Clostridium difficile from Australian cattle: all will be re(veal)ed!

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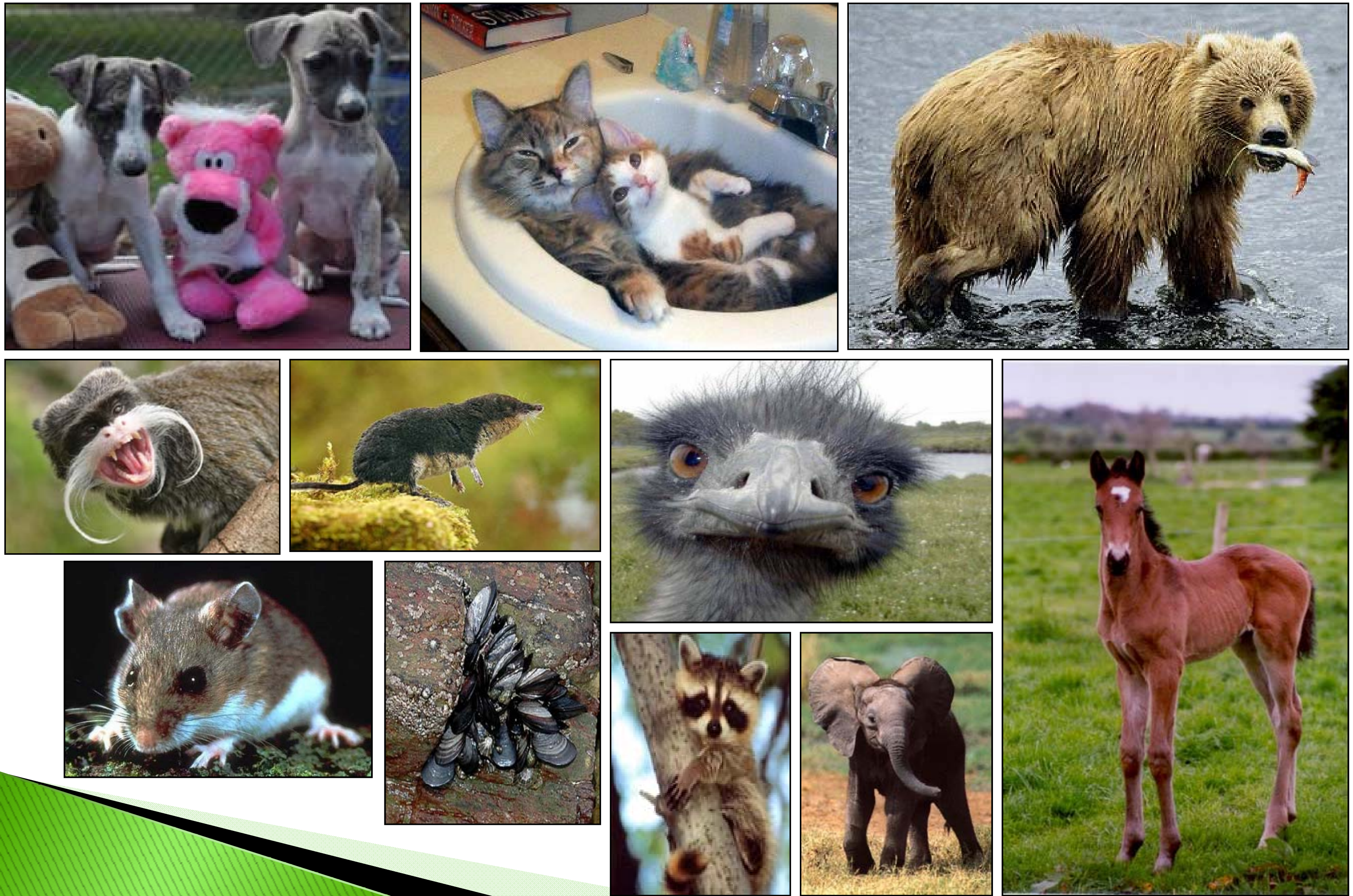
C. difficile in animals



C. difficile in swine

- Pre-weaning scour in neonatal piglets.
- Prevalence is typically highest in young piglets less than 10 days old and ranges from 25.9% to 100% , and declines with age.
- Predominant PCR ribotype in N. America and Europe is 078, toxinotype V (not found in AU).
- Recently reported genetic overlap between isolates from animals and human isolates suggests that animals may be a reservoir and *C. difficile* infection a potential zoonosis.

