Program

- Measures of disease frequency
- Introduction to clustered data
- Structured approach data-analysis
- Introduction software & computer exercises

- Tentative timetable
  - 09u00 – 10u30 Epidemiology part I (Theory)
  - 11u00 – 12u30 Epidemiology part II (Theory)
  - 12u30 – 14u00 Lunch
  - 14u00 – 17u00 Epidemiology part III (Practical exercises)

Handbook

Veterinary Epidemiologic Research
  - Dohoo, Martin, and Stryhn: 2nd edition
  - www.upei.ca/~ver

Today:
  - "Chapter 4: Measures of Disease Frequency"
  - "Chapter 20: Introduction to clustered data"
  - "Chapter 30: A structured approach to data-analysis"
  - "Chapter 3: Questionnaire design"
Who am I?
- Graduated as vet @ Ghent University in 1998 - Ambulatory clinic @ Ghent University 1998-2005
- Judicial expert @ Ghent University in 2003
- MSc vet. epidemiology and animal health economics @ Utrecht University in 2004
- PhD in appl. epidemiology @ Ghent University in 2004
- Diplomat ECVP, 2006
Currently associate prof. vet. law and practice management, head of M-team and co-founder of MEX
Keen interest in the veterinary profession

1. Services
   - Extension work
   - Training
   - Communication
   - Lab
   - Clinical trials

2. Teaching
   - Theoretical courses
   - Practical courses

3. Research
   - Projects and grants
   - Doctorates
   - Scientific publications

M-news

M²-magazine
Overview of this lecture

Mastitis, a classic example of a multifactorial disease
Epidemiology, an introduction (in relation to mastitis)

Measures of disease frequency
- Introduction and some terms
- Incidence
- Prevalence
- Prevalence and incidence
- Exercise
Mastitis
Introduction
-Inflammation of an udder quarter after infection usually caused by bacteria
Mastitis pathogen typically enters via teat canal

Multifactorial disease
- HOST
- PATHOGEN
- MANAGEMENT

Prevention should focus on different aspects

Prevention should focus on different aspects
Mastitis
Introduction

Multifactorial disease
- HOST
- PATHOGEN
- MANAGEMENT

Prevention should focus on different aspects

Mastitis
Prevention and control

Epidemiology

Aim of prevention and control program

• Reduction of duration of existing IMI (E)

Mastitis
Prevention and control

Epidemiology

Aim of prevention and control program

• Reduction of duration of existing IMI (E)
Mastitis
Prevention and control

Aim of prevention and control program

- Reduction of duration of existing IMI (E)
- Reduction of incidence of new IMI (N)

Prevalence ~ Duration & Incidence

1. Excellent milking technique (N)
2. Well functioning milking machine (N)
3. Excellent dry cow management (N+E)
4. Successful treatment of clinical and subclinical mastitis (E+N)
5. Culling of chronically infected animals (E+N)

6. Excellent comfort, hygiene and housing (N)
7. Good record keeping and monitoring
8. Putting forward aim – monitoring – evaluation and changes
9. Breeding policy (N) - Purchasing policy (N)
10. Sanitary measures/general herd health status (N)

Many factors have been identified through epidemiological studies
Mastitis
Prevention and control: it works!

Source: Barkema et al.

<table>
<thead>
<tr>
<th>Bulk milk SCC Management</th>
<th>&lt;150</th>
<th>151-250</th>
<th>&gt;250</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cows dried off with AB (%)</td>
<td>93</td>
<td>80</td>
<td>76</td>
</tr>
<tr>
<td>Number of years</td>
<td>12</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Teat dipping (%)</td>
<td>75</td>
<td>49</td>
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</tr>
<tr>
<td>Number of years</td>
<td>8</td>
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<td>N treatments per clinical case</td>
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<td>Cows in head-lock after milking (%)</td>
<td>32</td>
<td>29</td>
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"When we were not testing cows, we had less mastitis!"

