



Government  
of South Australia

SARDI



SOUTH AUSTRALIAN  
RESEARCH AND  
DEVELOPMENT  
INSTITUTE



# National Microbiological Database (NMD) Reporting System

**Michelle Lorimer**

# Background

- National Microbiological Database (NMD), also known as the ESAM program, established to help Australia meet market access requirements to the US.
- As export slaughter establishments – you collect and analyse carcass samples from all slaughter species for *E. coli* and *Salmonella*.
- AQIS then enter this data into the National Database,
  - can benchmark Australia's performance.
- Along with baseline data – very useful in market access negotiations.
- Australian results are very good, but there is still plant to plant variability.

## Background (cont.)

- Currently, no establishments are provided with regular feedback on their performance.
- A regular reporting system may assist you in:
  - Obtaining new export markets.
  - Demonstrate how “clean” your product is or has been over a long period of time.
  - Compare your establishment with the national average.
  - Improve data quality as anomalies will be identified

# Scope

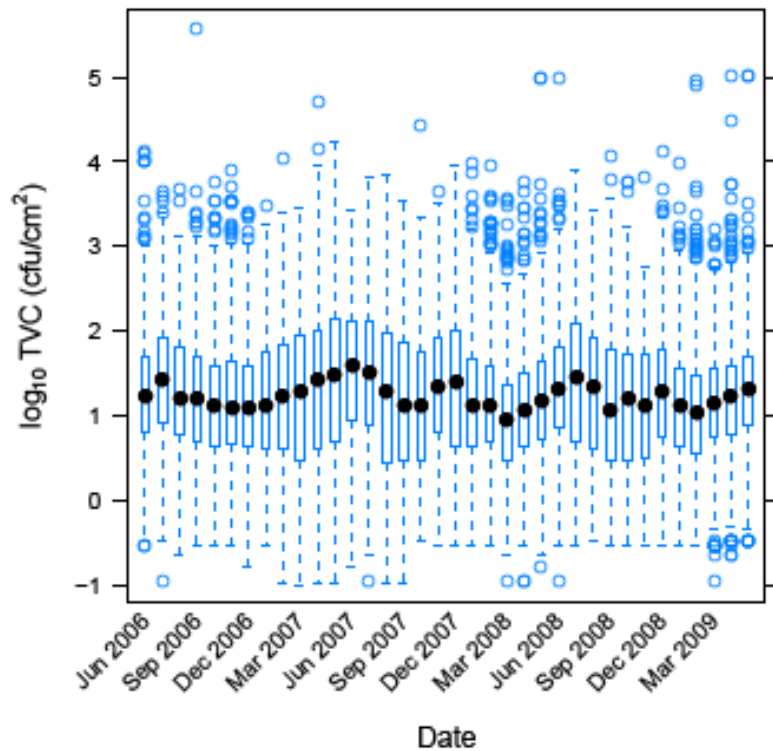
- Our role is to develop and provide an on-going reporting capability for you
  - Analysis of data from NMD,
  - Report regularly (monthly) to each Establishment so that you can compare your results with National benchmarks.
- We intend to use a moving 3-year window of data to generate the reports.
- Each species is considered separately.

By the end of this session you will...

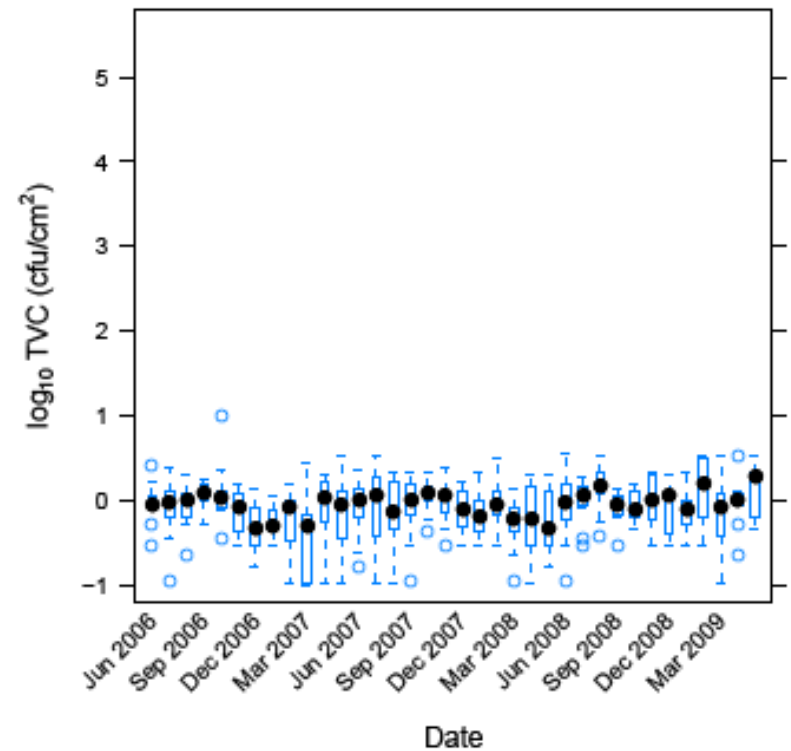
- Be able to interpret the tables and graphs in your report.
- Have a good understanding of the statistical terms used.
- Be able to assess where your plant is in terms of national benchmarks.