References

Debast *et al.*, 2009; Alvarez-Perez *et al.*, 2009; Hopman *et al.*, 2011; Songer and Anderson, 2006; Thakur *et al.*, 2010

C. difficile in poultry

- Link between colonization and disease not well understood.
- Prevalence reported: 1% – 60% and decreases with age and driven by use of cephalosporin antibiotics e.g. ceftiofur.
- Unlike the situation in pigs, there is a high diversity of PCR ribotypes, but no 078 or 027 reported so far.
- An initial small-scale look at chooks in Australia was negative.

References
Zidaric *et al.*, 2008; Harvey *et al.*, 2011

C. difficile in cattle

- Linked with calf enteritis and Neonatal Calf Diarrhea (NCD).
- Wide variations in prevalence reported: 0.5% - 50%.
- Age and seasonality effects.
- PCR ribotype 078 is prevalent in veal calves in Canada (67%) and the United States (94%).
- Other ribotypes known to cause disease in humans have been found; 012, 014, 017 and 027.

References
Avbersek *et al.*, 2011; Hoffer *et al.*, 2010; Houser *et al.*, 2012; Costa *et al.*, 2011; Rodriguez-Palacios *et al.*, 2006; Hammitt *et al.*, 2008

C. difficile in retail meat

- Europe – low levels, ~3%
- N. America – up to 42%
- Found in products of bovine origin
  - 11 (20.8%) of 53 beef samples
  - 1 (14.3%) of 7 ground veal samples
  - 3 (4.6%) of 65 veal chops
- Toxigenic strains 027 and 078

- Although *C. difficile* is present in food for human consumption, and overlapping of PCR ribotypes from animal and human have been found (by MLVA), food-borne infection caused by *C. difficile* has never been confirmed.

References

Rodriguez-Palacios et al., 2006, 2007 and 2009; Songer *et al.*, 2009

Objectives

- To undertake a survey of Australian cattle and calves at slaughter for the presence of *C. difficile*, and determine its prevalence.

- Characterize isolates to see if there is any relationship with humans isolates in Australia.

- Assess any risk of food-borne transmission of *C. difficile* from contamination.

Study design

- A total of 648 samples were collected and analysed

- Nov 2007 to Jan 2008
  - One abattoir in WA
  - 158 x adult cattle gut contents
  - 151 x adult cattle carcass washings

- Oct 2008 to May 2009
  - 25 abattoirs in NSW (5), VIC (4), SA (2) and QLD (11)
  - 225 samples of adult cattle faeces

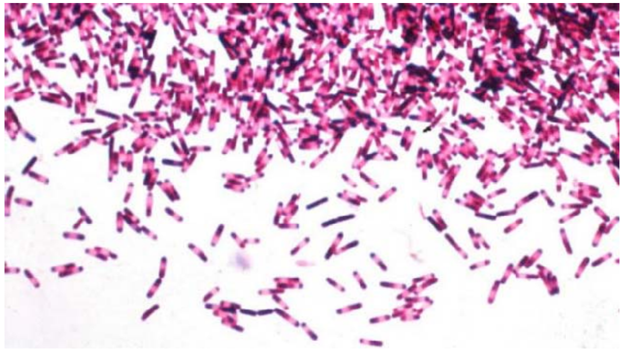
Study design

- **March and April 2012**
  - Two abattoirs in QLD (1) and VIC (1) comprising 17 'lots' or veal farms
  - 88 x 'bobby' calves aged <7 days
  - 5 x calves aged 2 months
  - 4 x calves aged 4 months
  - 17 x calves aged 6 months