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Epidemiology

Introduction

Epidemiology is the study of determinants of health, disease, and productivity in populations of humans, plants, or animal

Molecular epidemiology is the study of distribution and determinants of health and disease through the use of molecular biology methods” – goals:
- To identify the agents responsible for infectious diseases
- To determine their physical sources, their biological relationships, and their route of transmission
- To determine the genes responsible for their virulence, vaccine-relevant antigens and drug resistance

Epidemiology ~ disease prevention

- Basic concepts applied in herd health management

Epidemiologists
- Try to (1) QUANTIFY a situation, a problem, a disease ...
- Strive to (2) IDENTIFY EXPOSURES
- Want to (3) RECOGNIZE ASSOCIATIONS between exposure and disease: prevention (example: 10 point mastitis prevention program)

(1) Quantification

Mastitis examples:
- How many cows are subclinically infected?
- How many quarters are subclinically infected?
- What is the proportion of cows with a new elevated SCC
- What is the risk of cows to get CM during a year, a month, ...
- How many cases of CM are caused by S. aureus?
- How many cases of CM are recurring (versus reinfections)
- What is the monthly incidence rate of CM on herd X
- ...

Epidemiology ~ disease prevention

- Basic concepts applied in herd health management

Epidemiologists
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Epidemiology
(1) Quantification

Using available herd data:
- Dairy Herd Improvement Data (e.g. SCC)
- Farmers’ records
  - Cases of clinical mastitis
  - Bacteriological culturing results
  - CMT results
- Official milk quality parameters
- ...

Epidemiology
(2) Exposures (= risk factors)
- Teat dipping after milking
- Clean and dry housing
- Mineral/vitamin supplementation
- Parity
- Stage of lactation
- Breed
- Quarter position
- Teat damage

Epidemiology
(2) Exposures (= risk factors)

Hierarchy of the data (predictors):

- Herd
- Cow
- Quarter
Epidemiology

(2) Exposures

Bulk milk SCC

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Epidemiology

(3) Associations

Herd predictors

Source: Barkema et al.

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Measures of disease frequency

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Measures of disease frequency
Introduction

**Start and basis** for most epidemiological activities

As part of routine surveillance, research, outbreak investigations...

*Observational studies,* measuring the occurrence of disease (e.g. *Staphylococcus aureus* mastitis cases) and exposure (e.g. teat dipping) are the first steps in inferring causation.

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Measures of disease frequency
Introduction

**Morbidity & mortality**

- 2 main categories of events for which frequency measures are calculated
- Also:
  - Culling
  - Pregnancy
  - Survival
  - ...
- Calculated for different diseases, animal (sex, breed, purpose ... ) and herd (beef, dairy, clean, large ... ) attributes

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Measures of disease frequency
Introduction

**Factors affecting choice of frequency measure**

- **Study period**: period over which study is conducted
  - Calendar time - Point in time (e.g. at calving, slaughter, birth ...)
- **Risk period**: time during which animal can develop disease of interest
  - Short - Lifelong - ...
  - Disease with short risk period: calculate "risk measures"
  - Diseases with longer risk period: calculate "rate measures"
- Examples:
  - Mastitis: whole lactation + dry period
  - Ret. sec.: first 24 hours post partum
  - Hypocalcaemia: -1 D totD4 post partum

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Knowledge of the disease
Measures of disease frequency

Some terms: Endemic versus epidemic

**Endemic**
- Stable situation – predictable - also non-infectious diseases (hypocalcaemia, ketonaemia ...)

**Epidemic**
- Sudden increase in number of cases, fatalities... in a certain period – limited in time and space

**Count**
- Number of cases, number of diseased animals
- Population size is not considered
- Of very limited use for epidemiologic research
- Examples
  - 10 cats with meningitis
  - 12 lame cows
  - 7 sheep with BT antibodies
Measures of disease frequency
Some terms: count, proportion, odds, rate

Proportion
- Ratio with the nominator as a subset of the denominator
- \( \frac{A}{A+B} \)
- Example:
  - 15% of 100 cows in herd X have IBR antibodies: \( \frac{15 \text{ (AB+)}}{15 \text{ (AB+) + 85 \text{ (AB-)}} \)
  - “Prevalence” and “incidence risk” are both proportions

Odds
- Nominator is not a subset of the denominator
- \( \frac{A}{B} \)
- Examples:
  - 3 stillborn animals and 120 live births: odds of stillbirth is 3:120 = 0.025
  - Odds to have IBR antibodies in herd A: \( \frac{15}{85} = 0.176 \)

Rate
- Ratio in which the denominator is the number of animal-time units at risk
- Example:
  - 30 cases of kennel cough in a 100-dog kennel over a 3-month period: incidence rate is \( \frac{30}{100 \times 3} \) = 0.1 cases per dog-month (300 dog-months in the denominator)
Measures of disease frequency
Prevalence and incidence

