

Report of the

**Nordic Meeting on Advances in the Identification of Risk
Factors for *Campylobacter* Pre-harvest Infection and Post-
harvest Contamination of Chicken Broilers**

Uppsala, Sweden, 12-13 April 2005



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INTRODUCTION

Background

Foodborne illness due to *Campylobacter* is a widespread problem in all Nordic countries, and the Committee of Senior Officials under the Nordic Council has during the last years supported several projects related to this problem. Managing the problem requires a co-operation between people with different backgrounds and skills. In this respect, the organisation of workshops with plenty of room for discussions and interaction is useful to summarize the state of the art within a field, to exchange experiences, to create networks and to seed ideas for future work. In the fall of 2003, a workshop was held in Reykjavik with emphasis on risk management issues relating to *Campylobacter*. The present workshop was held in Uppsala 12-13 April 2005 and was focused on risk assessment and epidemiology issues in view of recent scientific studies and developments in the different Nordic *Campylobacter* Control Programs.

Objectives of the workshop

The objectives of the workshop were to review the results, the data and the methodology used in recent studies and control programs aimed at reducing the levels of *Campylobacter* in chicken broilers in order to summarize our present understanding and to identify future research priorities.

Workshop program and the present report

A programme committee with representatives from the Nordic countries issued a first call for abstracts based on the three main sessions that were identified. Based on the presentations that were submitted in response to the call the programme committee put together the final programme. The outcome of the meeting is presented in this report in the form of abstracts and slides of the presentations, and a summary extracted from the discussions during the workshop.

PROGRAM

Tuesday 12 April

Welcome and introduction, **Roland Lindqvist**

Session 1: Results of Nordic *Campylobacter* Control Programs - Hanne Rosenqvist **Moderator**

- The Danish *Campylobacter* control programme – results and evaluation, **Gudrun Sandø**
- Results and lessons learned from the Norwegian action plan against *Campylobacter* in broilers, **Merete Hofshagen**
- Finnish *Campylobacter* monitoring programme in broilers, **Marjaana Hakkinen**
- Swedish *Campylobacter* surveillance program, **Ingrid Hansson**

Session 2: Epidemiological studies (including typing studies) – Franklin Georgsson **moderator**

Risk factors

- Risk factors for *Campylobacter* infection in Danish broiler flocks, **Ole E. Heuer**
- The role of flies for *Campylobacter* infection of broiler flocks – a field study, **Birthe Hald**
- Post-chill *Campylobacter* contamination of broiler carcasses in relation to slaughter group colonization level and chilling system, **Mats Lindblad**

Molecular epidemiology

- Genotyping of *Campylobacter* from Broilers and Environment by AFLP, **Gro Johnsen**
- PFGE typing as a tool to trace the source of human infections, **Marja-Liisa Hänninen**

Session 3: Risk assessment studies - Merethe Hofshagen moderator

- Risk assessment model of *Campylobacter* in chicken in Iceland, **Jarle Reiersen**
- Evaluation of options at the producer and consumer stages for reducing the risk of *Campylobacter jejuni* illness from consumption of chicken, **Roland Lindqvist**

Group discussions

Group 1

Campylobacter control/surveillance programmes – Aim: Define an efficient program, identify interventions to be tested, identify knowledge gaps.

- What conclusions can be drawn?
- Which interventions have worked, not worked?

-
- How do you monitor effects of the program?

Group 2

Epidemiological studies – Aim: Identify and “rank” risk factors for *Campylobacter* in broilers, Identify pros and cons of current methods used for molecular epidemiological studies. Which are the most important ways of transmission of *Campylobacter* in chicken and in humans?

Group 3

Risk assessment studies – Aim: Identify primary knowledge and data gaps.

Wednesday 13 April

Session 2 (cont.): Epidemiological studies (including typing studies), – Marjaana Hakkinen moderator

- Ongoing Danish research activities on interventions to control *Campylobacter*, **Hanne Rosenquist**
- Designing challenge trials with *Campylobacter* in chickens, **Magne Kaldhusdal**
- Number of *Campylobacter* subtypes on individual chicken carcasses and slaughter groups postchilling, **Hans Lindmark**

Summary of group discussions

Plenary discussions (Lars Plym Forshell / Hans Lindmark)

Conclusions and recommendations of the workshop

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ABSTRACTS AND PRESENTATIONS

SESSION 1: RESULTS OF NORDIC *CAMPYLOBACTER* CONTROL PROGRAMS

The Danish Campylobacter control programme – results and evaluation

Gudrun Sandø

The Danish Veterinary and Food Administration has defined a voluntary programme for the reduction of *Campylobacter* in chicken meat in collaboration with the poultry industry and non governmental organizations, as a consequence of a steady increase in the incidence of human campylobacteriosis from 1993 to 2001, and based on the outcome of a Danish risk profile on *Campylobacter* and a risk assessment on *Campylobacter jejuni* in fresh chicken meat. The programme, which was initiated in 2003, comprised the following intervention strategies:

- 1) A strategy aimed at reducing the colonization of broiler flocks with *Campylobacter* by improving the bio security in and around the poultry house. In practice, the bonus for supplying *Campylobacter* free broiler flocks, which was introduced in 1998, was increased and the compliance with the industry code of practice was reinforced.
- 2) A strategy aimed at reducing the exposure of consumers to *Campylobacter* from chicken meat by reducing the number of *Campylobacter* on the products. In practice, *Campylobacter* contaminated broiler flocks were allocated to the production of frozen chicken meat, where possible.
- 3) A strategy aimed at educating consumers in proper kitchen hygiene by improving the understanding of how to avoid cross-contamination during food preparation. In practice, a consumer education campaign with focus on young people was launched.

Since, these interventions was introduced, there has been a marked decrease both in the number of *Campylobacter* positive broiler flocks (from 43 % in 2002 to 27 % in 2004) and in the prevalence of *Campylobacter* in Danish produced, fresh, chilled chicken meat (from 44% in 2003 to 20,1% in 2004). However, with regard to the number of human *Campylobacter* infections, after an initial decrease in 2002 and 2003 of approximately 25 %, the incidence has increased by 5 % in 2004. The reason for the limited effect on human campylobacteriosis in 2004 may be explained by an increased import of chicken meat, which is more contaminated with *Campylobacter* than the chicken meat produced in Denmark, an increased consumption of chicken meat or that other sources than chicken contribute to the human *Campylobacter* cases.



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and Food Administration

The Danish Campylobacter Program Results and evaluation

Nordic Meeting, 12 – 13 April 2005, Uppsala

Gudrun Sandø

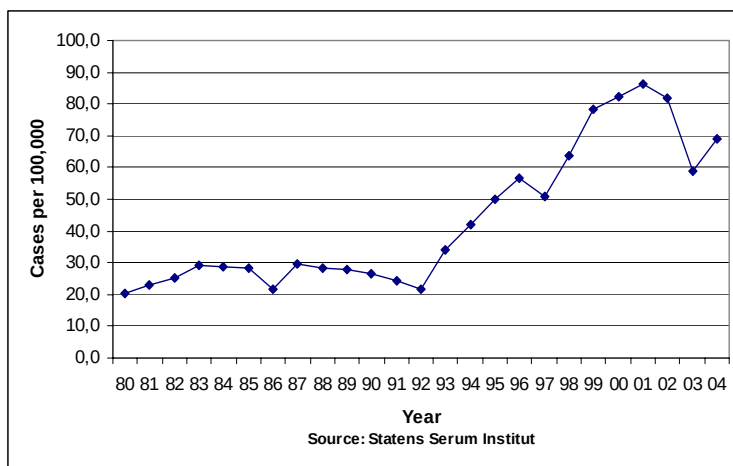
The Danish Veterinary and Food Administration

Division of Food Safety



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Human Campylobacter incidence 1980-2003





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The Danish Strategy against *Campylobacter*, Spring 2003

Developed in cooperation with:

- Danish Institute for Food and Veterinary Research
- The Danish Veterinary and Food Administration
- Consumers Board
- The Danish Poultry Meat Association



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The Danish Strategy against *Campylobacter*

Based on:

Risk Assessment on *Campylobacter jejuni* in chicken products,
January 2001.

Research on epidemiology and reduction in flocks.



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Reduction instead of elimination

- Reduction of *Campylobacter* in live chicken x 25
- Reduction of *Campylobacter* in poultry meat x 100
- Communication of information to consumers x 25