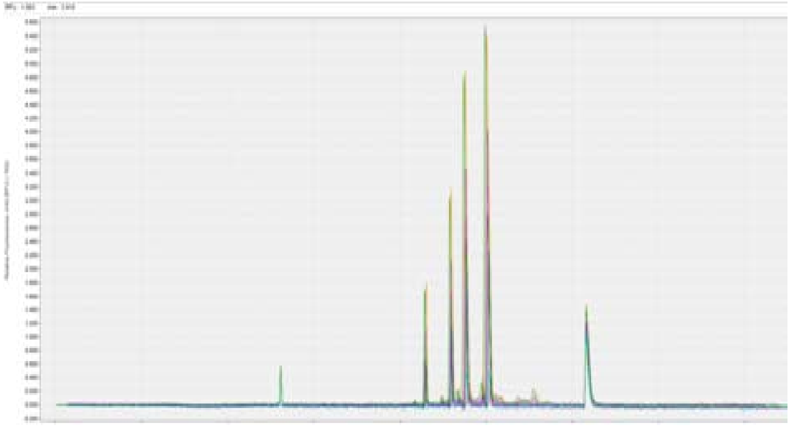
Geographically distinct?

- Slovenia 20,273 km2
- Australia 7,617,930 km2
- Distance between abattoirs 1,702.9 km

Methodology



- Direct and enrichment culture
  - CCFA direct and GCC broth/ ethanol shock
  - Bowman *et al*. 1988, Carroll *et al.* 1983
- Toxin A (*tcdA*) & toxin B (*tcdB*) and rep region of *tcdA*
  - Kato *et al*. 1991, Kato *et al*. 1998
- Binary toxin (*cdtA and cdtB*)
  - Stubbs *et al.* 2000
- Confirmation of PaLoc negative strains
  - Braun *et al*. 1996
- PCR Ribotyping
  - Capillary electrophoresis
  - Bionumerics band pattern analysis
  - Stubbs *et al*. 1999



Results – recovery of *C. difficile*

Source	State	Abattoir code	n	Age	Isolation of *CD (n/%)*
Gastrointestinal contents	Western Australia	W1	158	Adult	0 (0.0)
Carcass washings	Western Australia	W1	151	Adult	0 (0.0
Faeces	New South Wales	N1-N5	80	Adult	2 (2.5)
Faeces	Queensland	Q1-Q11	90	Adult	2 (2.2)
Faeces	South Australia	S1-S2	15	Adult	0 (0.0)
Faeces	Victoria	V1-V4	30	Adult	1 (3.3)
Faeces	Western Australia	W2-W3	10	Adult	0 (0.0)
Faeces	Victoria	V5	50	Calf (<7 days)	36 (72.0)
Faeces	Queensland	Q12	38	Calf (<7 days)	27 (71.1)
Faeces	Queensland	Q12	5	Calf (2 months)	1 (20.0)
Faeces	Queensland	Q12	4	Calf (4 months)	0 (0.0)
Faeces	Queensland	Q12	17	Calf (6 months)	0 (0.0)
Total			**648**		**69 (10.6)**

Age Group	A+B+CDT-	A+B+CDT+	A-B+CDT+	A-B-CDT-	A-B-CDT+
<7 days	2	51	1	-	9
2 months	-	1	-	-	-
4 months	-	-	-	-	-
6 months	-	-	-	-	-
Adult	1	-	1	3	-
Grand Total	**3**	**52**	**2**	**3**	**9**

Overall prevalence:
adults – 2.4% calves <7 days) – 72.0%*

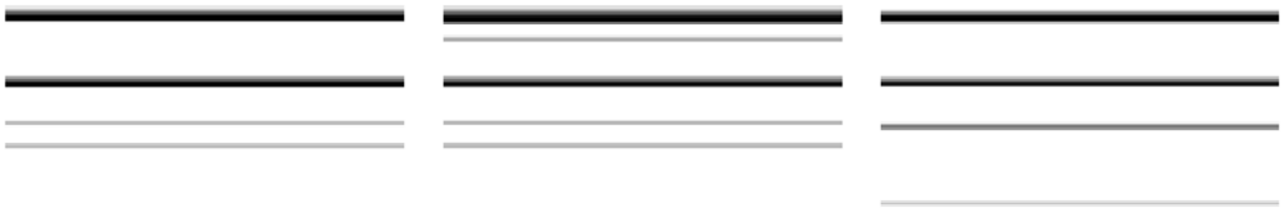
*12% by direct culture

Results – PCR Ribotyping

- From the 69 isolates,11 different PCR ribotype patterns were observed
- In calves, three predominant PCR ribotype patterns identified