**Prevalence:**
- Number of cases at a certain time point
- Photo

**Incidence:**
- Number of new cases during a period of time
- Movie

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**Measures of disease frequency**
**Incidence**

*Number of new events* (e.g. cases of clinical mastitis) in a certain population (e.g. a dairy herd) *during a certain period of time* (e.g. one month)

**MOVIE**

- New event is not always a first event
- Multiple events possible per animal
  - E.g. mastitis: cases in different quarters or recurrent cases in the same quarter
Measures of disease frequency

Incidence

Incidence times are times at which events happen

Incidence count is the count of the number of cases of disease observed in a population: of limited use

Incidence risk (R) is the probability that an animal contracts a disease in a defined period

Incidence rate (I) is the number of new events in a population per unit of animal-time during a given time period

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Incidence risk (R)

\[
R = \frac{\text{number of new cases during the study}}{\text{population at risk}}
\]

- Ranges from 0 to 1
- Time period should be specified (risk of CM in the next week < risk of CM in next month)
- Risk only calculated when working with closed populations (individual is observed for the full risk period)
- Calculated when making predictions is an objective: e.g. probability that a 7 yrs old boxer gets neoplasia is...
  - \( \approx \) Cumulative incidence

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Example: dairy herd with 55 dairy cows:
- 5 animals have sole ulcers at start of follow-up
- Follow-up during 6 months: 4 new cases
- \( R = \frac{4}{55-5} \times 100 = 8\% \)
- Risk that a cow gets a sole ulcer in 6 month period = 8%
Measures of disease frequency –

Incidence risk (R)

New case
- Good definition needed of what “a new case” is
- Usually at the animal level - could be at a higher level (herd becoming BVD positive) or a lower level (quarter)

Population at risk
- Sometimes difficult to estimate
- Only animals free of the disease at the start of the study period are considered to be at risk
- Can be “closed” or “open”

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Measures of disease frequency –

Incidence risk (R)

Population at risk
- Closed population
  - No additions to the population for the duration of the study and few to no losses
  - Animals which are lost to follow-up during the study period = withdrawals
    - Simplest way of dealing with withdrawals: subtract half of the number of withdrawals from the population at risk (this assumes that, on average, the withdrawals leave halfway through the study period)

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Measures of disease frequency –

Incidence risk (R)

Population at risk
- Open population
  - One in which animals are leaving and entering the population throughout the study period
  - It is not possible to compute risk directly from an open population but it can be estimated
    - From incidence rate \( R = I \times \Delta t \)
    - Or
      - Using survival analysis
Measures of disease frequency

Incidences rate (I)

\[ I = \frac{\text{number of new cases during the study}}{\text{animal time units at risk during the study}} \]

- Unit of I: 1/animal-time
- Always positive, no upper bound

Animal time-unit: “one animal for a defined period of time” (e.g. Cow-month, dog-day...)

Example: 30 cows followed during 12 months

- 10 never sick, stay 12 months
- 10 sick and die after 3 months
- 10 sick and die after 9 months

\[ I = \frac{20}{1200} = 0.083 \text{ cases per cow-month} \]

Exact: each animal attributes a certain amount of time to the denominator
Measures of disease frequency

Incidence rate (I)

\[ I = \frac{\text{number of new cases during the study}}{\text{animal time units at risk during the study}} \]

Example: 4 healthy cows being followed during 1 month
- 1 cow stays healthy (1 cow-month at risk)
- 1 cow sick after 10 days (0.33 cow-month at risk)
- 1 cow sick after 20 days (0.66 cow-month at risk)
- 1 sold at day 15 (0.5 cow-month at risk)

\[ I = 2 / (4 - (0.5*2) - (0.5*1)) \times 1 \text{ month} = 2 / 2.5 = 0.8 \text{ cases per cow-month} \]

Estimated: \[ \left[ \text{#start} - \left(1/2 \times \# \text{sick}\right) - \left(1/2 \times \# \text{with}\right) + \left(1/2 \times \# \text{add}\right) \right] \times \text{study period} \]

Calculating R from I

- If complete data are available for a closed population then:
  \[ R = \frac{A}{N} \text{ and } I = \frac{A}{N \times \Delta t} \]
  \[ R = I \times \Delta t \]