= 25 % reduction of risk to consumers

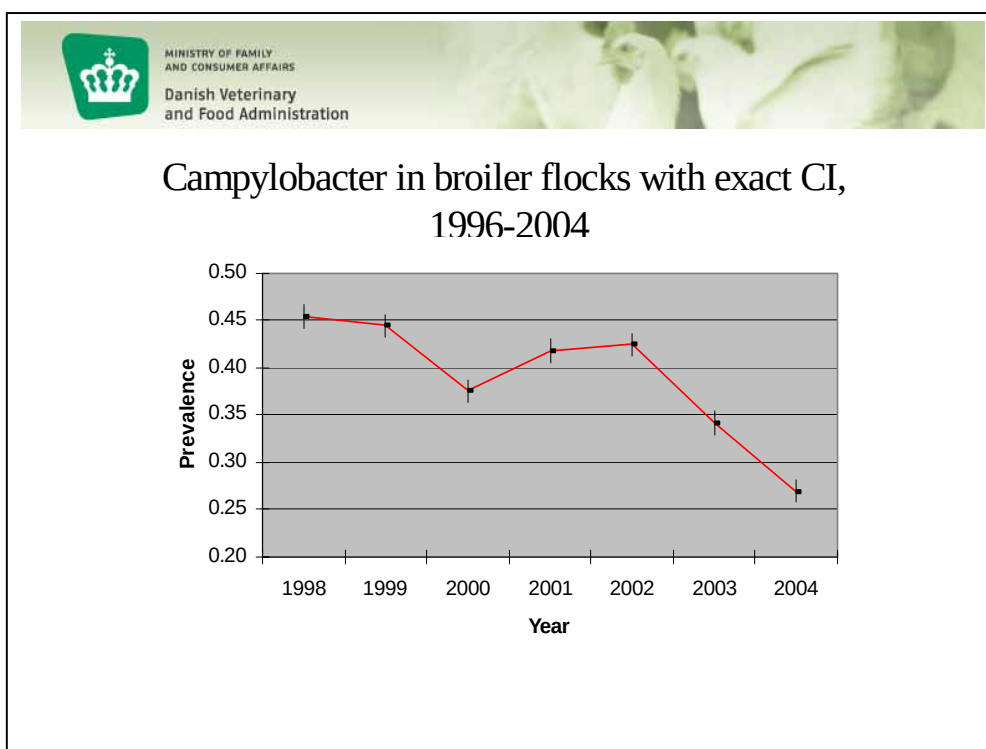


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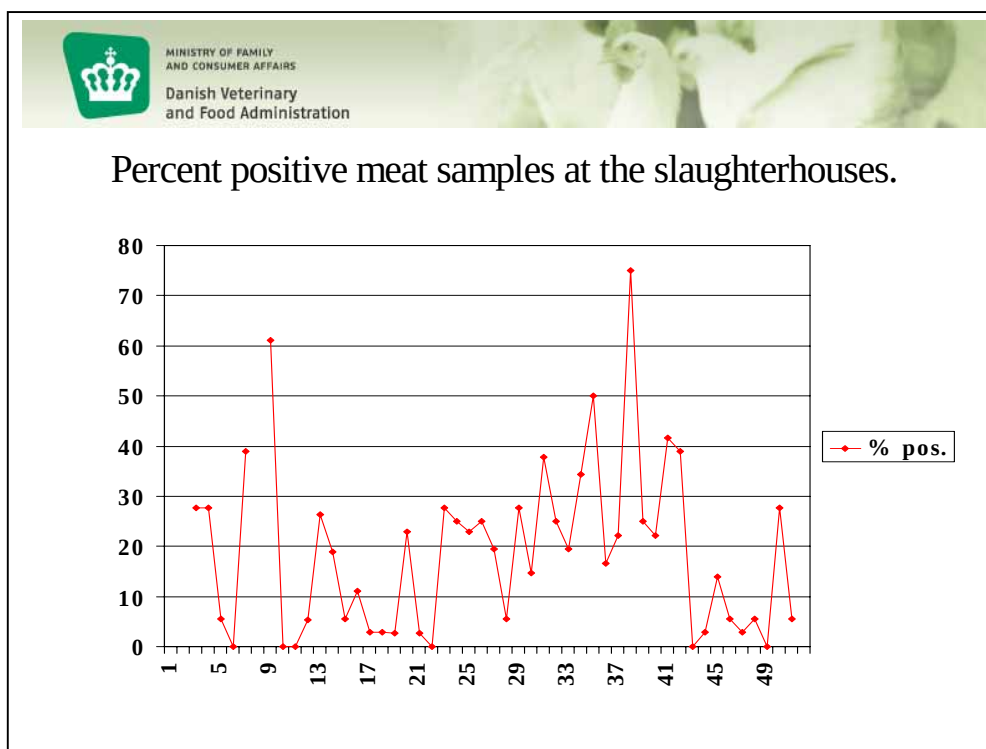
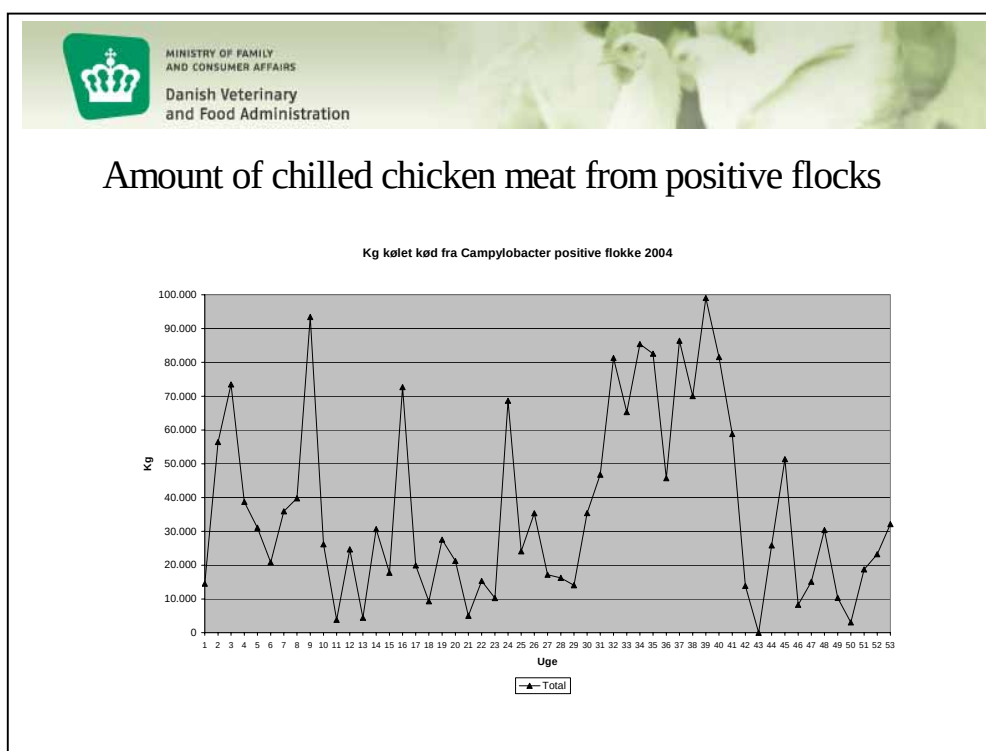


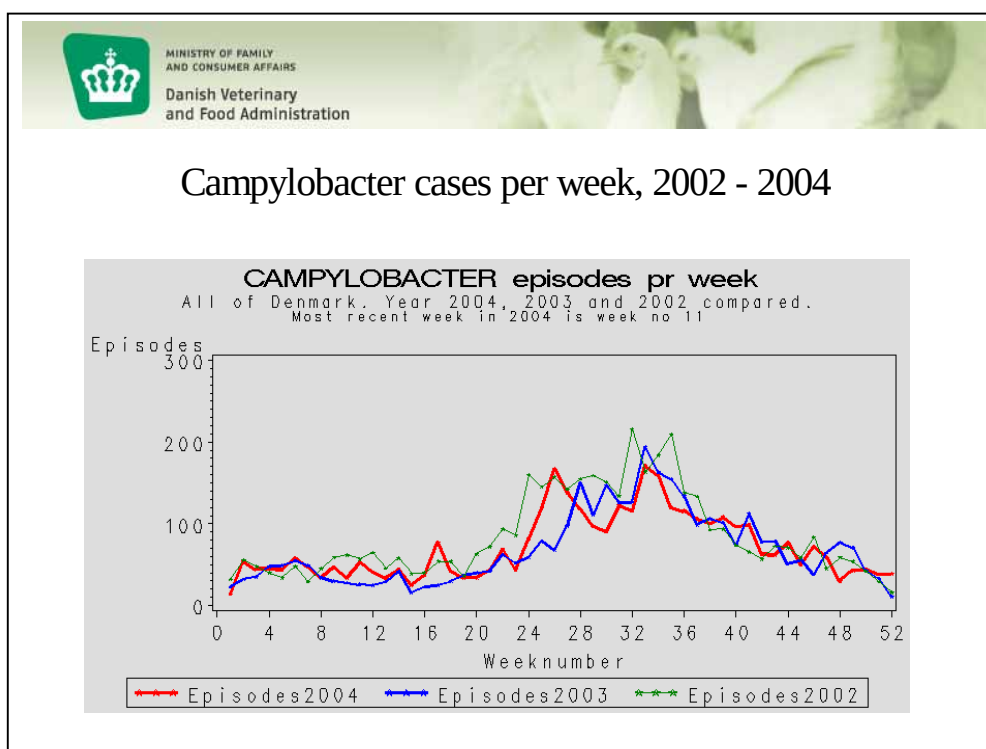
Reducing infection at the farm level

- Improving bio security in and around houses.
- Economic incentive introduced by the industry in 1998, rewarding farmers supplying *Campylobacter*-negative flocks.
- Increase of bonus in 2003 rewarding compliance with the industry code.



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- ### Reduction at the slaughterhouse
- Allocating *Campylobacter*-positive flocks to frozen products where possible
 - Investigating other methods of reducing the number of *Campylobacter* on carcasses.





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Consumer information

- Information campaigns, how to handle poultry in domestic kitchens.



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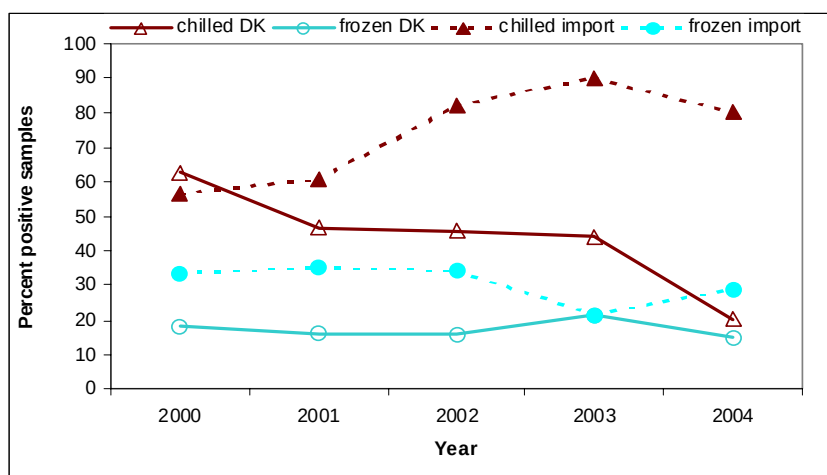
Projects & investigations, 2004 Campylobacter and poultry

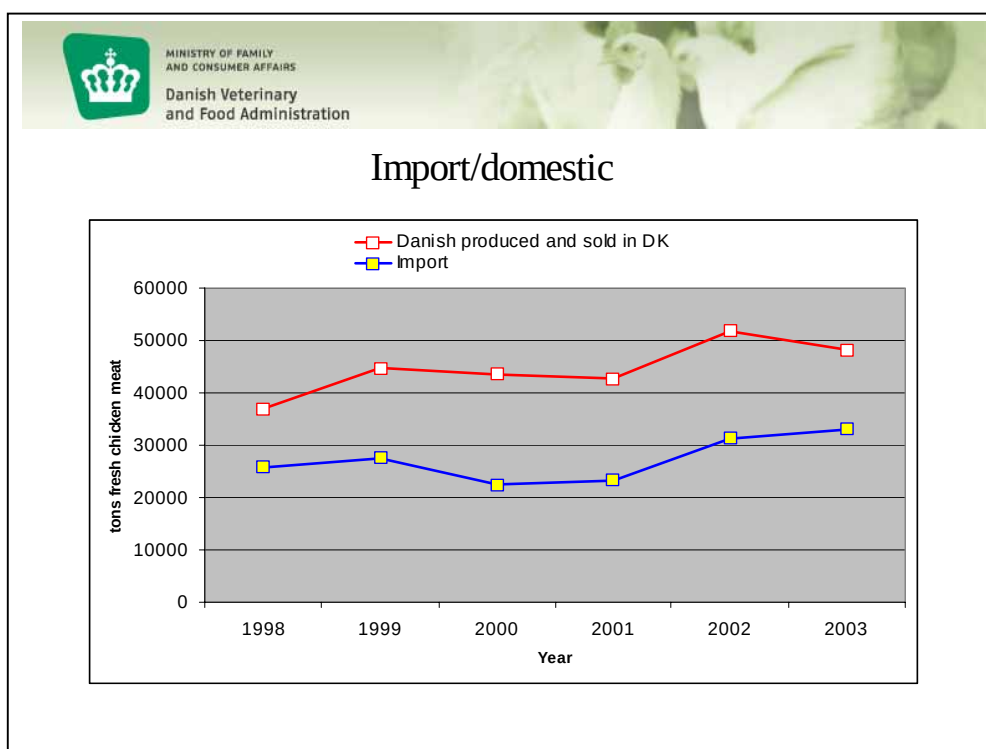
- Surveillance of Campylobacter in fresh chilled meat, at slaughterhouse level, 2000 samples, 18 samples/week. (2005, 2006).
- Surveillance of Campylobacter in chilled & frozen poultry, domestic and import, retail level, 1050 samples. (2005, 2006).
- Screening of imported fresh chilled chicken and poultry, 170 samples, nov. - dec.



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Results





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Status

- Reduction in flocks since 2002 ☺
- Reduction in prevalence in fresh chilled meat in 2004 ☺
- No reduction in human incidence since 2003 ☺

A cartoon illustration of a man in a suit sitting at a desk, looking at papers. A large orange triangle is positioned behind him, pointing upwards.



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- Increase in the consumption of imported poultry meat ?
- Channelling campylobacter free flocks to chilled products not efficient enough?
- Increase of travel associated cases?
- Other sources?