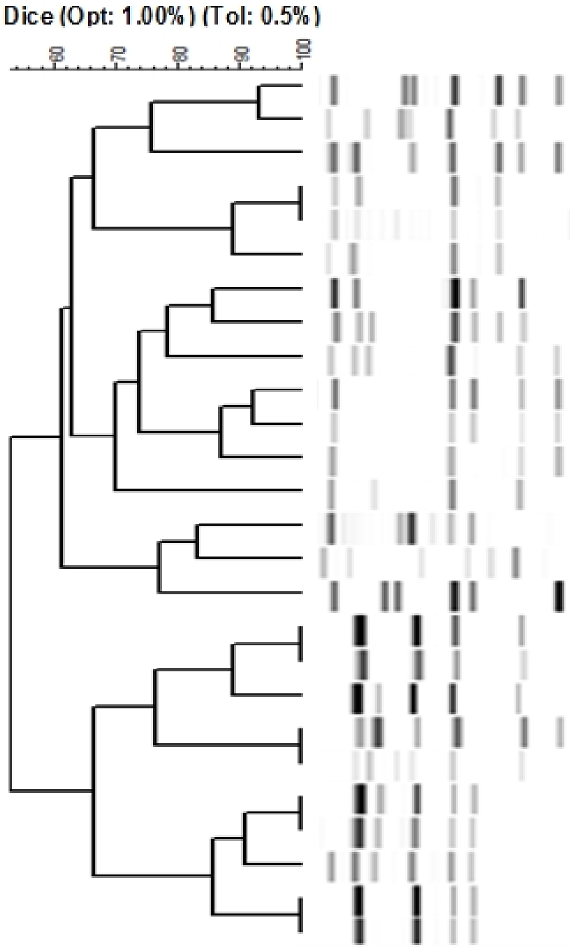
	A	B	C
n (%)	41 (59.4)	11 (15.9)	9 (13.0)
Location (n)	VIC (n=31), QLD (n=10)	QLD	VIC (n=3), QLD (n=6)
Toxin profile	A+B+CDT+	A+B+CDT+	A-B-CDT+
Source	40 x <7 days, 1 x 2mo	Calf <7 days	Calf <7 days

PCR Ribotype pattern



- CDT+ strains are associated with increased severity of disease in humans and are often found in animals. Avbersek *et al*., 2009; Barbut *et al*., 2005

Results – PCR ribotyping



PCR Ribotype	Toxin profile: *tcdA*	Toxin profile: *tcdB*	Toxin profile: *cdtA/cdtB*	n (%)	Age	Specimen type	Abattoir (n)	
AU258	+	+	-	1 (1.4)	Adult cow	Faeces	Q5†	
AU259	-	-	-	1 (1.4)	Adult cow	Faeces	V4‡	
UK103*	+	+	-					
UK002*	+	+	-					
UK010*	-	-	-					
AU255	-	-	-	1 (1.4)	Adult cow	Faeces	N1§	
AU261	+	+	-	1 (1.4)	Veal calf	Faeces	Q12†	
UK005*	+	+	-					
UK244*	+	+	+					
UK014*	+	+	-					
UK020*	+	+	-					
UK001*	+	+	-					
AU256	-	-	-	1 (1.4)	Adult cow	Faeces	Q4†	
UK070*	+	+	-					
AU257	-	+	+	1 (1.4)	Adult cow	Faeces	N2§	
AU260	+	+	-	1 (1.4)	Veal calf	Faeces	V5‡	
UK033	-	-	+	9 (13.0)	Veal calf	Faeces	Q12† (6) / V5‡ (3)	C
UK033*	-	-	+					
AU262	-	+	+	1 (1.4)	Veal calf	Faeces	V5†	
UK027*	+	+	+					
UK054*	+	+	-					
UK126	+	+	+	11 (15.9)	Veal calf	Faeces	Q12‡	B
UK126*	+	+	+					
UK078*	+	+	+					
UK127	+	+	+	41 (59.4)	Veal calf	Faeces	Q12† (10¶) / V5‡ (31)	A
UK127*	+	+	+					

*Reference strain, Abattoir(s) in Queensland (†),Victoria (‡), New South Wales (§). ¶one isolate from 2 month old calf