- A = # cases
- N = population at risk
- \( \Delta t = \text{length study period} \)
Measures of disease frequency

**Incidence rate (I) and Incidence risk (R)**

**Calculating R from I**

- If the population can only be considered closed for short subintervals of the study period, and incident risks or rates in those subintervals are known and small:
  \[ R = 1 - \exp \left( - \sum_k I^* \Delta t_k \right) \]

- If only average I over all subintervals is available, then assuming I is constant over the time period:
  \[ R = 1 - \exp \left( - I^* \Delta t \right) \]

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**Measures of disease frequency**

- Introduction and some terms
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- Prevalence
- Prevalence and incidence
- Exercises

**Prevalence**

This measure of frequency relates to cases of disease existing at a **specific point in time** (rather than new cases occurring over a time period)

PICTURE
Measures of disease frequency

Prevalence

\[ P = \frac{\text{number of cases at certain moment}}{\text{number of animals at risk at that moment}} \]

Example: Blood samples taken from 75 beef cows for BVD-antibodies today
- 3 out of 75 cows are positive
- \( P = \frac{3}{75} \)
- \( = 0.04 \)
- \( = 4\% \)

Measures of disease frequency

Prevalence

Reduction in the Aujeszky's disease seroprevalence in the Netherlands

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Measures of disease frequency
Prevalence and incidence

Relation between prevalence and incidence
- In a stable population in which I of a disease remains constant (which it rarely does for contagious diseases):

\[ P = \frac{I \times D}{(I \times D) + 1} \]

- D = duration of the disease

Example:
I of subclinical mastitis in a dairy herd is 0.3/cow-year (i.e., 30 new infections/100 cows per year) and the mean duration of an infection is 3 months (0.25 year), then we would expect P to be:

\[ P = \frac{0.3 \times 0.25}{0.3 \times 0.25 + 1} = 0.07 = 7\% \]

If I and D would be constant than at any given day 7% of cows would have subclinical mastitis
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Exercise (p82)

You are interested in determining the frequency of new intramammary infections (IMI) with Staphylococcus aureus in dairy cattle so you randomly select cows in a dairy herd, follow them for one full lactation (10 months) and culture milk samples at months 0, 2, 4, 6, 8, and 10 (60-day-off). The results are presented in the table below. A cow is only considered to have a new intramammary infection if it was negative on the preceding sample.

<table>
<thead>
<tr>
<th>Sampling times</th>
<th>Total months at risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First case only</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>X</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
</tr>
</tbody>
</table>

where:
- X = positive culture
- X = positive culture that represents a new IMI
- O = negative culture
- = cow removed from herd
- = population at risk

Exercise

- Risk of infection during first 2 months of lactation (2-month R)?
- Risk of infection during lactation (lactation R)?
- Rate of IMI considering first cases only?
- Rate of IMI considering all new IMI?
- Lactation risk estimated from lactation rate (first cases only)?
- Prevalence of IMI at dry off?
Exercise

• Risk of infection during first 2 months of lactation (2-month R)?
  • 1 new IMI
  • 4 cows at risk at beginning of study
  • $\frac{1}{4} = 0.25 = 25\%$ risk of getting new *S. aureus* IMI during the first two months of lactation

Exercise

• Risk of first infection during lactation (lactation R)?
  • 2 new IMI
  • 4 - 0.5 (1 withdrawal) cows at risk
  • $R = \frac{2}{3.5} = 0.57 = 57\%$ risk of getting new *S. aureus* IMI during lactation

Exercise

• Rate of IMI considering first cases only?
  • 2 new IMI
  • 20 cow-months at risk
  • $I = \frac{2}{20} = 0.1$ cases per cow-month = 1 case per cow-lactation
Exercise

- Rate of IMI considering all new IMI?
  - 5 new IMI
  - 30 cow-months at risk
  - $I = \frac{5}{30} = 0.17 = 0.17$ per cow-month = 1.7 cases/cow-lactation

Exercise

- Lactation risk estimated from lactation rate (first cases only)?
  - $R = 1 - \exp(-I\Delta t)$
  - $I (= 0.1$ cases per cow-month) * $\Delta t$ (10 months) = 1
  - $1 - \exp(-1) = 0.63$

Exercise

- Prevalence of IMI at dry off?
  - 3 IMI
  - 4 cows present
  - $P = \frac{3}{4} = 0.75 = 75\%$ of all cows have IMI at dry-off