# Example – TVC ( $\log_{10}$ cfu/cm<sup>2</sup>)

National

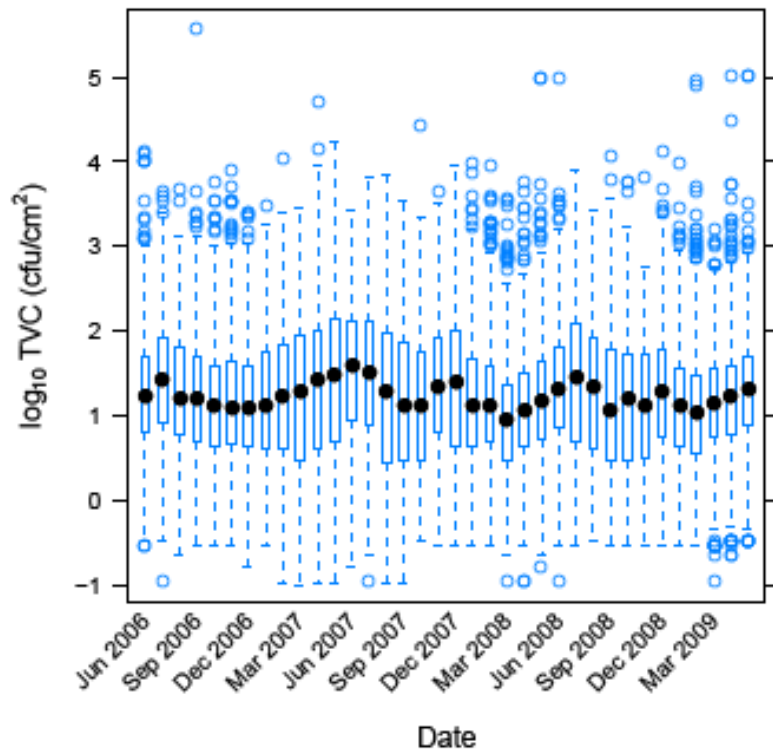


Establishment A

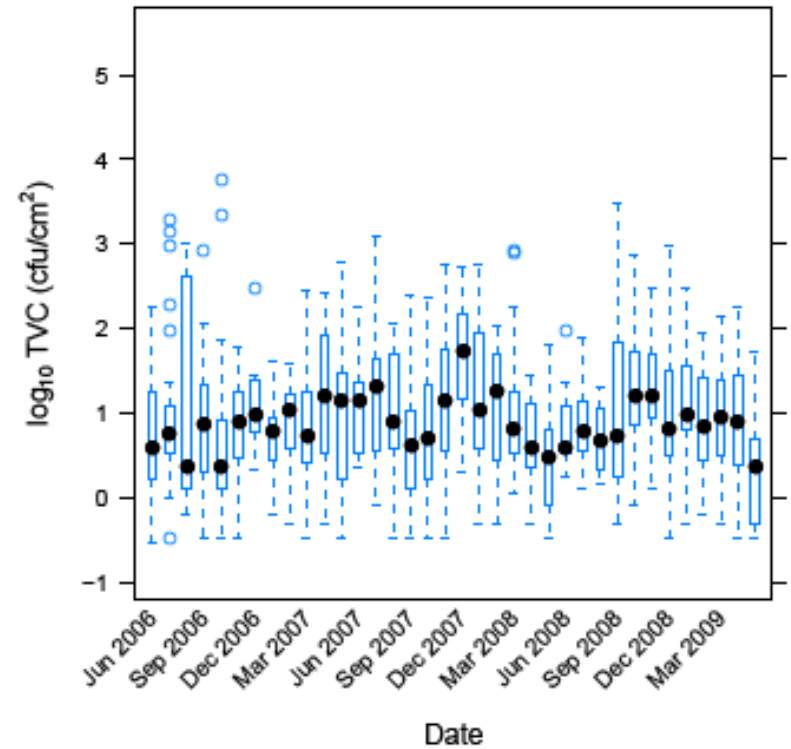


# Example – TVC ( $\log_{10}$ cfu/cm<sup>2</sup>)

## National

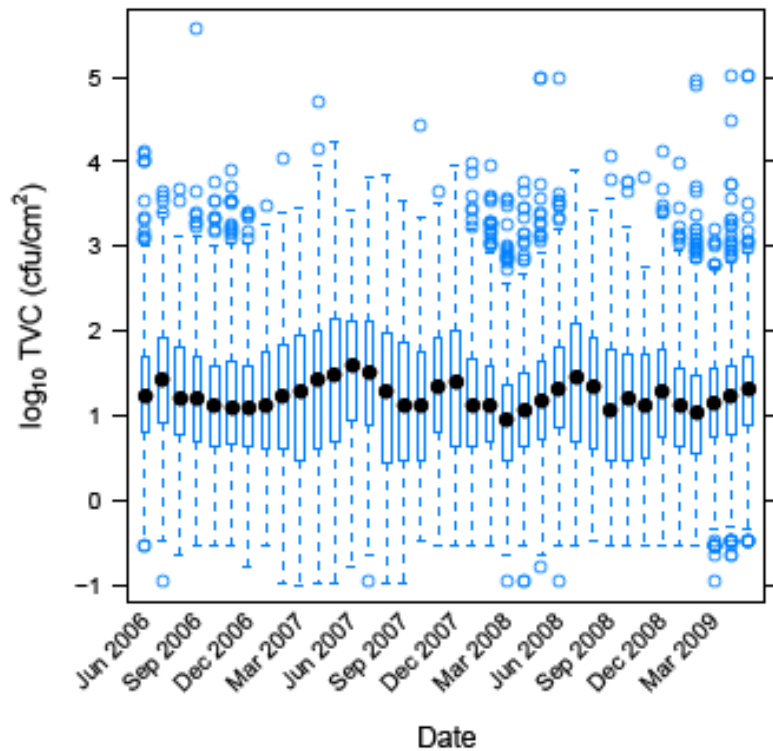


## Establishment B

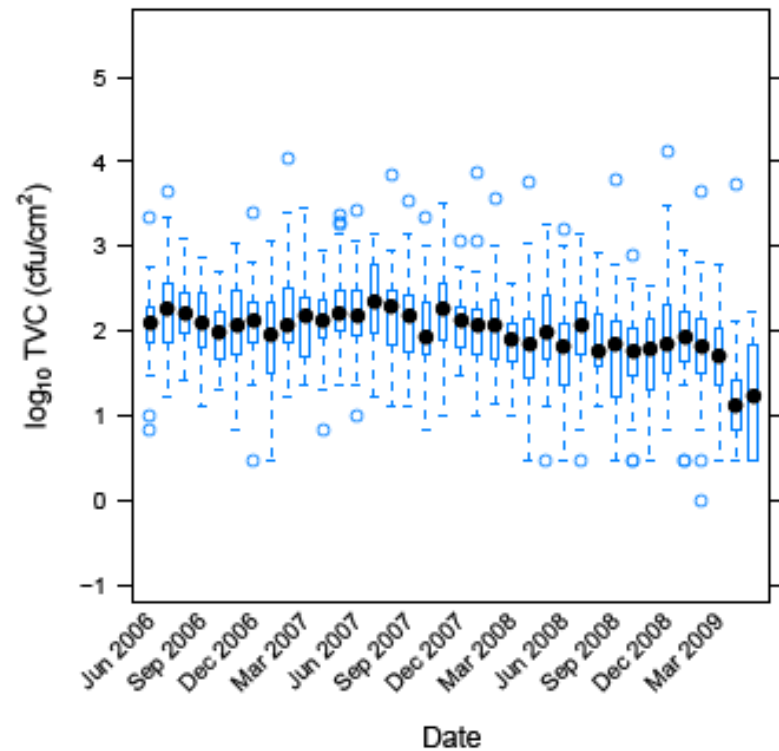


# Example – TVC ( $\log_{10}$ cfu/cm<sup>2</sup>)

National



Establishment C



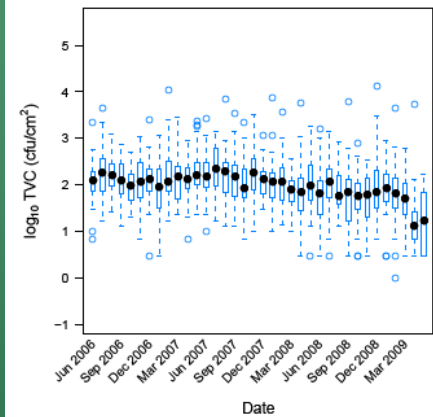
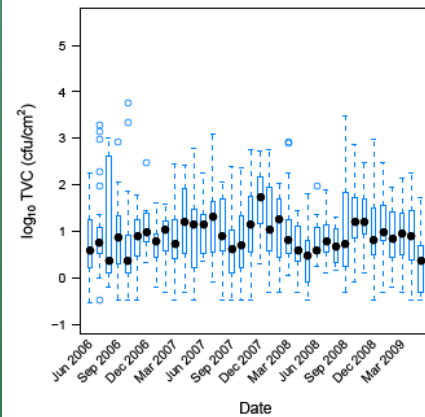
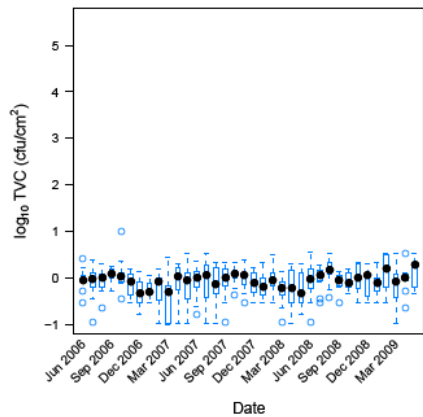
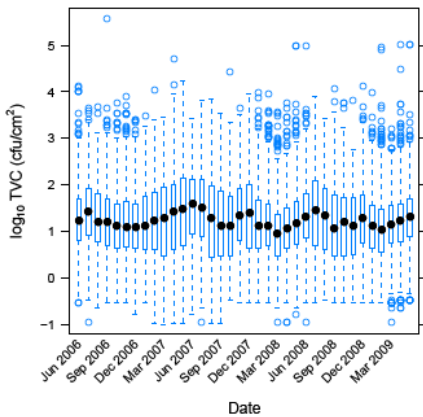
# Example – TVC ( $\log_{10}$ cfu/cm<sup>2</sup>)