Results and lessons learned from the Norwegian action plan against Campylobacter in broilers

Merete Hofshagen

Results from the start of the action plan in 2001 and up to March 2005 will be presented. Also, the changes made in the action plan, and the reasons behind the changes will be presented.



Results and lessons learned – Norwegian action plan against *Campylobacter* in broilers

Merete Hofshagen, DVM, PhD
Norwegian Zoonosis Centre
National Veterinary Institute
Zoonoses Workshop, Uppsala April 2005

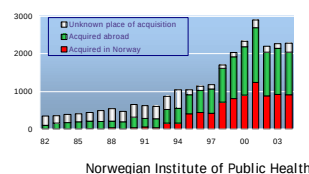


Outline

- Action Plan
 - Short description
 - Results
- Lessons learned
 - Time for sampling before slaughter
 - Sampling
 - Method
 - Laboratory performance
 - Cross-contamination
 - Freezing
 - +++



Norwegian Action Plan



■ Background

- Human campylobacteriosis increased
- Study 1999-2000¹ identifying risk factors
 - Eating poultry purchased raw
 - Drinking non-disinfected water
 - Professional contact with animals
 - Attending outdoor barbeques
- *Campylobacter* present in Norwegian poultry
 - 1990s: Flocks: 4 - 18%, Fresh products: 5 - 10%
- Increase in consumption of poultry bought fresh
- Limited import of poultry

¹Kapperud *et al.* 2003. Am J Epid 158, 3

Norwegian Action Plan – cont.

- Action plan established May 2001
 - Reduce human exposure to *Campylobacter* from Norwegian poultry
 - Authorities, scientific institutions, poultry industry
 - Coordinated by the Norwegian Zoonosis Centre

Norwegian Action Plan – cont.

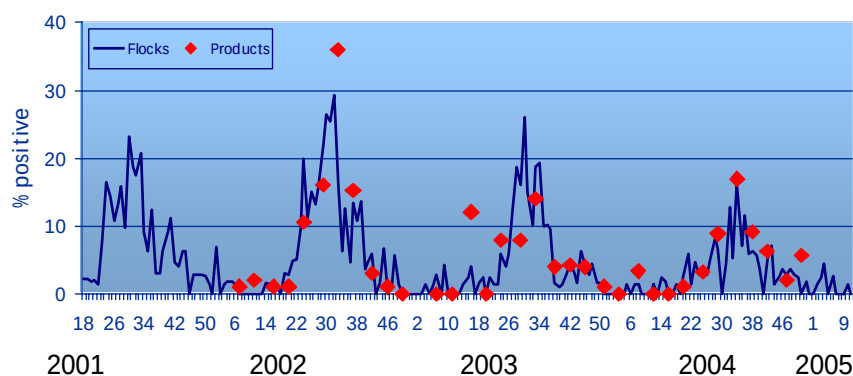
- Surveillance of all broiler flocks
 - Sampling before slaughter (10 faecal swabs)
 - Positive flocks: Carcasses heat treated or frozen (min. 3 weeks)
 - Re-sampling at slaughter (10 caecae)
 - No consequences for current flock
- Follow-up of positive farms
 - Standardized consultations
 - Introduce measures to reduce infection of flocks
- Survey of poultry meat products
 - Retail level, four cities, 100 samples/month



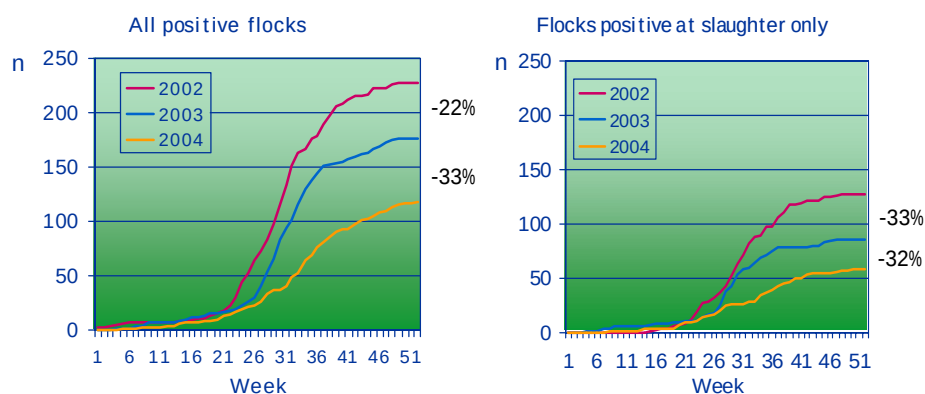
Results - overview

Positive flocks (May 2001 – Feb. 2005): 5.2% (696 / 13401)

Positive products (2002 – 2004): 6.1% (196 / 3229)



Comparing years



Veterinärinstitutet
National Veterinary Institute

Results - farms



N = 580, Positive = 302 (52%)

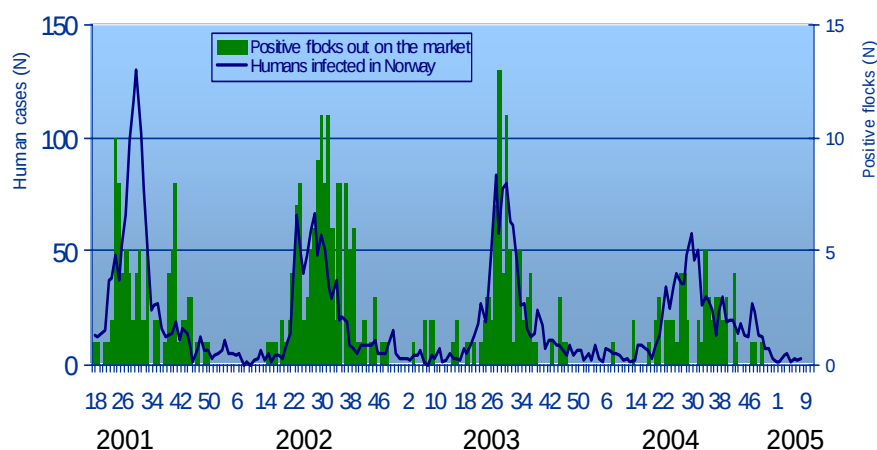
No. pos. flocks	N	Contribution to positive flocks
0	278	0
1	155	1/4
2	63	1/4
>2	84	1/2
>3	44	1/3

Farms with >1 positive flocks:

- 2001: 30
- 2002: 36
- 2003: 32
- 2004: 16

Veterinärinstitutet
National Veterinary Institute

Broilers - Humans



Data from humans: Norwegian Institute of Public Health



Lessons learned - 1

- Time for sampling before slaughter important
 - 8 days – 50% discovered
 - Approx. 10 – 15% increase per day closer to slaughter
 - Response
 - Sampling before slaughter: max 8 days (2001)
 - Sampling before slaughter: max 4 days (1.3 2005)
 - Cultivation: Drop enrichment step (2001)
 - Farm samples analysed with PCR (1.1 2005)
 - No logistic slaughter (1.3 2005)



Lessons learned - 2

- Optimizing sampling
 - Studies in 2003
 - 10 swabs by owner (pooled to 2): Discover 89% (84-95)
 - 10 cloacal swabs at slaughterhouse (pooled to 1): Discover 78% (56-89)
 - 10 caecae at slaughterhouse: Discover 100% (88-100)
 - Problems with slaughterhouse sampling
 - Cloacal swabs prone to cross-contamination
 - Flocks (farms) being identified as positive when negative
 - Response
 - Caecal samples at slaughter (instead of cloacal swabs) (1.5.2004)



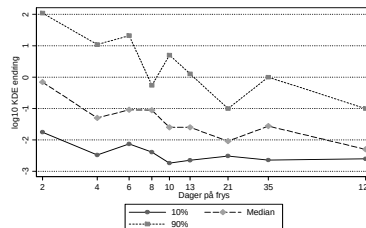
Lessons learned - 3

- Optimizing methods
 - 41.5°C slightly better than 37°C
 - Response
 - Incubation at 41.5°C (instead of 37°C) (1.5.2004)
- Laboratory performance a problem
 - False negatives, false positives, cross-contamination, keeping strains alive
 - Response: Training – constant process



Lessons learned - 4

- Freezing is expensive
 - Study in 2003



- Response
 - Freezing positive flocks 3 weeks (instead of 5) (1.7.2004)

Lessons learned - 5

- Information to all stakeholders important
 - Results updated regularly on the Internet (2002)
 - Regular meetings
 - Yearly reports (Internet, e-mail)
- Some farms have a recurrent problem (n=44)
 - Special focus summer 2005 (authorities/industry)
 - CC study

Lessons learned - 6

- Product surveys are difficult
 - 2001: Surveys Feb/March and Aug/Sept at plants
 - 2002: Monthly surveys in shops
 - 2003 + 2004: Quantitative surveys (May- Sep)
 - 2005: Only qualitative surveys
- Optimal design??



Conclusions



- Excellent cooperation
- Prevents a large number of positive broiler flocks from entering the market fresh
 - So far approx. 4.5 million broilers
- Positive public health effect
- Generates data for further research



Further information



www.zoonose.no



Finnish Campylobacter monitoring programme in broilers

Marjaana Hakkinen

In Finland there are ca. 210 broiler farms that are mainly located in western parts of the country. They produce annually ca. 3300 slaughter batches that are slaughtered in three slaughterhouses. The broiler meat production is ca. 69 million kg/year. The *campylobacter* monitoring programme was implemented in June 2004. All broiler slaughter batches between June and October are examined for thermophilic *campylobacters*. Between November and April random samples are taken according to a sampling scheme designed by the National Food Agency. One pooled sample of ten caeca from each slaughter batch is examined by direct plating on mCCDA in local approved laboratories. *Campylobacter* isolates are sent to EELA for confirmation and antimicrobial sensitivity testing.

If two consecutive *campylobacter* positive flocks are detected in the same holding, the management practices are checked and improvements in the production hygiene are carried out. The flocks from the holding are slaughtered at the end of the day until two consecutive flocks are *campylobacter* negative.

In 2004 the proportion of *campylobacter* positive broiler slaughter batches varied between 3.1 and 10.1% from June to October. No *campylobacters* were detected in samples taken between November 2004 and March 2005.



Finnish campylobacter monitoring programme in broilers

Marjaana Hakkinen
**National Veterinary and Food
Research Institute**



Finnish broiler production

- ca. 210 broiler farms
- three broiler slaughterhouses
- broiler meat production ca. 69 million kg / year
- ca. 3300 slaughter batches / year



Monitoring programme (1)

- implemented in June 2004
- compulsory for all broiler slaughterhouses
- primary sample size
 - all broiler slaughter batches between June and October
 - random testing during the rest of the year
 - target prevalence: 5 %, confidence level 95%, accuracy 5 %



Monitoring programme (2)

- secondary sample size: 10 intact caeca / batch
- pooling into one sample in the laboratory
 - swab samples from caecal contents into 5 ml of peptone water
- direct culture on modified CCDA
- analysis performed by local approved laboratories
- confirmation of strains and antimicrobial sensitivity testing at the National Veterinary and Food Research Institute (EELA)



Control measures

- two consecutive campylobacter positive flocks from the same holding
 - checking and improvement of production hygiene in the holding
 - slaughtering of flocks from the holding at the end of the day
 - until two consecutive negative flocks



Costs of the monitoring

- paid by the broiler industry
- confirmation and antimicrobial sensitivity testing paid by EELA



Results from 2004 -2005

	Batches tested	Batches positive	Positive %
June	258	8	3.1
July	269	24	8.9
August	268	27	10.1
September	271	14	5.1
October	250	9	3.6
November –March	52	0	0

Swedish Campylobacter programme

Hansson, I., Olsson Engvall, E., Vågsholm, I.

