliferate. Three pasture beef properties had no or low levels of *Campylobacter*, but one property had a prevalence of 56 per cent. From the questionnaire a possible risk factor was identified for this property: the cattle grazed near the house septic tank-absorption trench.

Campylobacter prevalence has been reported to be seasonal, with both humans and animals^{3,18} having higher levels during the warmer months. Our study was conducted in the cooler months of the year (between May and October), so we could anticipate higher levels during warmer months. Nielsen *et al*²¹ demonstrated an overlap between serotypes of *C. jejuni* found in humans, poultry, and cattle, indicating that poultry and cattle should be considered in the transmission via food to humans. This 'snapshot' study was too small to demonstrate statistically significant differences ($p < 0.05$) between production systems but the trends observed indicate that cattle, and in particular feedlot cattle, must be considered a potential source of *Campylobacter* for humans. The animals in this study were healthy animals still on-farm and ready for slaughter, and as such would be carrying *C. jejuni* into the abattoir environment.

It is interesting to note that, from the results of the questionnaire, diarrhoea in humans was recorded from three of the six dairy properties, one of three pasture beef properties, but not from the other production systems. The causative agents for the diarrhoea in humans were unknown and there was no statistically

significant association between diarrhoea in humans and any of the pathogens isolated from animals on the same property. Nonetheless, it was found that the three dairy properties with cases of human diarrhoea also had animals shedding *Campylobacter*. This organism is the most common bacterial cause of diarrhoea in humans and Thompson²² found a strong association between human campylobacteriosis and living on a farm. Our report of diarrhoea in dairy workers warrants further investigation. The management of dairy cattle exposes the workers to cattle faeces much more commonly than in any other production system.

L. monocytogenes was not isolated from any property in this study. *L. ivanovii* was cultured from one dairy cow but was not isolated from any sheep.²³ The dairy cow was on pasture and fed supplementary pellets but no silage. It had been stressed by drought followed by recent rain. In Australian sheep, outbreaks of clinical listeriosis have been reported following both floods²⁴ and drought.²⁵ *L. ivanovii* is a rare human pathogen²⁶ and is considered to be less virulent than *L. monocytogenes*.²⁷ Mutton is considered the most common food associated with the presence of *L. ivanovii*.²⁸

Studies in other countries have found relatively high levels of *L. monocytogenes* in cattle and sheep. These levels may be a reflection of more intensive production systems, housing inside in winter and supplementary feeding. Housing animals is not a common practice in Australia. High prevalence rates in cattle faeces have been reported in Yugoslavia 19 per cent,^{29,30} Germany 33 per cent,³¹ Denmark 51 per cent,³² Canada 14.5 per cent,³³ Scandinavia 3.1 per cent (spring to autumn on pasture) to 9.2 per cent (winter indoors).³⁴ One study in Hungary,³⁵ showed 90 per cent of 50 healthy sheep investigated during summer were excreting *Listeria* in their faeces, nasal mucus, vaginal mucus or milk. For the properties in our study, cattle and sheep production systems in Australia did not favour the carriage of *Listeria*, although silage was used in half the feedlots and dairies.

No *Yersinia* associated with human foodborne disease (*Y. enterocolitica*) was isolated from any of the properties. This result is in keeping with other studies that indicate that cattle and sheep are an unlikely source for human infection.^{15,16}

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