National

Establishment A

Establishment B

Establishment C



N (+ve) = 19,246

N (+ve) = 473

N (+ve) = 719

N (+ve) = 1444

Mean (+ve) = 1.21

Mean (+ve) = -0.07

Mean (+ve) = 0.94

Mean (+ve) = 1.98

SD = 0.77

SD = 0.33

SD = 0.77

SD = 0.58

# The database

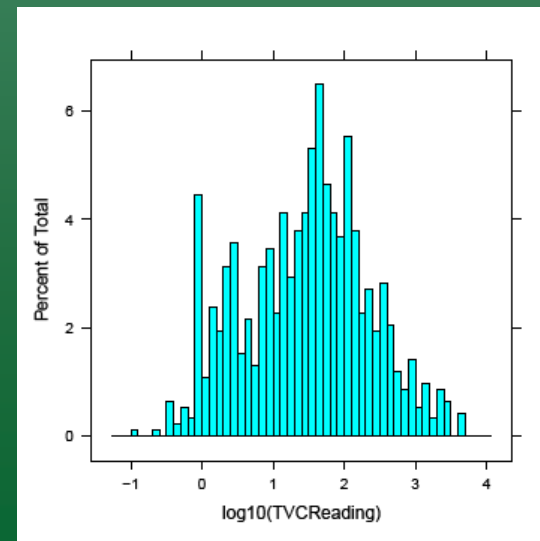
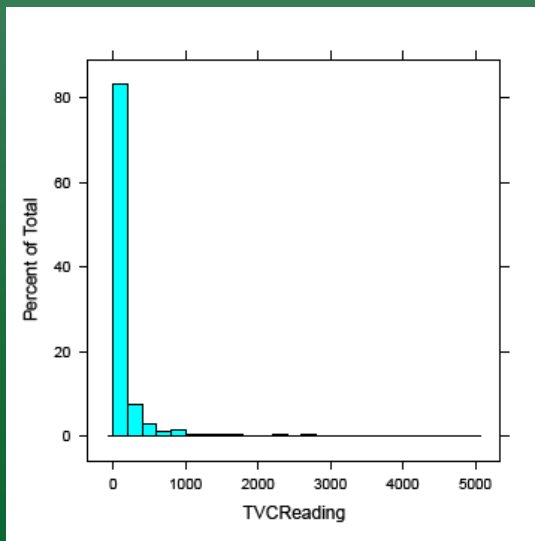
- Establishment ID
- Species
- Date (of test)
- TVC Reading (cfu/cm<sup>2</sup>)
- *E. coli* Reading (cfu/cm<sup>2</sup>)
- *Salmonella* Result (Pass or Fail)
- *Salmonella* Serotype
- Dressing (Bed, Conventional, Gravity Rail, Inverted)
- Chain Number (1 to 7)
- Shift (First, Second, Third)
- Swabbed (Hot, Cold)
- TVC Regulatory (& Voluntary)
- *E. coli* Regulatory (& Voluntary)

# The Reports

- Separate reports are generated for each species at each establishment.
- Species: Calf, Cow/Bull, Steer/Heifer, Sheep, Lambs, Goat Skin on, Goat Skin off
- Includes summary tables and graphs:
  - For the establishment
  - Nationally.
- In the tables, the data are summarised over the whole sampling period – 3 years.
- In the box plots, the data are summarised monthly over the sampling period.
- In the time plot, individual observations are considered.

# Transformation

- All positive concentration data are converted into logarithms with base 10, given by  $\log_{10}$  cfu/cm<sup>2</sup>.
- Standard micro practice – lots of research reports and published papers use it.
- Distribution of concentrations is made more symmetrical.



# Transformations (cont.)

Conc		$\log_{10}$
0.1	$1 \times 10^{-1}$	-1
100	$1 \times 10^2$	2
1,000	$1 \times 10^3$	3
2,000	$2 \times 10^3$	3.3
10,000	$1 \times 10^4$	4
20,000	$2 \times 10^4$	4.3
35,000	$3.5 \times 10^4$	4.5
100,000	$1 \times 10^5$	5
200,000	$2 \times 10^5$	5.3

- A 1 log reduction means a 90% reduction in concentrations on the original scale.
- A 2 log reduction means a 99% reduction in concentrations on the original scale.
- A 3 log reduction means a 99.9% reduction in concentrations on the original scale.

# Prevalence Summaries

- Table 1.
- Tests:
  - The total number of samples in the NMD during the reporting period (for this establishment and species).
- Positives:
  - The number of samples with positive concentrations/test.
- Percent + ve:
  - $\text{Positives/Tests} * 100$

# Prevalence Summaries (cont.)

## Lower Bound and Upper Bound

- Describe the bounds of a 95% Confidence Interval.
- The range or the ‘ballpark’ of where the “true” prevalence may be.
- The “true” prevalence is what you would get if you tested all carcasses.

## TVC – Prevalence Summary

	Plant	National
# of Tests	239	1069
Positives	163	785
Percent + ve	68.20	73.43
Lower Bound	61.89	70.68
Upper Bound	74.06	76.06

- Table 1.
- At this Plant, a lower proportion of samples with positive TVC were found when compared to the National prevalence.

Table 3: *E. coli* Prevalence Summary

	Plant	National
Tests	891	16142
Positives	268	1135
Percent +ve	30.08	7.03
Lower Bound	27.08	6.64
Upper Bound	33.21	7.44

- Table 3.
- At this plant the prevalence of *E. coli* is much higher than that found nationally.
- This Plant accounts for 24% of all *E. coli* positive samples.

Table 5: *Salmonella* Prevalence Summary

	Plant	National
Tests	980	5370
Positives	2	39
Percent +ve	0.204	0.726
Lower Bound	0.025	0.517
Upper Bound	0.735	0.991

- Table 5.
- Plant prevalence (%) is lower than National prevalence.
- The width of the lower and upper bounds is wider for plants than national – indicative of the difference in sample size (number of tests).

## Table 6: *Salmonella* serotypes

Test Date	Serotype
2007-09-13	S. typhimurium
2007-11-28	

## TVC and *E. coli* concentration (positive samples only) summaries

- Table 2.
- Sort/order values from smallest to largest.
  - Minimum concentration.
  - 1<sup>st</sup> Quartile (Q1):
    - 25% of the data are less than this value and 75% are greater.
  - Median:
    - The midpoint of the data.
    - 50% of data are less than this concentration and 50% are greater.
  - 3<sup>rd</sup> Quartile (Q3):
    - 75% of the data are less than this value and 25% are greater.
  - Maximum concentration.

## TVC and *E. coli* concentration (positive samples only) summaries (cont.)

- 90<sup>th</sup> Percentile: 90% of the data are less than this value, 10% are greater.
- 95<sup>th</sup> Percentile: 95% of the data are less than this value, 5% are greater.
- 99<sup>th</sup> Percentile: 99% of the data are less than this value, 1% are greater.



## TVC and *E. coli* counts (positive samples only) summaries (cont.)

- Mean
  - The average
- Standard Deviation (SD)
  - A measure of spread (variability) about the mean.
  - *LARGE* if observations are widely spread about the mean.
  - *SMALL* if observations are close to the mean.
    - A small SD indicates more consistent carcass hygiene.

Example: Consider two sets of data (each with 5 samples)

0.8	0.9	1.0	1.1	1.2
-----	-----	-----	-----	-----

Mean = 1  
SD = 0.16

0.4	0.7	1.0	1.3	1.6
-----	-----	-----	-----	-----

Mean = 1  
SD = 0.47

# Median versus Mean

- Median is known as a resistant measure of centre, but the mean is not.
- Median is not influenced by extreme observations.
- The mean is easily influenced by extreme observations.

0.8	0.9	1.0	1.1	1.2
-----	-----	-----	-----	-----

Median = 1

Mean = 1

0.8	0.9	1.0	1.1	6.5
-----	-----	-----	-----	-----

Median = 1

Mean = 2.06

## Example: Total Viable Count Summary

	Plant	National
Positives	1444	19246
Minimum	0	-1.00
Q1	1.66	0.70
Median	2.01	1.19
Mean (+ve)	1.98	1.21
Q3	2.35	1.70
90 <sup>th</sup> Percentile	2.66	2.22
95 <sup>th</sup> Percentile	2.92	2.51
99 <sup>th</sup> Percentile	3.41	3.16
Maximum	4.13	5.57
SD	0.58	0.77

- Table 2.
- Data integrity
  - Sampling factor for this species is 0.33 cfu/cm<sup>2</sup> (-0.48 log<sub>10</sub> cfu/cm<sup>2</sup>).
  - This should be the minimum (positive) concentration observed.
  - But the minimum TVC Reading nationally for this species is 0.1 cfu/cm<sup>2</sup> (-1.0 log<sub>10</sub> cfu/cm<sup>2</sup>).

# Data Integrity

- AQIS Meat Notice – Procedures to calculate concentrations in cfu/cm<sup>2</sup> of carcass surface.
- Cows & Bulls and Steers & Heifers:
  - A 300cm<sup>2</sup> area is swabbed in 25 ml of diluent.
  - Each ml of undiluted sample represents 12cm<sup>2</sup> (300/25) of swabbed surface
  - For an undiluted sample, the number of bacteria is divided by 12 (or multiplied by 1/12=0.08) which gives the concentration in cfu/cm<sup>2</sup> of carcass surface.
  - 1/12 or 0.08 is called the *sampling factor*.
- Sheep, lambs, calves and goats,
  - Three 5x5 cm<sup>2</sup> (or 75cm<sup>2</sup> total) is swabbed in 25 ml of diluent.
  - Each ml represents 3cm<sup>2</sup> of swabbed surface
  - 1/3 or 0.33 is the *sampling factor*.