Background

In Sweden, a revised *Campylobacter* programme in broilers was initiated in 2001 based on the Swedish Board of Agriculture's regulation on organised health control. The purpose is to reduce the occurrence of *Campylobacter* in the food chain through preventive measures, starting with the primary production. The Swedish Poultry Meat Association runs the current *Campylobacter* programme. The overall prevalences of *Campylobacter* positive slaughter groups (cloacal samples) during the last three years have been 20% (2002), 18% (2003) and 14% (2004). A decreasing trend has also been noted in other Nordic countries.

During May to December 2004, a study was done at broiler farms aiming identifying risk factors for introducing *Campylobacter* into the broiler houses. About 30 farms were sampled at least once a week during a maximum of four rotations. The samples consisted of sock samples from the ground outside, sock samples from the floor in the stables and anterooms, and samples from insects, water, feed, ventilation shaft and air. Both farms with high and low incidence of *Campylobacter*, according to earlier results of the Swedish *Campylobacter* programme, were included.

Results

Four scenarios were identified: (I) no *Campylobacter* found in the surroundings or in the stables, (II) *Campylobacter* isolated from the surroundings, but not from the stables, (III) *Campylobacter* found in the surroundings and in the stable. (IV) *Campylobacter* found only in the stables. For clarification of transmission routes, genotyping by pulsed-field-gel-electrophoresis will be carried out on isolated *Campylobacter* strains.

When *Campylobacter* was found at the ground outside the stables, it had more likely been raining during the last (two) days. *Campylobacter* was also more frequently found in the environment during cloudy/rainy days compared with clear/sunny days.

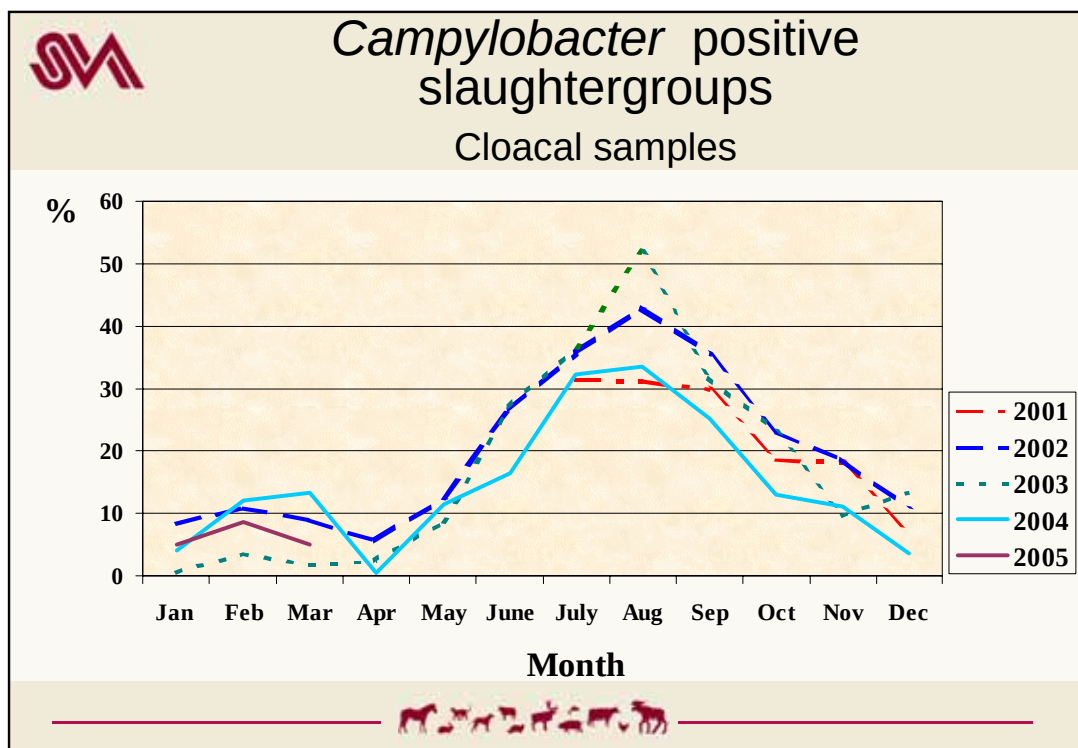
One of the aims of the study was to investigate if there is a relationship between environmental *Campylobacter* presence and flock incidence. The results of the study did not indicate any significant statistical difference regarding environmental contamination between producers that often deliver *Campylobacter* positive slaughter groups (> 30% positive slaughter groups/year) and those that rarely deliver *Campylobacter* positive slaughter groups (<10%). Hence, it appears that hygiene barriers are of importance for preventing *Campylobacter* in the environment to be transferred into the broiler houses.


STATENS VETERINÄRMEDICINSKA ANSTALT

Swedish *Campylobacter* programme



Ingrid Hansson Department of Bacteriology
National Veterinary Institute Sweden





Campylobacter in broilers in Sweden

	<u>2001</u> (6 months)	<u>2002</u>	<u>2003</u>	<u>2004</u>
Slaughtergroups, cloacal	2 140	3 842	3 224	3 019
Pos slaughtergroups, cloacal	489 (23%)	760 (20%)	566 (18%)	429 (14%)
Slaughtergroups, neckskin	2 110	3 764	3 144	2 940
Pos slaughtergroups, neckskin	579 (27%)	917 (24%)	659 (21%)	566 (19%)




Swedish broiler producers 1st of July 2001 to 30th of June 2004

Categories of producers in relation to <i>Campylobacter</i> frequency	Number of producers	Fraction of total no of slaughter groups	Fraction of total <i>Campylobacter</i> load
Never; 0% pos	13 (10%)	3%	0%
Sporadic; 1-9% pos	43 (31%)	29%	8%
Seasonally; 10-30% pos	58 (42%)	50%	51%
Often ; >30% pos	24 (17%)	18%	41%
Total	138 (100%)		





Critical points ?

Studies at farm level (2004)



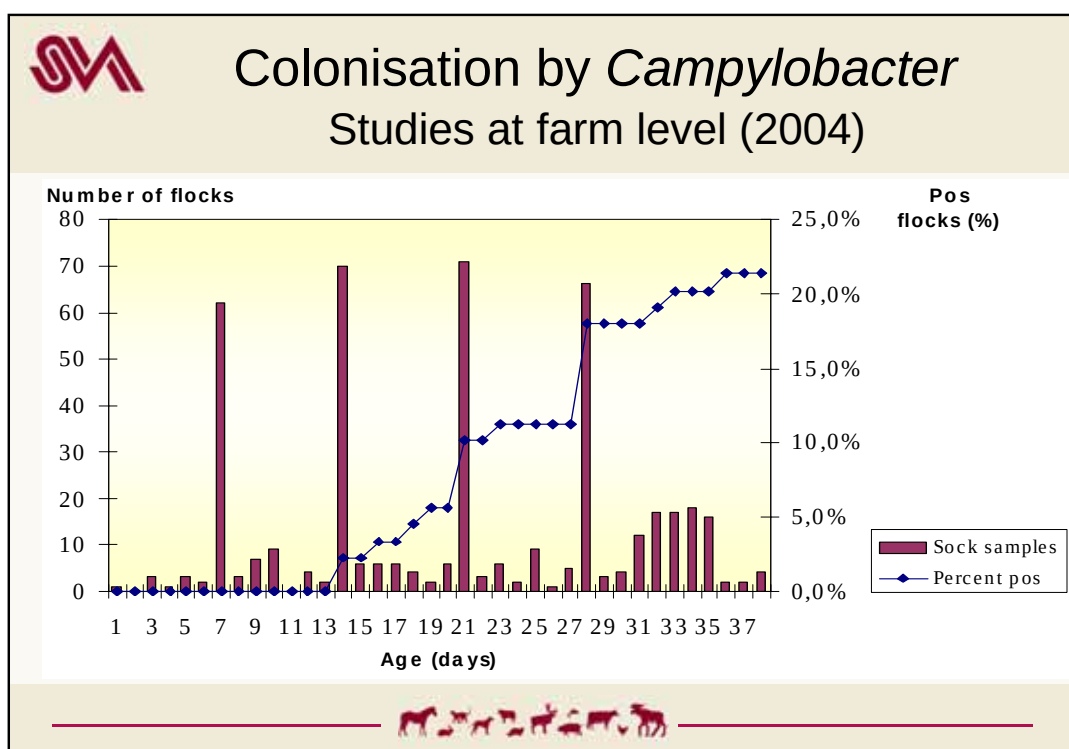


- Stable
- Anteroom
- Ventilation
- Surroundings
- Feed,
- Water
- Insects









 Why are some never affected? Studies at farm level (2004)							
Categories of producers <i>Camp</i> frequency 2001-2004	No of rotations	Campylobacter results (No of rotations)					
		neg env	stable	pos env .	pos env. stable	pos stable	
Sporadic; < 10% pos (N=11)	27	15		10	1		1
Seasonally; 10-30% pos (N=9)	27	17		7	3		0
Often; >30% pos (N=11)	35	13		7	9		6
Total (N=31)	89						

 Campylobacter – A pain in the rain? Studies at farm level (2004)			
Sock samples outside the stables (n=2-6/occasion)			
Weather conditions	Camp positive		
Sunny	21/404	(5%)	
Cloudy	42/423	(10%)	
Rain	22/174	(13%)	
Snow	0/5	(0%)	



Campylobacter in broilers in Sweden



Thank you for
your attention



ABSTRACTS AND PRESENTATIONS

SESSION 2: EPIDEMIOLOGICAL STUDIES (INCLUDING TYPING STUDIES)

Risk factors for Campylobacter infection in Danish broiler flocks

O. E. Heuer, D. M. A. Lo Fo Wong, M.E. Patrick, M. Wainø, A. Wedderkopp and M. Madsen

Ole E. Heuer, DVM, Ph.D., Research scientist, Department of Epidemiology and Risk Assessment, Danish Institute for Food and Veterinary Research, Mørkhøj Bygade 19, DK-2860 Søborg, DENMARK

Based on *Campylobacter* surveillance data and standardised questionnaire interviews with Danish broiler farm owners, a study was conducted to identify risk factors for the occurrence of *Campylobacter* in broiler flocks. Two hundred forty-four farms, comprising 543 broiler houses, were included in the study.

Farm owners were interviewed between May 1999 and October 2000. The questionnaire addressed farm specific as well as house specific variables. For each farm all broiler flocks slaughtered within 12 months before and 12 months after the interview were included in the analysis. All flocks were tested for *Campylobacter* at slaughter. Combining data from all houses, a total of 2,497 (37.2%) of 6,704 flocks slaughtered during the study period tested positive for *Campylobacter*.

The outcome variable was defined as the proportion of *Campylobacter* positive flocks out of the total number of broiler flocks produced in each broiler house during the study period. Initially, explanatory variables were screened for relationship with the outcome in univariate analysis. Variables fulfilling inclusion criteria were subjected to multivariate logistic regression using the PROC GENMOD procedure in SAS. Preliminary results suggested that slaughterhouse, hatchery, presence of other animals on the farm, number of gates to the broiler house and the brand of ventilation system employed, were significantly associated with the *Campylobacter* status of the flocks. Furthermore, the preliminary results suggested that use of whole wheat in the broiler feed (the percentage of wheat used and age at which wheat was introduced), and the presence of rodent baits (no of baits and frequency of checking them) was significantly associated with the *Campylobacter* status of the flocks.