Discussion

Genotypic characterisation of *C. difficile* from Australian calves at slaughter

PCR Ribotypes

- 126 ($A^+B^+CDT^+$)
- 127 ($A^+B^+CDT^+$)
- 033 ($A^-B^-CDT^+$)

Sequence type 11 (by MLST) Which falls into clade 5, the same clade as the epidemic ribotype 078

Stabler *et al.*, 2012

PCR ribotype 078 ($A^+B^+CDT^+$)

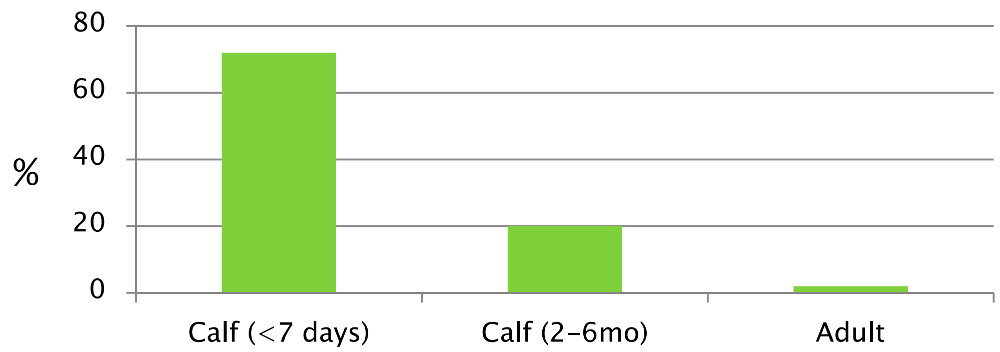
- The most common animal ribotype worldwide Rodriguez-Palacios *et al.,* 2006
- Prevalent in veal calves in: Canada, 67% (102/152) Costa *et al.*, 2011
  USA, 94% (31/33) Keel *et al.*, 2007
- Similar hypervirulent attributes to the RT 027. Rupnik *et al.*, 2008; Avbersek *et al.*, 2009
- 3rd most common human isolate in European hospitals and is increasingly associated with community-acquired CDI (CA-CDI). Jhung *et al.*, 2008; Bauer *et al.*, 2011; Weese *et al.*, 2010; Kuntz *et al.*, 2011; Riley *et al.,* 2009

Strains belonging to ribotypes 033, 126 and 127 have all been isolated from humans with disease in Australia in the last decade. Riley *et al*, unpublished.

Discussion

C. difficile prevalence in Australian calves declines with age

- Significant declines in *C. difficile* prevalence was observed with increasing age



- Costa *et al*., 2011 – noted high prevalence (32% to 51%) at 1 week after arrival at a veal farm followed by a decline to 2% at 21 weeks after arrival.
- Age related effect also seen with pigs Hopman *et al*., 2011
  - Neonatal animals have underdeveloped intestinal micro flora
  - *C. difficile* is better able to colonize, proliferate and produce toxins

Rodriguez-Palacios *et al*., 2006

Discussion

C. difficile prevalence in Australian cattle at slaughter

- Adults: ~2% – low level, consistent with other studies
- Calves: ~72% – higher prevalence than reported in any other study

Country	Prevalence	Reference
Canada	11.2% (31/278)	Rodriguez-Palacios *et al*., 2006
Canada	32% - 51%	Costa *et al*., 2011
USA	9% (18/200)	Houser *et al*., 2012
Slovenia	9% (4/42)	Avbersek *et al*., 2009
Switzerland	0.5% (1/204)	Hoffer *et al*., 2010

Discussion

Can the contrasting levels of *C. difficile* be explained by differences in slaughter practices between countries?