## Data Integrity (cont.)

Serial Dilutions	Number of colonies on a plate	Sampling Factor	Cfu/cm <sup>2</sup>
No dilution (10 <sup>0</sup> )	0	0.08	< 0.08
No dilution (10 <sup>0</sup> )	0	0.33	<0.33
No dilution (10 <sup>0</sup> )	1	0.08	0.08
No dilution (10 <sup>0</sup> )	100	0.33	33
1:10 dilution (10 <sup>1</sup> )	10	0.33	33
1:10 dilution (10 <sup>1</sup> )	100	0.08	80
1 in 100 dilution (10 <sup>2</sup> )	100	0.08	800
1 in 1000 dilution (10 <sup>3</sup> )	100	0.33	33,000

- Concentration in cfu/cm<sup>2</sup> = no. colonies x sampling factor x dilution factor
- The minimum concentration is < 0.08 or < 0.33 and these are our limits of detections.
- All other concentrations should be multiples of the sampling factor – not always the case.

## Example: Total Viable Count Summary (cont.)

	Plant	National
Positives	1444	19246
Minimum	0	-1.00
Q1	1.66	0.70
Median	2.01	1.19
Mean (+ve)	1.98	1.21
Q3	2.35	1.70
90 <sup>th</sup> Percentile	2.66	2.22
95 <sup>th</sup> Percentile	2.92	2.51
99 <sup>th</sup> Percentile	3.41	3.16
Maximum	4.13	5.57
SD	0.58	0.77

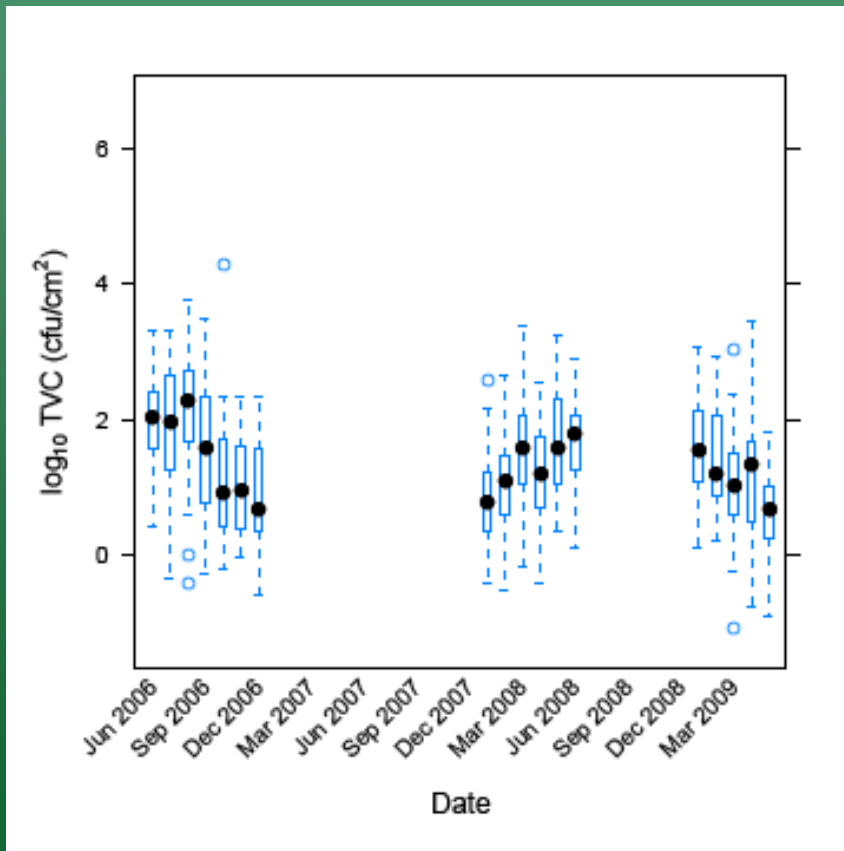
- At this plant, TVC's are generally higher, on average, compared to those found nationally
- TVC's were higher than those nationally at Q1, Median, Mean, Q3 and at 90<sup>th</sup>, 95<sup>th</sup>, 99<sup>th</sup> percentile.
- But the maximum TVC is 1.4 log less at this Plant than was found nationally.
- Indicates that the levels are higher at this Plant, but the variability is less than that found nationally.

## Table 4: *E. coli* summary

	Plant	National
Positives	268	1135
Minimum	-1.097	-1.097
Q1	-1.097	-1.097
Median	-1.097	-1.097
Mean (+ve)	-0.716	-0.799
Q3	-0.495	-0.620
90 <sup>th</sup> Percentile	0.058	-0.097
95 <sup>th</sup> Percentile	0.442	0.246
99 <sup>th</sup> Percentile	1.120	1.186
Maximum	2.146	2.413
SD	0.553	0.525

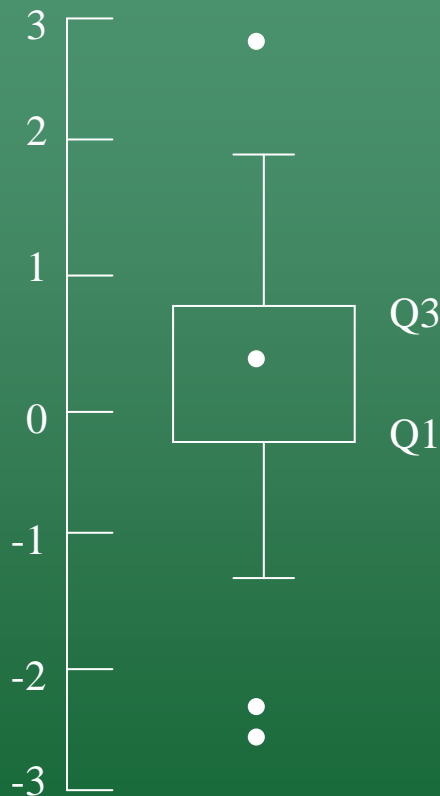
- Table 4.
- Same Plant that had 30% of samples positive compared to 7% nationally presented previously.
- Although this Plant had a high prevalence of *E. coli*, the concentrations are low and similar to those found nationally.

# Box plot - Used to assess the central tendency and spread



- Illustrate the spread of data within each sampling month.
- Seasonal trends, extreme or unexpected concentrations can be identified.