Risk factors for Campylobacter in Danish broiler flocks

Ole E. Heuer, Danilo M. A. Lo Fo Wong, Mary E. Patrick,
Michael Wainø, Alice Wedderkopp and Mogens Madsen

Danish Institute of Food and Veterinary Research
Copenhagen, Denmark

Campy RF study

Objective:

- To identify RF for the occurrence of Campylobacter i Danish broiler flocks

The study was based on:

- Results from continuous Campylobacter surveillance in broiler flocks
- Data from interviews with farm owners 1999/2000

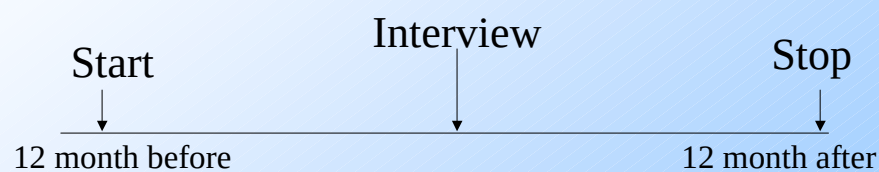
Campy RF study

Mat/Met:

- 244 broiler farms were included in the study (543 broiler houses, range 1-16, median: 2)
- Campylobacter status of 6704 flocks slaughtered during the study period were included (37,2% Campylobacter positive flocks)

Campy RF study

Mat/Met:



For each house at each farm the proportion of positive flocks produced within the study period was calculated

Campy RF study

Mat/Met:

- Farm owners were interviewed between May 1999 and October 2000
- Questions addressed >50 variables concerning:
 - Management
 - Feeding/water supply
 - House construction
 - House surroundings
 - House interior/equipment

Campy RF study

Mat/Met

- Outcome variable: The proportion of *Campylobacter* positive flocks out of the total number of broiler flocks produced in each broiler house during the study period
- Explanatory variables were screened for relationship with the outcome in univariate analysis.
- Variables fulfilling inclusion criteria were subjected to multivariate logistic regression using the PROC GENMOD procedure in SAS.

Campy RF study

Multivariate analysis:

- slaughterhouse
- hatchery
- age of broiler house
- presence of other animals on the farm
- number of gates to the broiler house
- brand of ventilation system employed
- use of whole wheat in the broiler feed (the percentage of wheat used and age at which wheat was introduced)
- presence of rodent baits (no of baits and frequency of checking them)

were significantly associated with the *Campylobacter* status of the flocks.

Campy RF study

Discussion:

- Variables related to biosecurity are present in the final model, but not dominating
- The significance of hatchery and slaughterhouse may be related to the management-regime of each broiler company
- Significance of ventilation system to be further explored.
- Rodent control and some indication that litter storage is of significance to be further explored

Insect screen against Campylobacter in chickens - an intervention study

Birthe Hald, Henrik Skovgård, Karl Pedersen, Henrik Bunkenborg, and Mogens Madsen

Study design

An intervention trial in pairs of houses. One broiler house at each of five Danish broiler farms was protected from insects from 1. April to 31. October, 2004. The other unprotected house was studied for the load of insects on the farm. Broiler flocks reared under the study (18 in the screened houses, 18 in the unprotected houses) were surveyed for *Campylobacter* infection.

A descriptive study of insects. The load of insects on the houses was studied by the capture of insects (n = 7004) in net bags placed in ventilation air inlet channels, and (n = 1951) on sticky traps in the broiler room. All insects were speciated.

Campylobacter prevalence of flies. Flies (n = 2816) and pools of other insects than flies (n = 72) were collected in the farm surroundings and cultured for *Campylobacter*. *Campylobacter* isolates were tested in PFGE to investigate their clonality and epidemiology (results are not quite ready).

Results

The broiler flocks were significantly more often and earlier *Campylobacter* positive in unprotected houses than in screened houses ($P < 0.0279$). The screen caused a lag in *Campylobacter* infection time of two weeks ($P < 0.0001$), when an unprotected *Campylobacter* infected house was present in parallel. When an unprotected house was infected early in the rotation, a spread of *Campylobacter* to the corresponding screened house was seen late in the rotation, probably due to insufficient hygiene barriers. At slaughter, 8 of 18 unprotected flocks, and 5 of 18 screened flocks were positive, a 37% decrease in positive flocks in screened houses. However this effect was not statistically significant, possibly due to the few positive flocks and few participants.

The estimated load of insects on the houses was estimated from 2,233 to 180,300 insects per rotation in unprotected houses, of which 688 to 49,500 were flies. Flies constituted 21.1% of the insects collected in broiler houses, the family *Muscidae* alone 5.5%. The amount of flies that entered the unprotected houses correlated with the outdoor day temperature and with the broiler flock's need for ventilation air (no. m³/h). The insect screen reduced the number of insects in screened houses by 83.4% and the number of flies by 94.4%. A weak correlation was seen between the number of flies in unprotected houses and the risk of the particular broiler flock to become *Campylobacter* positive.

Vector flies in surroundings Totally 32 flies out of 2816 tested were found *Campylobacter* positive. The *Campylobacter* prevalence in flies varied considerably between fly species, farm and season (from 0.0 to 16.3%). Flies of *Muscidae*, including the common house fly *M. domestica*, which are associated with faeces, constituted 87.5% of all *Campylobacter* positive flies, and 5.5% of the load of insects on the broilers as mentioned above. In contrast, *Calliphorids*, which are associated with putrefaction such as in carcass containers, constituted 6.3% of the positive flies in the surroundings, and only 0.07% of the load of insects on the broilers houses. The majority (75%) of the positive flies was found on a farm with additional

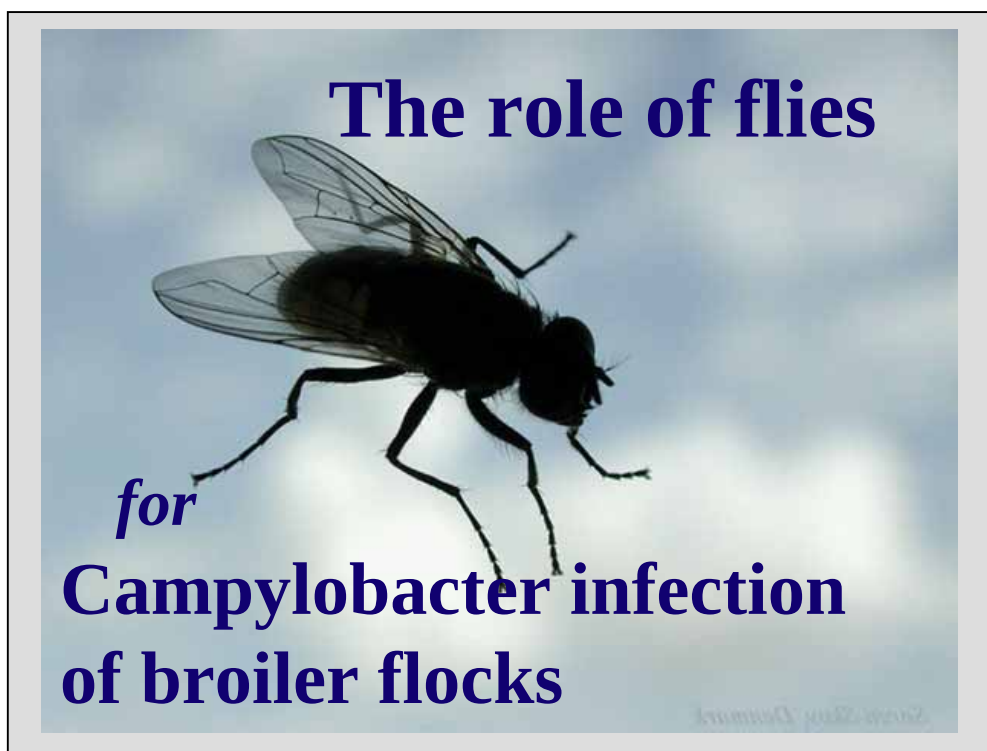
pig production, the rest was distributed on the other four farms, which had no production animals in the surroundings. The season was significant for *Campylobacter* prevalence of flies, 84% of positive were found in July and August.

Future steps

Insect screening might well be a significant future tool against *Campylobacter* in summer, but the assumption needs further consolidation. As indicated by our results, it seems crucial that all houses on a broiler farm are screened, and stress the importance of excellent hygiene barriers between broiler house and surrounding as well.

The variation in prevalence of vector flies, may well be the major cause of the variation in the seasonal distribution of *Campylobacter* infection and campylobacteriosis.

The intervention study was carried out in excellent cooperation with the private companies OK Miljø, Skov A/S, DACS A/S, Danpo A/S, Rose Poultry A/S, Lemberg APS, MT sprayteknik A/S, DanHatch A/S, and five broiler farmers in Jutland, Denmark.



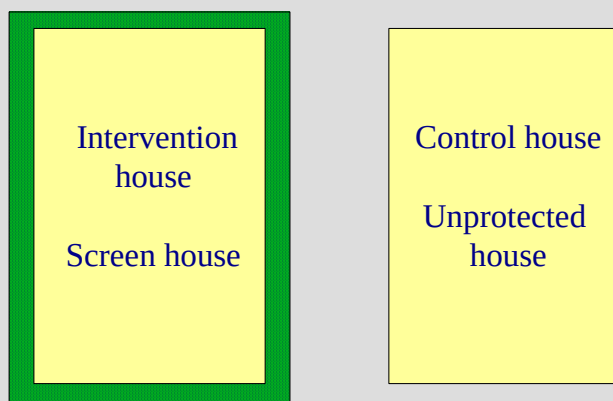
Intervention trial: insect screening, 2004

—Participants

- *Dansk Slagtefjerkræ*
- *Danish Institute for Food and Veterinary Research*
- *Danish Pest Infestation Laboratory,*
- *OK Miljø,*
- *Skov A/S,*
- *DACS A/S,*
- *Danpo A/S,*
- *Rose Poultry A/S,*
- *Lemberg APS,*
- *MT sprayteknik A/S,*
- *DanHatch A/S,*
- *Five broiler farmers in Jutland, Denmark.*