Juvenile or 'Stirk' calves

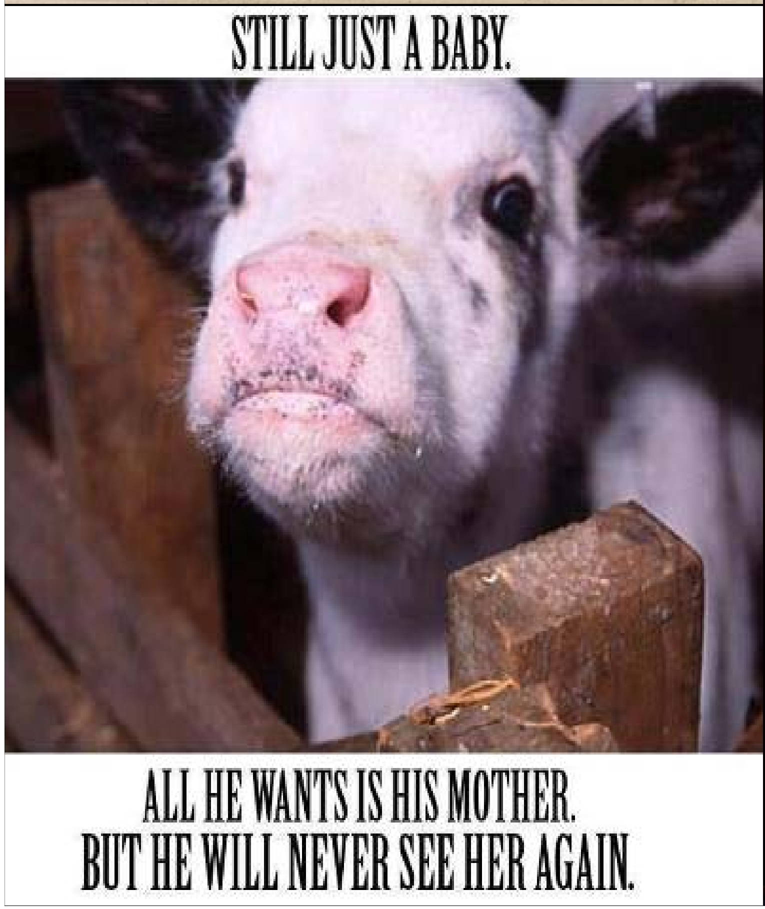
- 4-5 months/ weighs about 300 lb (135 kg)
- Sold as veal to general consumer market
- Lower prevalence of *C. difficile*
- Usually grain or milk-fed

Baby 'Bobby' calves

- Up to a month old (often under 10 days)
- Carcass weighs about 60 lb
- Not routinely sold via retail chains, meat is more delicate and white with little fat
- Much higher prevalence of *C. difficile*
- Only practiced by a small group of countries Great Britain, New Zealand and Australia

Why so controversial?


LIFESPAN: 14 DAYS
LIFESPAN: 5 DAYS
Every year 1 million five-day old calves are slaughtered to supply the veal market. Please reconsider your oder of veal products. www.animal-lib.org.au
ANIMAL LIBERATION


STILL JUST A BABY.
ALL HE WANTS IS HIS MOTHER.
BUT HE WILL NEVER SEE HER AGAIN.


THE DARK TRUTH ABOUT DAIRY
He's all alone. He just wants his mother.
But he will never see her again.
EVOLVE! Campaigns
This innocent baby is a dairy industry reject.
~ GO VEGAN ~
Take a stand. Boycott cruelty. Make a difference.

Discussion

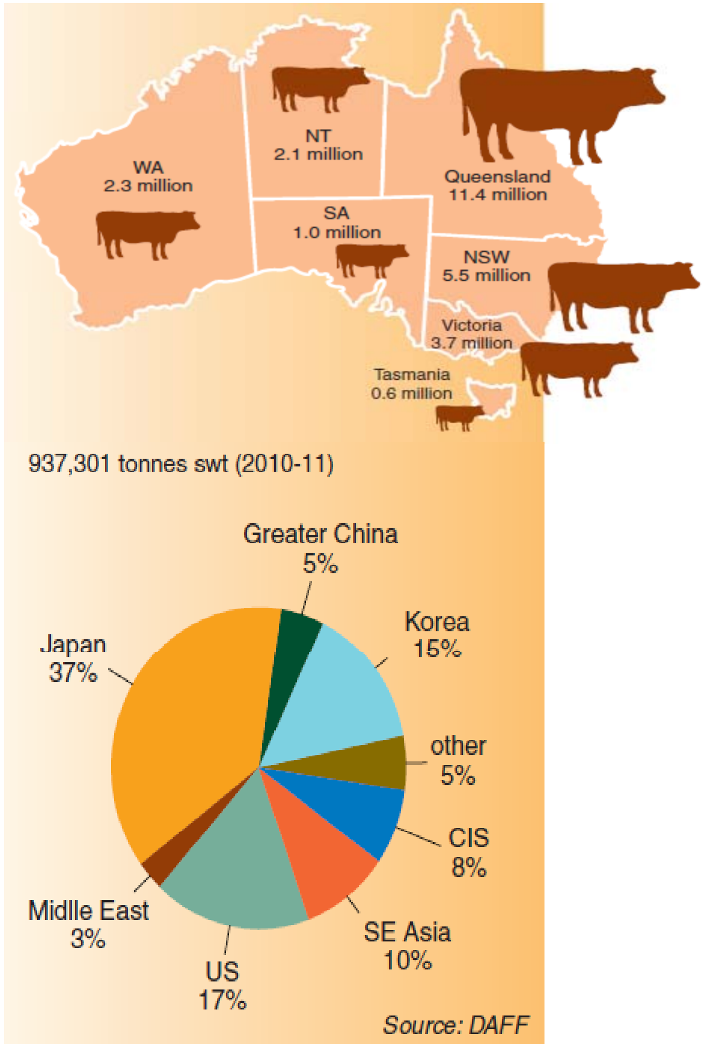
In 2012 700,000 Australian calves will be slaughtered, rising to just under 1 million in 2016.