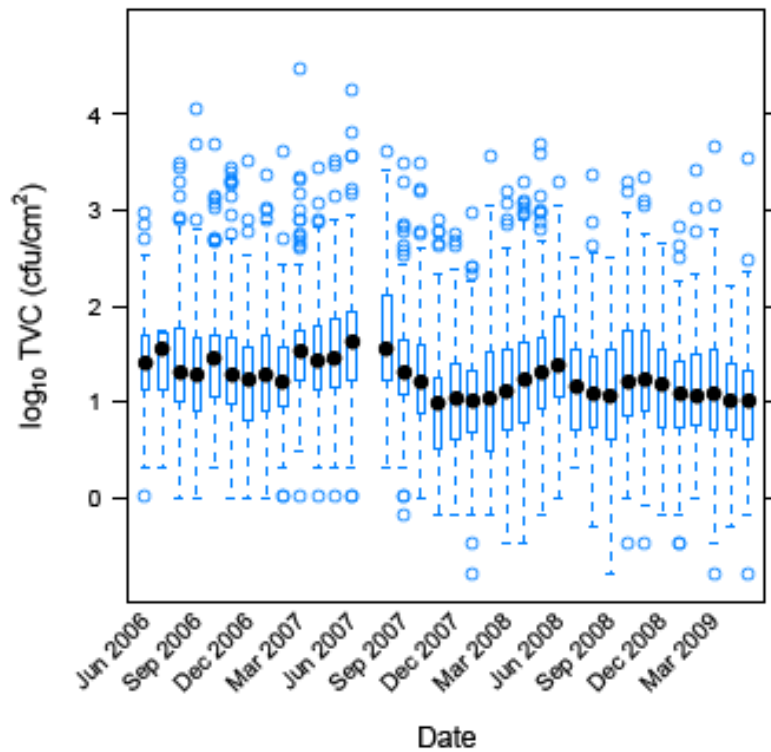
# Box plot Construction



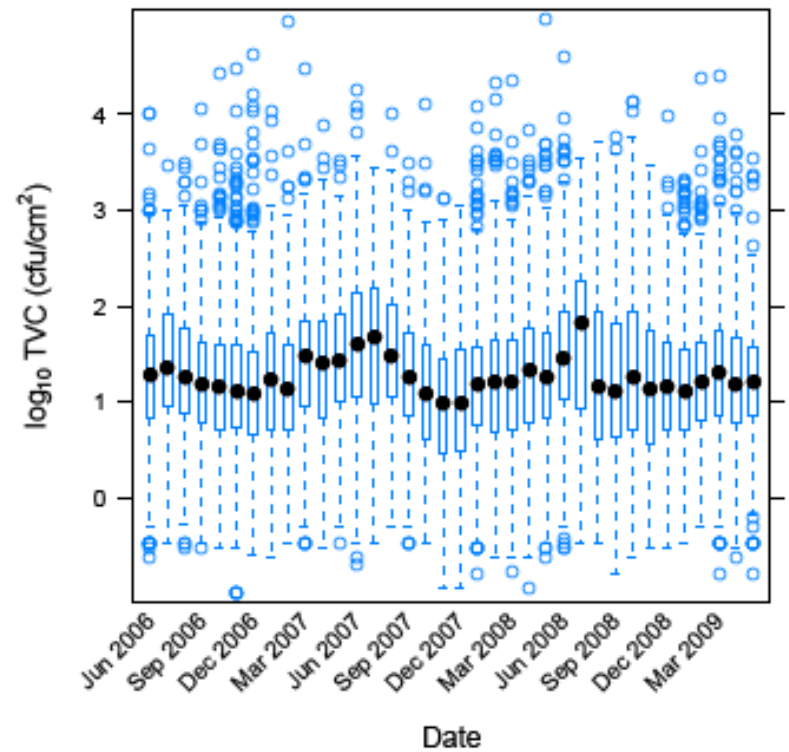
- Draw a box from Q1 to Q3
  - Half (50%) of the data fall within the box
  - Inter-quartile Range (IQR) =  $Q3 - Q1$
- Draw the median
- Draw the whiskers
  - The length is determined by  $1.5 \times \text{IQR}$ , ending on an observation
- Other observations are indicated separately – indicate extreme observations or unexpected concentrations.

# Example – Figure 1: TVC

Plant



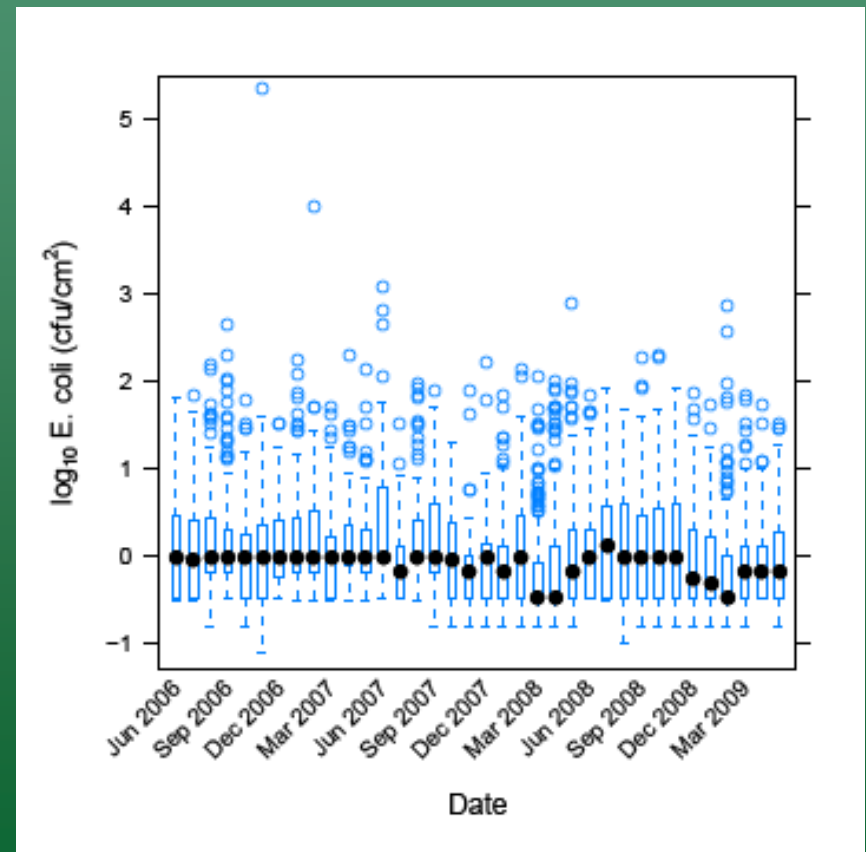
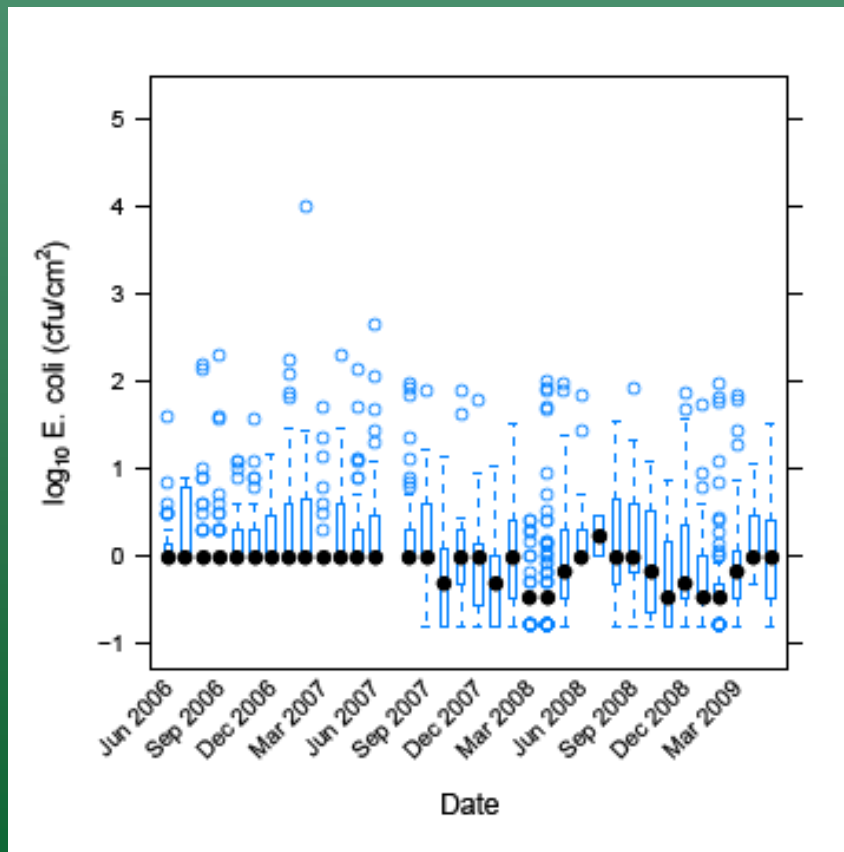
National



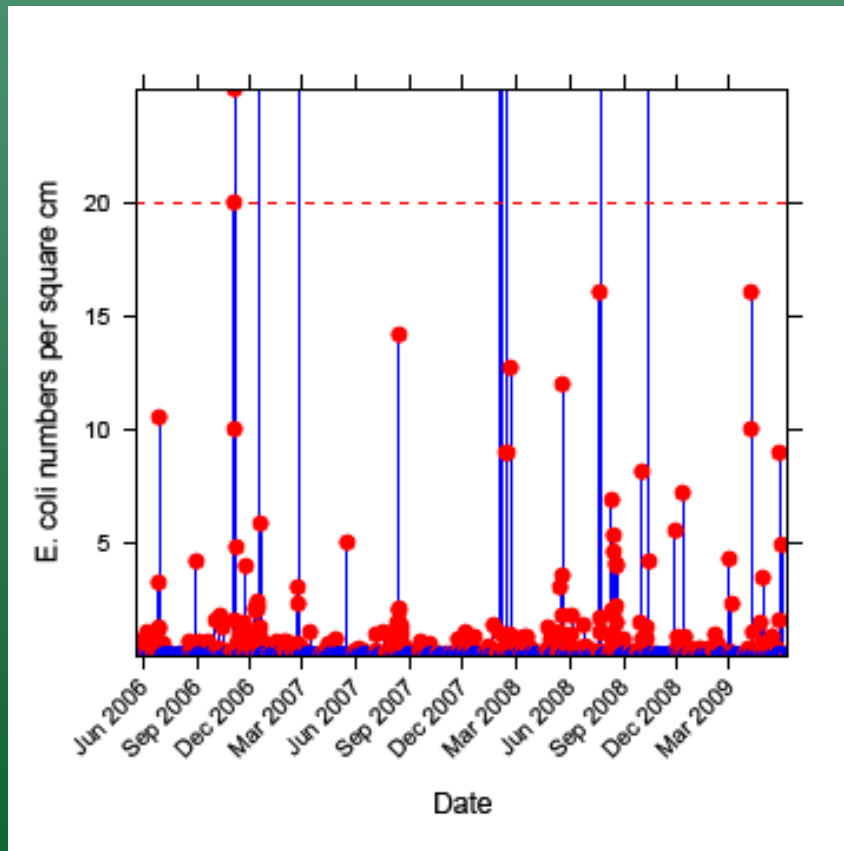
# Figure 2: Box plot of positive *E. coli* concentrations

Plant

National



# Time plot of *E. coli* concentrations (original scale)



- Useful to compare individual plant's level of *E. coli* over time to National level
- Positive tests – red dots.
- Negative – blue open circles.
- Red dashed horizontal lines show 'm' and 'M'.
  - Defined as part of the Three-class sampling plan.