Intervention trial: insect screening, 2004

Design

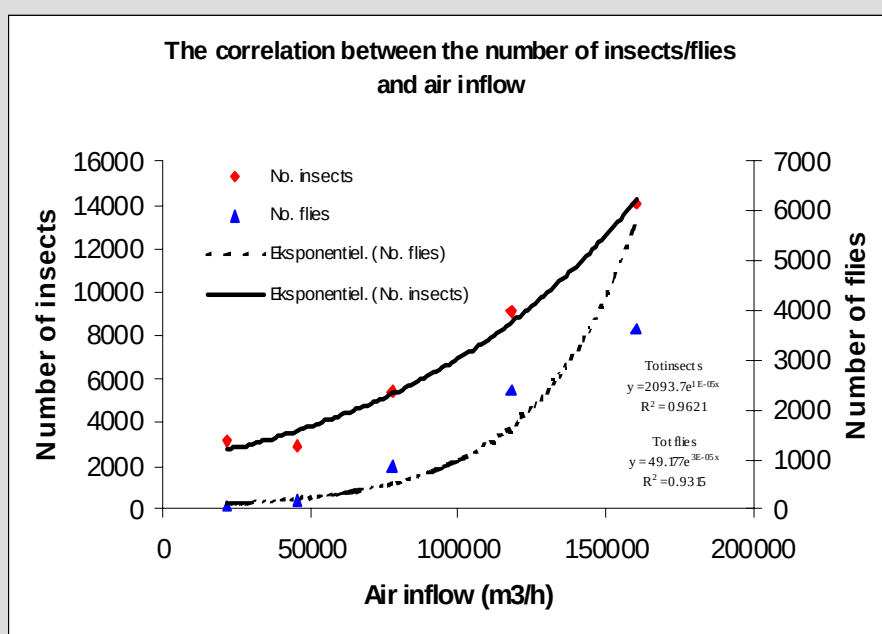


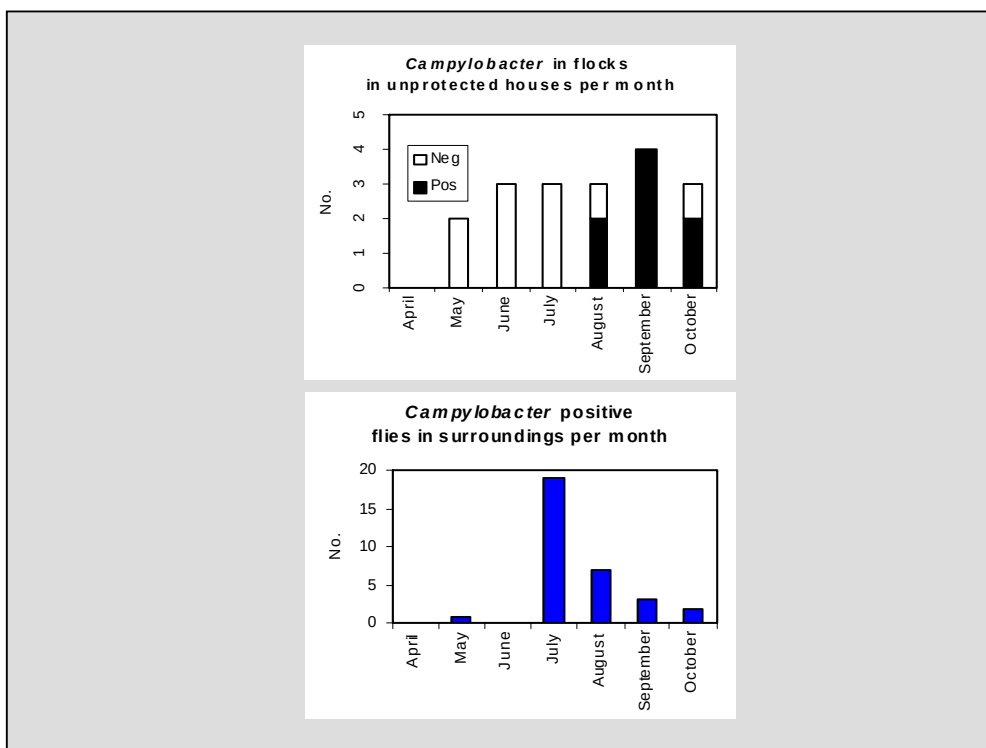
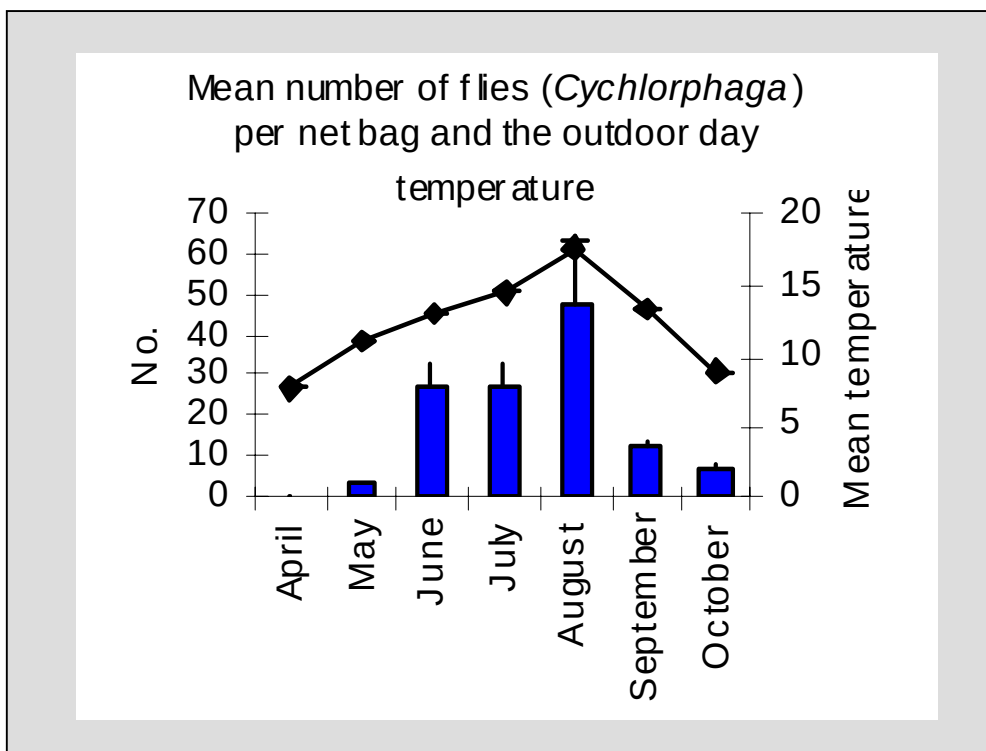
Campylobacter of flies in surroundings

Fly species	Total	+ Camp	Pos %
<i>Muscidae</i>			
House fly	782	21	2.7
Stable fly	271	4	1.6
False Stable fly	109	1	
Guldvinge	7	1	
Black dump fly	69	1	
<i>Calliphoridae</i>			
Sheep blow fly	338	2	0.6
Unspeciated	361	2	0.2
Negative sp.	879	0	
Total	2816	32	1.1

Total number of flies in surroundings on the 5 farms and % with *Campylobacter*

Farm	No. flies	No. Camp+	% Camp+
1	562	3	0.4
2	601	2	0.3
3	652	24	3.7
4	599	1	0.3
5	402	2	0.5
Total	2831	32	1,1





Cumulative % of infected flocks						
Day	0-7	8-14	15-21	22-28	29-35	35-S
Insect-screen houses	0	0	0	8.3	30.8	38.5
Unprotected houses	0	8.3	25.0	33.3	53.8	61.5

Results 2004

- Flies in surroundings
 - 0 – 16% Camp. Positive
 - Prevalence correlated with
 - Season - Peak in July/August
 - Fly species - The main risk flies were House fly and Stable fly
 - Type of farm - Additional production of pigs increased the prevalence

Results 2004

- Flies in broiler houses
 - 2,233 to 180,300 insects per broiler rotation of which 21.1% were flies and 5.5% *Muscidae*
 - The number correlated with
 - M3/h of inflow ventilation air
 - outdoor temperature

Results 2004

- Broilers *Campylobacter* infection
- Statistically significant effect of the interventions
 - Delay of 2 weeks in insect screened houses
 - 37% decrease in number Camp. Pos
 - A (weak) correlation between the number of flies in a rotation and *Campylobacter* positive flock at slaughter

Post-chill Campylobacter contamination of broiler carcasses in relation to degree of slaughter group colonization and chilling system

Mats Lindblad, Ingrid Hansson, Ivar Vågsholm, Roland Lindqvist

Data from an ongoing national surveillance program of *Campylobacter* prevalence in broiler slaughter groups were related to results from a one-year baseline study of broiler carcasses post-chill. The aims were to establish the relation between *Campylobacter* prevalence in slaughter groups and on carcasses, to evaluate the results of the surveillance programme compared to baseline study data and to determine the effect of different chilling systems on *Campylobacter* prevalence. Pooled cloacal and neck-skin samples were analyzed after enrichment in the surveillance programme. Carcass rinse samples from the baseline study were analyzed after enrichment and by direct plating. Data were available for 614 carcasses. The results show that surveillance data at a flock level are relevant for estimation of *Campylobacter* carcass prevalence, although lower within-flock prevalence than 100% can be expected especially in low-colonized slaughter group. No consistent differences between different chilling systems in their effect on *Campylobacter* prevalence post-chill were observed, but the prevalence was significantly reduced at one slaughterhouse using an air-chilling system.



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Post-chill *Campylobacter* contamination of broiler carcasses

Mats Lindblad, Ingrid Hansson, Ivar Vågsholm,
Roland Lindqvist

Nordic meeting, Uppsala, 12-13 april 2005



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Data from two studies

Surveillance
programme

Baseline study
2002-2003

Arrival Pre-chill
per slaughter group

Post-chill
per carcass





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Aims

- ➡ Establish the relation between slaughter group prevalence and carcass prevalence
- ➡ Examine the effect of different chilling systems on *Campylobacter* prevalence post-chill



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Analysis methods

- Surveillance programme:
 - cloacal and neck skin samples
 - enrichment in Preston broth
 - plating on Preston agar
- Baseline study:
 - carcass rinse samples
 - enrichment in Preston broth
 - plating on CBFS
 - direct plating on CBFS



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Species identification of carcass isolates

1. Real time PCR → *C. Jejuni*



2. Therm PCR → Therm. *Campylobacter*



Neg PCR



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Number of samples

Data from both studies available for 614 carcasses, mainly from six slaughterhouses

Colonization:
none / low / high

Pos/neg
Level



Chilling:
water / air /
evaporative





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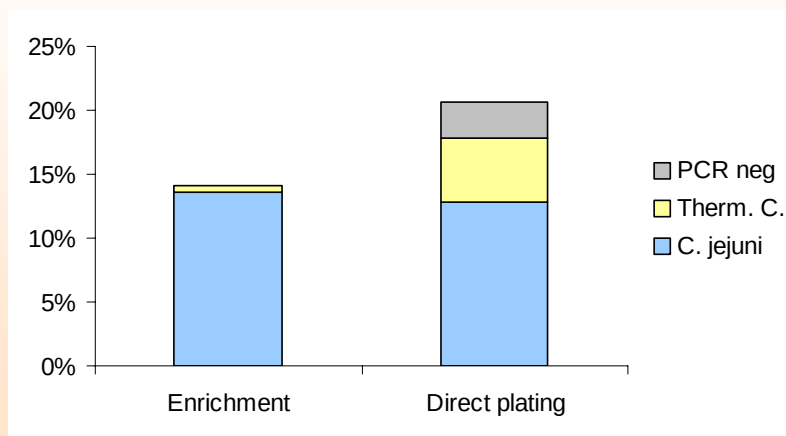
Overall *Campylobacter* prevalence

- 17 % of the 614 carcasses originated from slaughter groups with positive cloacal or neck skin samples
- 14 % tested positive after enrichment
- 21 % tested positive after direct plating



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Species distribution of carcass isolates

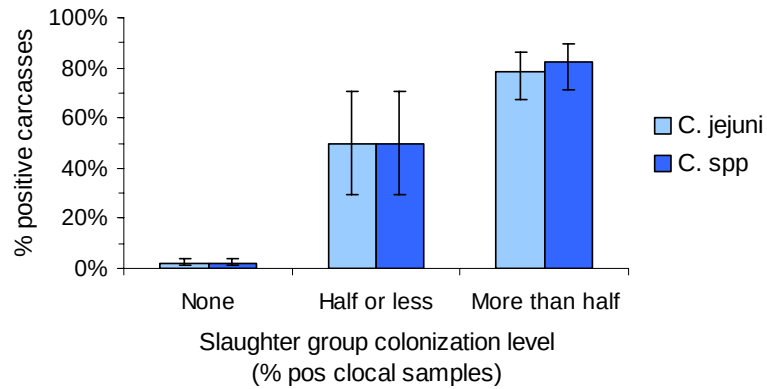




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Post-chill prevalence in relation to slaughter group prevalence