Domestic consumption?

- Status unsure, however, clearly a potential threat to Australian consumers
- Specialty butchers and smallgoods manufacturers

Export?

- Status – high risk to consumers:
- In 2010-11 Australia exported 65% of its total beef and veal production to more than 100 countries.
    - The value of total beef and veal exports in 2010-11 was $4.5 billion

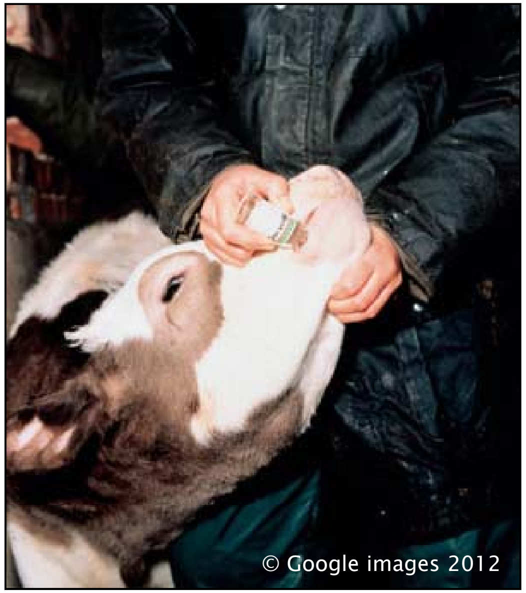


References
All figures based on MLA/DAFF/ABS/ABARE 2010-11

Discussion

What's driving *C. difficile* in veal calves?

- Shift in antibiotic prescribing practices by Australian veterinarians in recent years (Personal communication)



- Availability of once-daily antimicrobial agents like ceftiofur could be driving amplification of *C. difficile* in production animal populations leading to an outbreak of community-acquired CDI in Australia

smh.com.au
The Sydney Morning Herald

Antibiotic banned in US still used by Australian vets

Limitations

- Lacking in demographic and pathological information regarding calf movement and health prior to slaughter
- Use of more discriminatory typing method e.g. MLVA or WGS
- Future studies of a similar nature should include sampling of the abattoir environment
- Bacterial enumeration

In Progress

- Large scale surveillance of *C. difficile* in Australian calves
  - >500 calves
  - multiple abattoirs
  - Carcass washings and faeces
  - Seasonality effect, should we expect to see higher rates in winter months?

- Whole Genome Sequencing of animal/human ribotype matches

- Retail meat surveillance
  - To date no *C. difficile* has been identified in retail meats in Australia.
    Foster *et al*, unpublished data

- *C. difficile* in Australian sheep and lambs

Conclusions – *C. difficile* in Australian veal calves

- Potentially a major reservoir of *C. difficile* known to cause disease in humans.
- Clear potential for toxigenic *C. difficile* to be contaminate retail veal for human consumption both domestically and exported.
- The age related affect is a unique risk factor in food-borne transmission.
- Injudicious use of cephalosporin antibiotics could be driving this amplification.
- Farms, abattoirs and veal products appear well placed to become niches for CA-CDI.

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