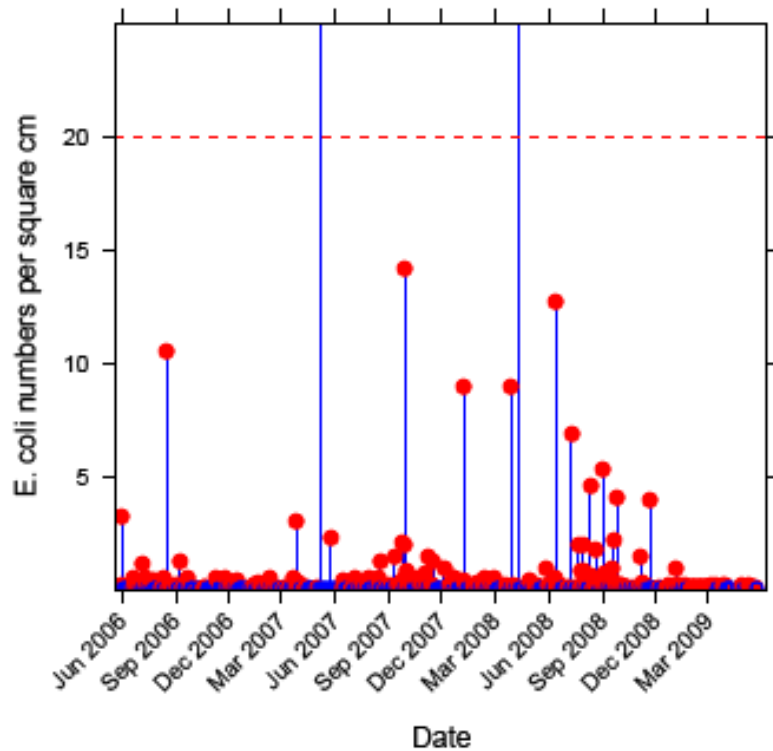
# What are 'm' and 'M'?



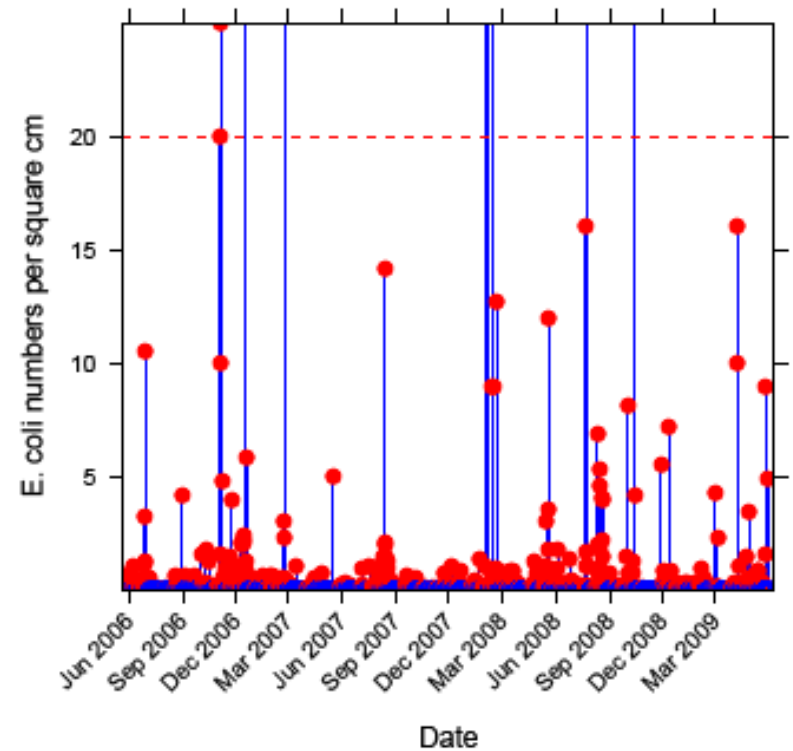
- Defined in AQIS Meat Notice 2003/6 in Appendix 1.
- Separate the concentration data into 3 classes: Acceptable, Marginal and Unacceptable.
- Samples below 'm' are considered to have Acceptable levels of *E. coli*.
- Samples above 'M' are considered to have Unacceptable levels of *E. coli*.
- Samples between 'm' and 'M' are considered to have Marginal levels of *E. coli*.
- Are there specific times of the year where there are more "high" positives?