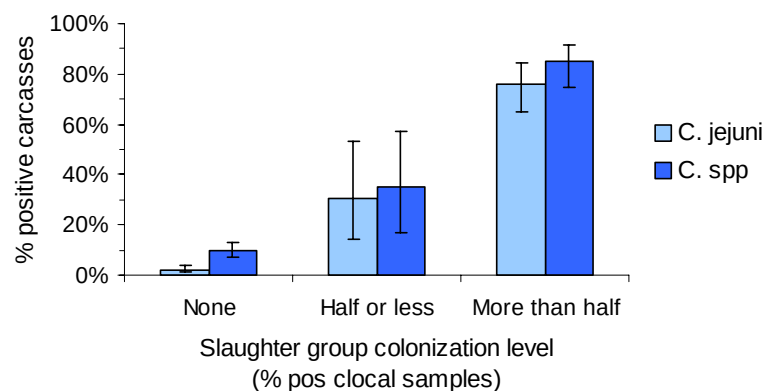
Enrichment analyses



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Post-chill prevalence in relation to slaughter group prevalence

Direct plating

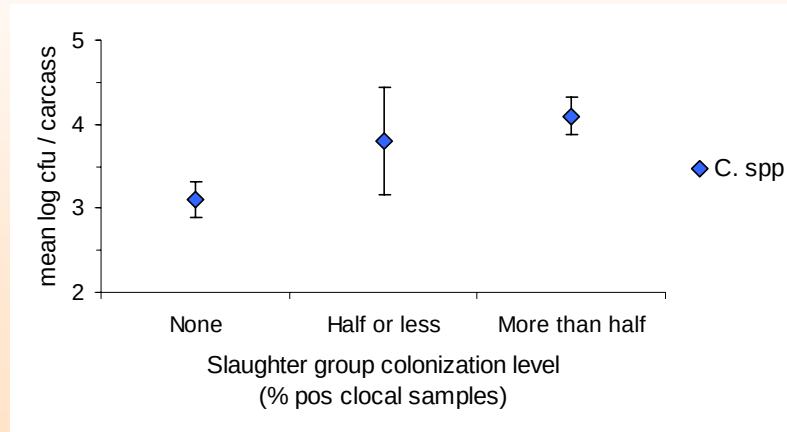




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Campylobacter levels in relation to slaughter group prevalence

Direct plating, levels on positive samples



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Prevalence pre- and post chill at different slaughterhouses

Null hypothesis:

Carcasses originating from positive slaughter groups are positive after chilling

Effect of interest:

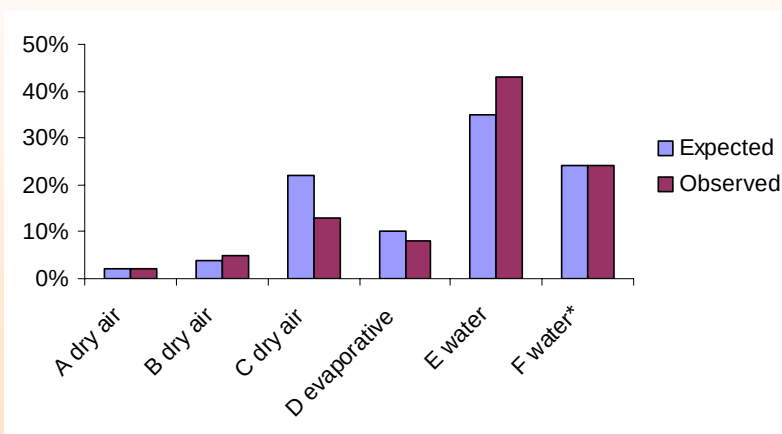
Chilling at different slaughterhouses

Data from enrichment analyses only



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Prevalence pre- and post chill at different slaughterhouses



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Summing up

- the prevalence of *Campylobacter* on carcasses from slaughter groups with negative cloacal samples was low
- increasing prevalence with increasing slaughter group colonization levels – up to about 80 % contaminated carcasses
- no consistent effect of chilling on post-chill prevalence – differed between slaughterhouses

Genotyping of Campylobacter from Broilers and Environment by AFLP

Gro Johnsen

During the summer season 2004, *Campylobacter* were isolated from broilers and different sites in the broiler house and outdoor environment of four broiler farms. The genetic diversity and clonal patterns of the isolates were investigated using amplified-fragment length polymorphism (AFLP). A high number of different genetic profiles were found. Only two out of eight broiler profiles were isolated from outdoor environment prior to infection. *Campylobacter* bacteria were regularly present in the drinking water and the environment surrounding the chicken farms. Isolates belonging to the same clone were present for at least six weeks. This shows that drinking water and farm environment constitute a constant source of infection, and stresses the importance of water disinfection and sufficient hygienic barriers between the environment and chickens. Detailed results will be presented at the meeting.

Genotyping of *Campylobacter* from Broilers and Environment by AFLP

Norwegian Zoonosis Centre
National Veterinary Institute
Gro Johnsen
Zoonoses Workshop, Uppsala April 2005



Introduction

- Background
 - *Campylobacter* an emerging human pathogen
 - Poultry a known risk factor
 - Environment significant infection source for broiler
- Aim
 - Characterize the genetic diversity of *Campylobacter* in
 - broilers
 - broiler house
 - outdoor environment
 - Describe transmittance routes
 - Suggest preventive measures



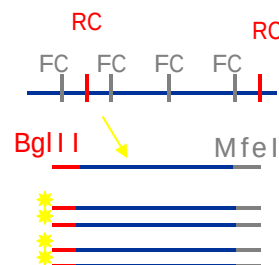
Material and methods

- Sampling
 - Place: 4 farms in Hedmark County
 - Time: 9 visits in May – September 2004
 - Sampling:
 - Broilers: One July flock at each farm
 - 5 sites inside broiler-house
 - 9-13 sites in outdoor environment
- Methods
 - Culturing: NMKL 19 (2002), ISO-DIS 17995 (2003)
 - Fingerprinting by AFLP

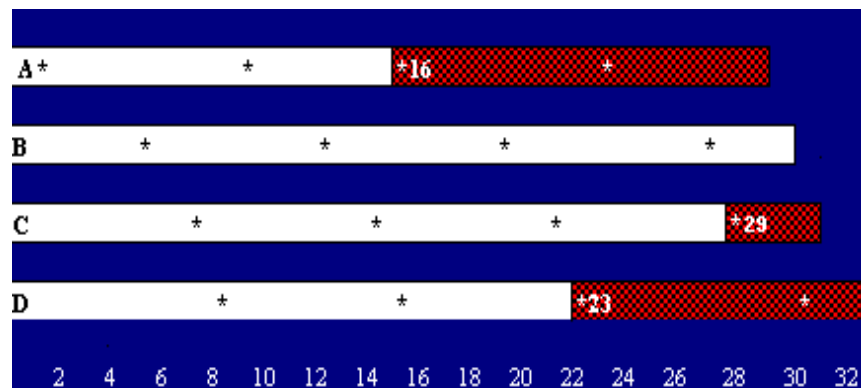


Amplified Fragment Length Polymorphism

- Cutting: BglIII (RC) and MfeI (FC) restriction endo-nucleases
- Adaptor ligation: 0.1% BglIII RC-adaptor, 1% MfeI FC-adaptor and T4 DNA ligase
- Amplification: FAM labelled BglII-primer, unlabelled MfeI-primer, Taq-polymerase
- Detection: Capillary electrophoresis on ABI-310 Genetic Analyzer (PE Biosystems) with TAMRA-500 as internal standard: 22 fragments in range 50 – 500 bp
- Analysis: Computer program: GelCompar II (Applied Maths, Belgium)

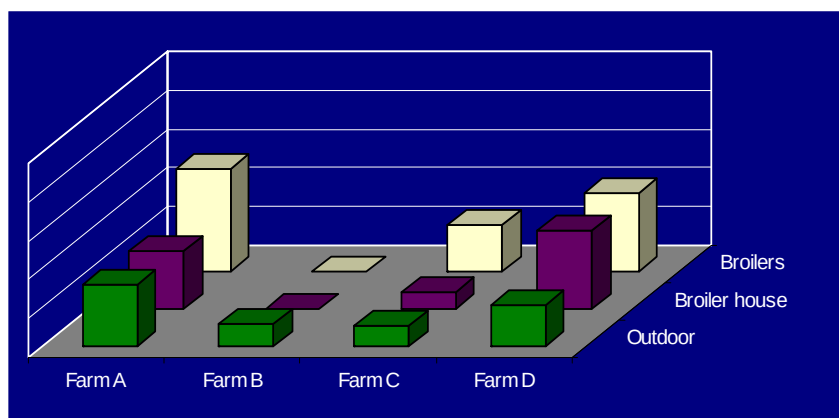


Campylobacter infection of broilers

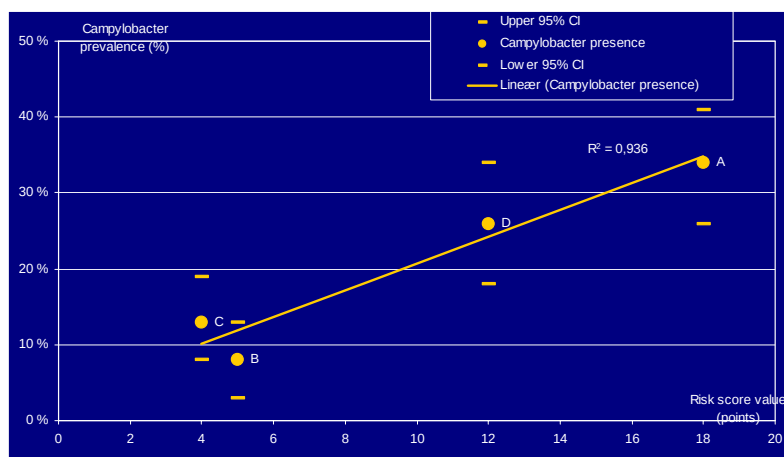


* Sampling day

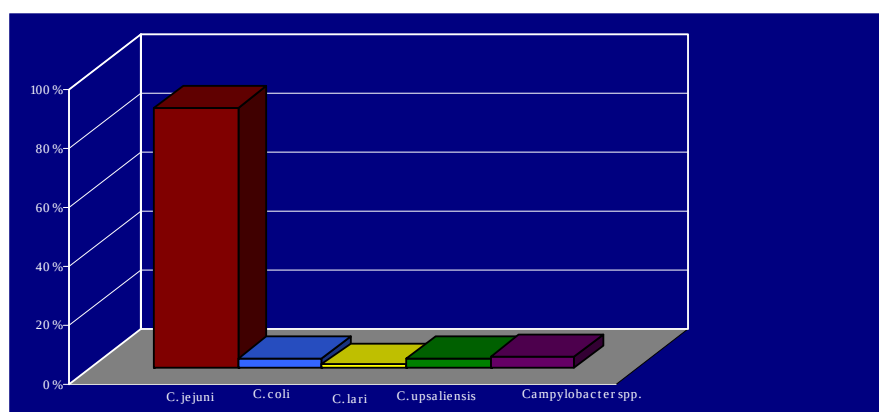
Campylobacter frequency (%)