# Figure 3: Time plot of *E. coli* concentrations

Plant



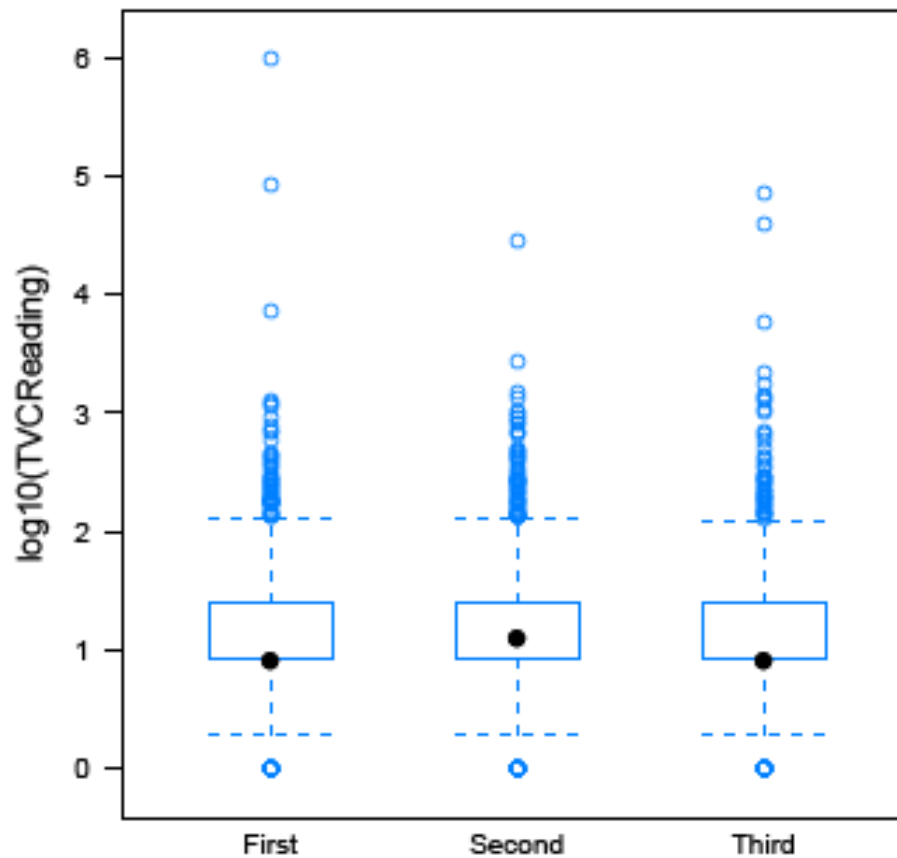
National



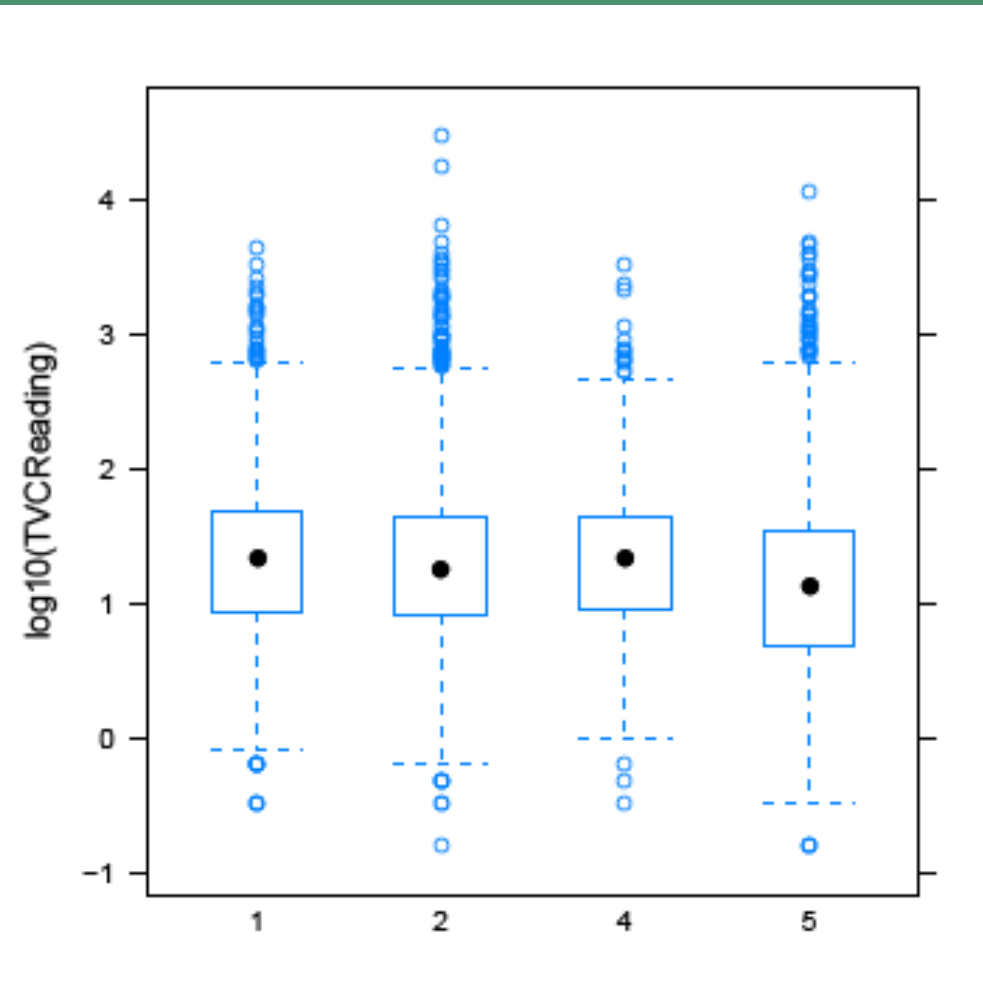
# Additional Information

- Establishments that slaughter species on multiple chains, shifts or by different dressing methods
- Hot versus Cold Swabbing?
  - Is there variability in the level of hygiene?

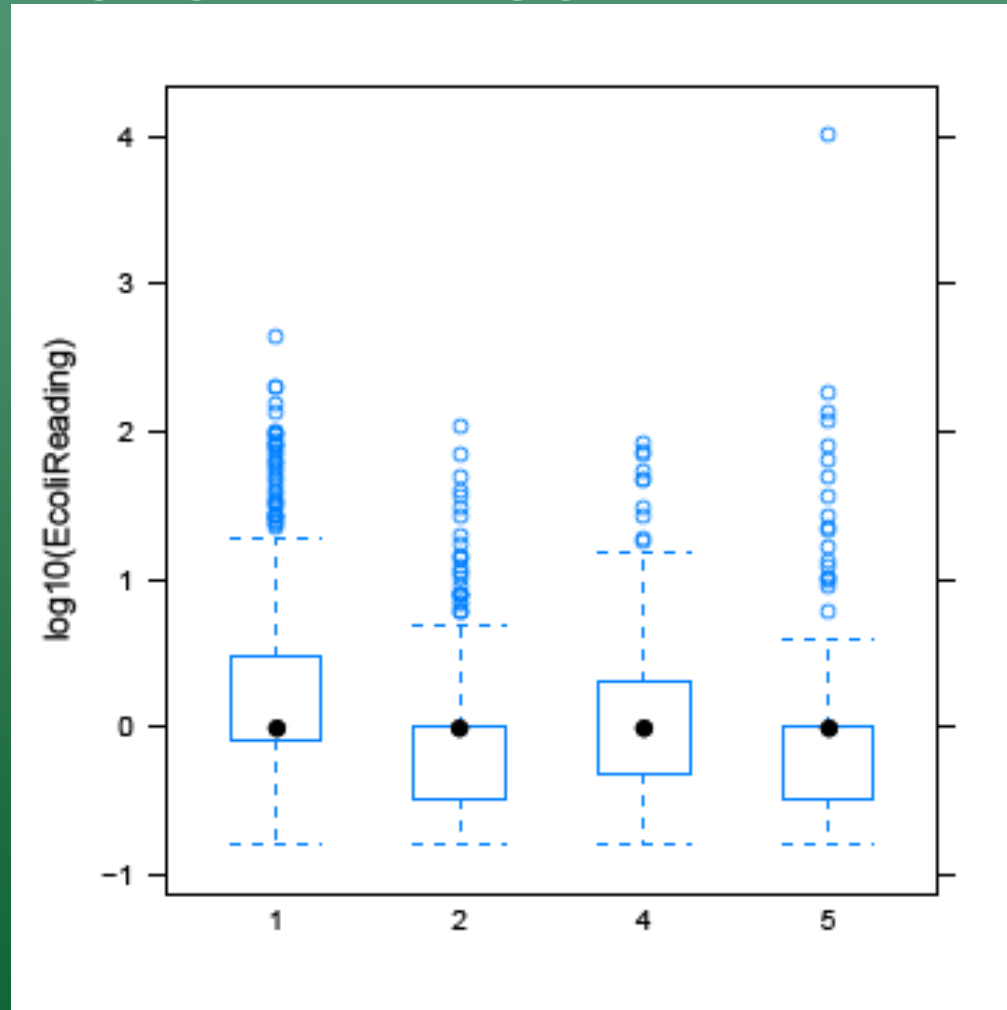
# Example: Shift



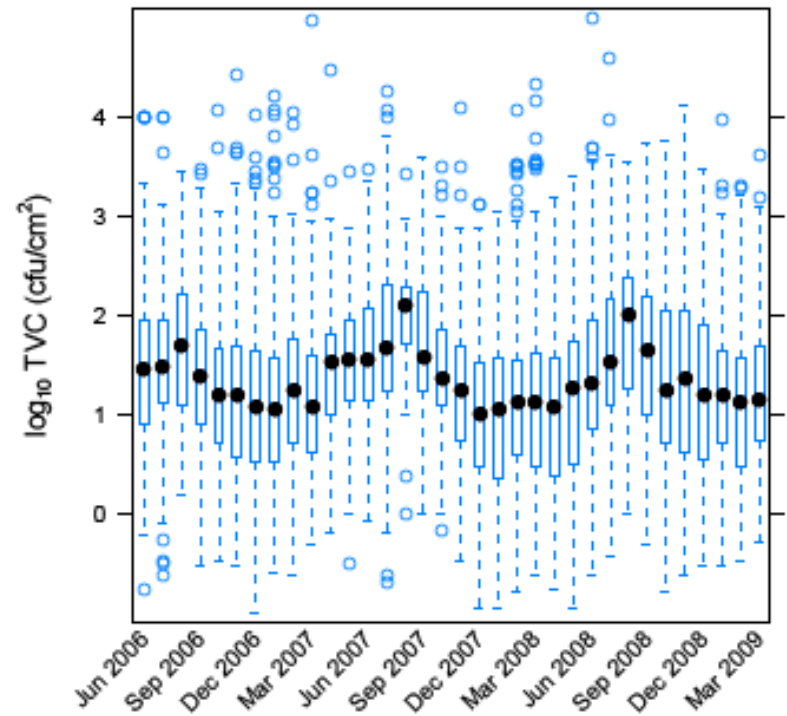
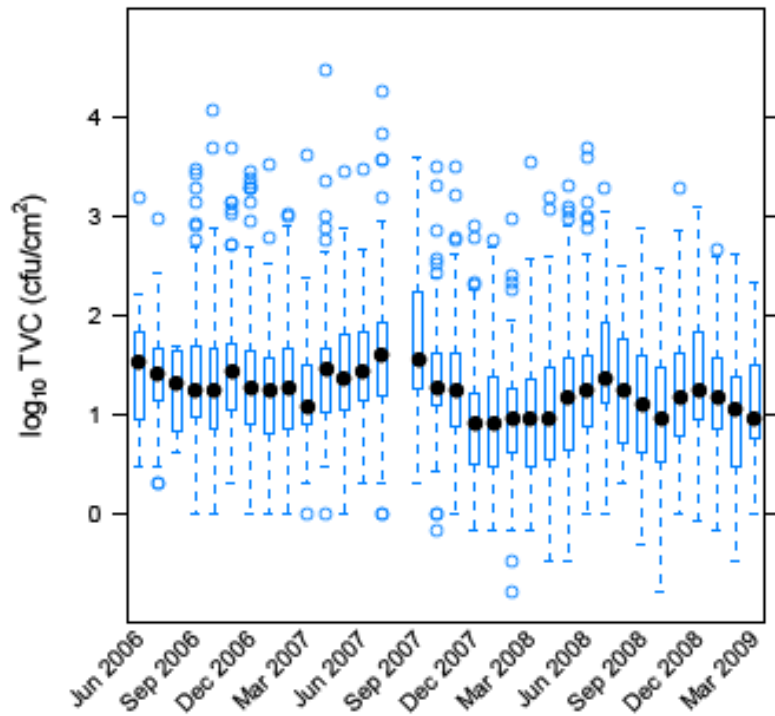
# Example: Chain – TVC



# Example: Chain – *E. coli*

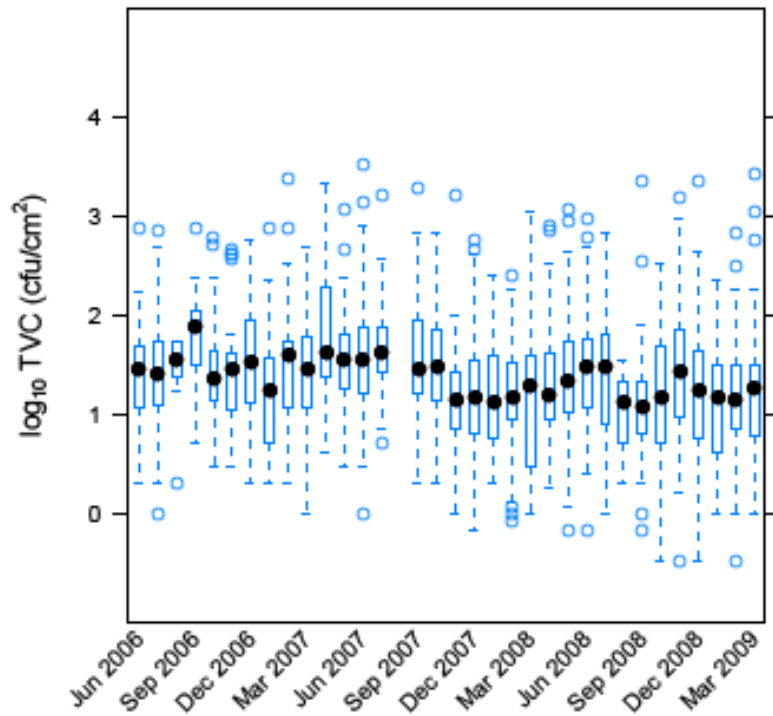


# Cold Swabbing

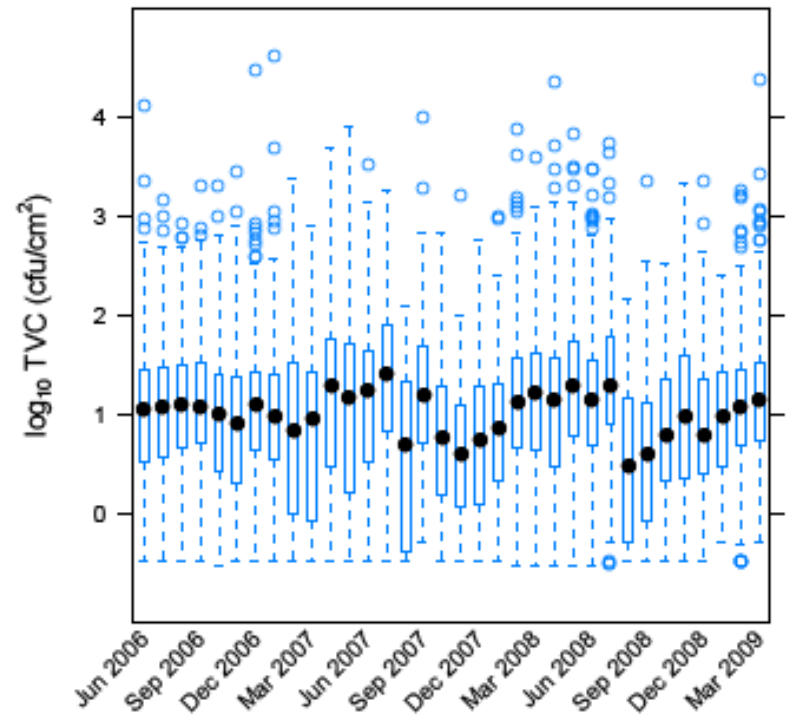


# Hot Swabbing

Plant



National



## What to look for...within my plant.

- For prevalence:
  - Do we have enough information to obtain an accurate estimate of prevalence?
    - Large number of tests performed?
    - How wide is the Confidence Interval?
- For concentrations:
  - Are the mean and median similar?
  - Is the SD small?
  - Is there any evidence of extreme (or unusual) observations?
- Over time:
  - Is there evidence of extreme observations?
  - Trend over time?

## What to look for...Plant versus National.

- How do we compare our Plant with the National benchmarks?
- For prevalence:
  - Is the prevalence higher at this Plant?
- For concentrations:
  - Is the mean difference between Plant and National concentrations greater than 1 log?
  - Is the Plant SD higher than the National SD?
- Over time:
  - From the box plots, is there any evidence of seasonality over time?
  - Are there times of the year, when concentrations are higher?

# Statistical difference versus Practical difference

- Statistical difference

- Based on the data, can you differentiate between the two groups?
  - Can get statistical difference with very small differences if the sample size is large enough.
- Also known as statistical significance.

- Practical difference

- Is the difference large enough to be of value in a practical sense?
  - Often consider mean log differences  $> 0.5$  or  $1$ .

## Where to from here?

- Reports will be sent monthly to participating establishments from soon after this meeting
- Reports will be sent monthly until mid next year
- You can call me (08 8207 7945) or email me ([michelle.lorimer@sa.gov.au](mailto:michelle.lorimer@sa.gov.au)) to answer questions or assist with interpretation.
- You can suggest changes to the report (improve for everyone)
- You can request additional analysis of your data (fee for service)
- After mid next year the reporting will become fee for service
- Discussions/help at MINTRAC QA Manager meetings

# Want to improve? Looking for ideas?

- General

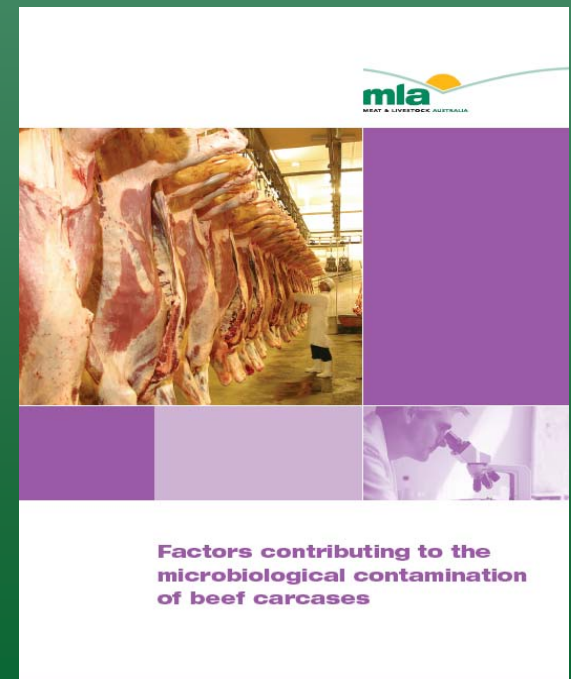
- Meat Industry Services [www.meatupdate.csiro.au](http://www.meatupdate.csiro.au)
- Red Meat Innovation for processors  
[www.redmeatinnovation.com.au](http://www.redmeatinnovation.com.au) (launching in October)
- Quality Assurance resource disc (MLA)



# Want to improve? Looking for ideas?

- Beef

- Report on factors contributing to micro contamination (MLA)
- Process Assessment Tool (coming)



# Want to improve? Looking for ideas?

- Sheep
  - Report on processing variables and micro quality (MLA)
  - Process Assessment Tool (coming)