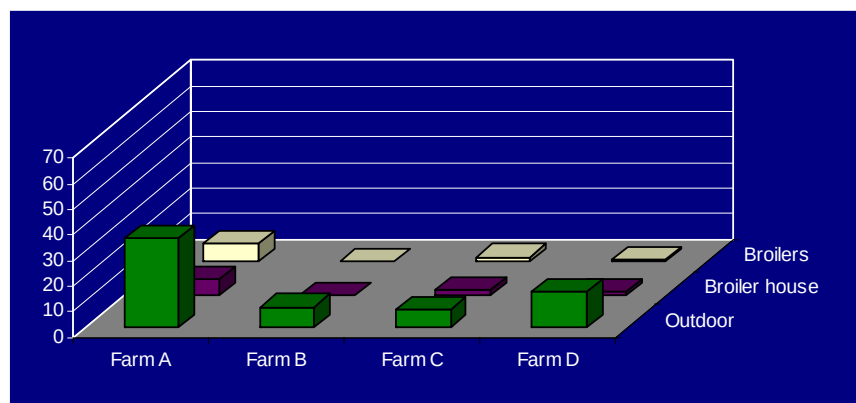
Campylobacter frequency as function of risk score values



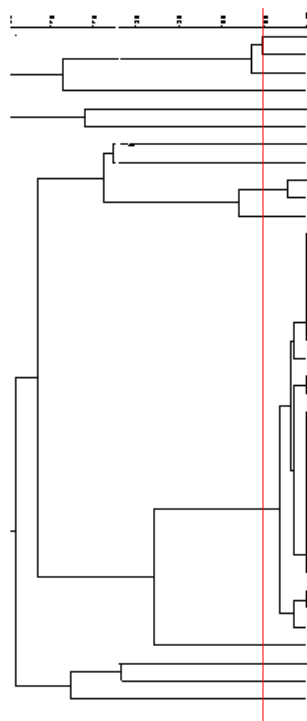
Distribution of species



Number of different profiles



Veterinärinstitutet
National Reference Laboratory



Farm D	Soil-houl	Week 38
Farm D	Wall water	Week 38
Farm D	Standing water	Week 38
Farm D	Bird Dropping	Week 27
Farm D	Soil-houl	Week 24
Farm D	Standing water	Week 24
Farm D	Soil-houl	Week 22
Farm D	Bird Dropping	Week 22
Farm D	Environmen	Week 38
Farm D	Environmen	Week 38
Farm D	Wall-Water	Week 38
Farm D	Chicken caecal dropp	Week 31
Farm D	Chicken caecal dropp	Week 32
Farm D	Chicken caecal dropp	Week 32
Farm D	Wheel uddide	Week 32
Farm D	Chicken caecal dropp	Week 32
Farm D	Cleanpartiale room	Week 32
Farm D	Feeding device	Week 32
Farm D	Chicken caecal dropp	Week 31
Farm D	Anle room environmen	Week 31
Farm D	Feeding device	Week 31
Farm D	Cleanpartiale room	Week 31
Farm D	Chicken caecal dropp	Week 31
Farm D	Risale water	Week 31
Farm D	Insects in chickenhouse	Week 31
Farm D	Chicken caecal dropp	Week 31
Farm D	Anle room floor	Week 31
Farm D	Risale water	Week 31
Farm D	Environmen	Week 31
Farm D	Drinking device	Week 31
Farm D	Farmers inhouse shoe	Week 31
Farm D	Farmers inhouse shoe	Week 32
Farm D	Drinking device	Week 32
Farm D	Chicken caecal dropp	Week 31
Farm D	Bird Dropping	Week 24
Farm D	Risale water	Week 30
Farm D	Risale water	Week 30
Farm D	Bird Dropping	Week 29

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Common profiles between farms

- Profile i) *C. jejuni*
 - Farm B: wild birds week 29
 - Farm C: wild birds week 29
 - Farm A: water week 30
- Profile ii) *C. jejuni*
 - Farm B: environment week 29
 - Farm C: wild birds week 24 and 26
- Profile iii) *C. jejuni*
 - Farm C: dog week 32
 - Farm A: dog, environment and water week 29, 27 and 31
- Profile iv) *C. jejuni*
 - Farm A: broiler-house and broiler week 31-33, environment week 33
 - Farm C: broiler-house and broiler week 32-33
- Profile v) *C. jejuni*
 - Farm D: water week 30
 - Farm A: wild birds week 31



Interesting findings

- Broiler house: Same profiles at the same time as in broilers
- Water: Brook Farm A (n=25): 21 different profiles
- Dog (n=9): 7 different profiles (4 *C. upsaliensis*, 3 *C. jejuni*)
 - Within farm: 1 profile with 2 isolates (Farm C)
 - Between farm: 1 profile with 2 isolates (Farm A & C)
- Wild birds (n=16):
 - Within farm : 1 profile with 2 isolates (Farm C)
 - Between farm : 1 profile with 2 isolates (Farm B & C)
- Environment:
 - Mainly different profiles
 - A few profiles also found in dog (n=2), wild birds (n=4), water (n=3)
- Transmittance to broilers
 - Broiler-profiles only found in environment simultaneously or later than in broiler
 - Farm A: Outdoor boots week 27 and broilers (and dog) week 32
 - Farm A: Courtyard puddle week 27 and broilers week 31



Conclusion

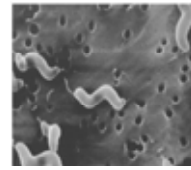
- Earliest infection of broilers on farm with highest risk score value
- Common profiles between environment and wild bird / environment and dog
- One profile found in environment after 6 weeks
- High number of profiles present in water and environment
- Water and environment constitute a constant infection stress with regard to Campylobacter
- Important preventive measures
 - Disinfection of drinking water
 - Hygienic barriers between environment and broilers

Genotyping of Campylobacter to understand contamination sources and kinetics of chicken flocks and role of chicken campylobacters in human infections

Marja-Liisa Hänninen

Genotyping of chicken *Campylobacter jejuni* and *C. coli* strains/isolates isolated from various sources has been applied for approximately ten years period. The methods used vary from pulsed-field gel electrophoresis (PFGE) and amplified fragment length polymorphism (AFLP) which measure diversity at whole genome level to methods (e.g. PCR RFLP of *flaA* and *flab* genes) which measure diversity at a gene level. Application of these typing methods has increased very much our understanding on the general diversity of *C. jejuni* populations occurring in various niches, such as in the intestines of various animal hosts or human patients. We have been able to identify the similarity of genotypes occurring in the intestinal contents of several animal species and those isolated from human patients confirming the significance of animals as sources in human infections. *C. jejuni* is commonly associated with waterborne outbreaks in the Nordic countries and genotyping has been enabling us to show the similarity of the isolates from human patients and drinking water sources. We have also been able to show that each year some of the genotypes are predominant at simultaneously in certain animal sources, especially in chickens, and in human patients acquired their infections from domestic sources. In addition, some of the uncommon genotypes occur only in animals, never seen in among human isolates, suggesting a strict host microbe association for these genotypes. Systematic collection of domestic *Campylobacter* strains which have both spatial and temporary association from various sources should continue and genotyping studies of these collections should continue. Using this approach a more extended picture on the epidemiology of *Campylobacter* infections in humans as well as on transmission and sources of contamination of chicken flocks will be achieved. Comparative studies on the temporary similarity of *C. jejuni* genotypes in the Nordic countries are needed in order to understand the similarity/dissimilarity of the epidemiology of *Campylobacter* infections in the Nordic countries.

Molecular typing – Campylobacter jejuni



– Ten years perspective

Marja-Liisa Hänninen

Department of Food and Environmental Hygiene B.O. Box 66
Helsinki University

Reviews on topic

- Wassenaar & Newell, 2000
 - Maiden (MLST) 2003
 - Feil et al. (eBUST) 2004
 - vanBelkum 2003
-

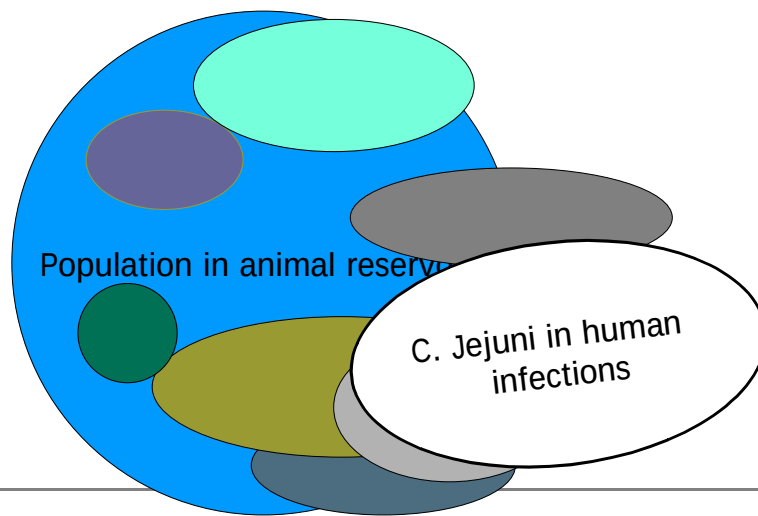
Short history

- 115 outputs on PubMed (molecular typing/Campylobacter)
- 1993: Flagellin gene typing (Nachamkin)
- 1993: Ribotyping (Owen et al)

IMPACT OF GENETICS ON EPIDEMIOLOGY and virulence

- BIOLOGY – POPULATION BIOLOGY
 - adaptation to a wide spectrum of hosts
 - adaptation of virulence
 - all all strains similar potential in virulence ?
- CHARACTERISTICS OF STRAINS VIRULENT TO HUMANS

C. jejuni population biology

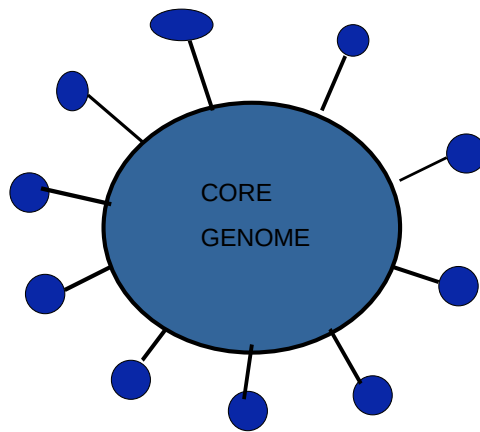


C. jejuni

- high genetic diversity in C. jejuni population
 - a. intraspecies homologous recombination
 - b. interspecies recombination
 - c. point mutations
 - d. natural competence to transformation



Structure of genome –interpretation of the results



- a. CORE GENOME
- b. ACCESSORY GENES

appr. 20% of genes are strain specific (Dorrell et al.)
appr. 36% (599) of the genes of *C. jejuni* NCTC 11168 were variable at least in 1 of 97 strains studied (Taboda et al. 2004)

Targets of typing and analysis

- 1. **IN OUTBREAKS** SHOWING SIMILARITY OF STRAINS ISOLATED FROM SUSPECTED SOURCE AND HUMAN PATIENTS
- 2. COMPARISON OF GENOTYPES OF STRAINS ISOLATES FROM VARIOUS SOURCES/RESERVOIRS TO REVEAL A POTENTIAL IMPACT OF VARIOUS SOURCES IN THE EPIDEMIOLOGY OF A. **HUMAN INFECTIONS (ZOOONOTIC TRANSMISSION)** B. **CONTAMINATION OF FOOD PRODUCTION CHAINS (CHICKEN)**
- 3. POPULATION BIOLOGY: SPATIAL AND TEMPORAL CHANGES IN POPULATION AND ASSOCIATION TO CHANGES HOSTS ?

Requirements for studies

- 1. Sampling
- - number and origin of strains
- - temporary and spatial association
- -background knowledge of the strains
- - method development: outbreak associated and non-associated strains

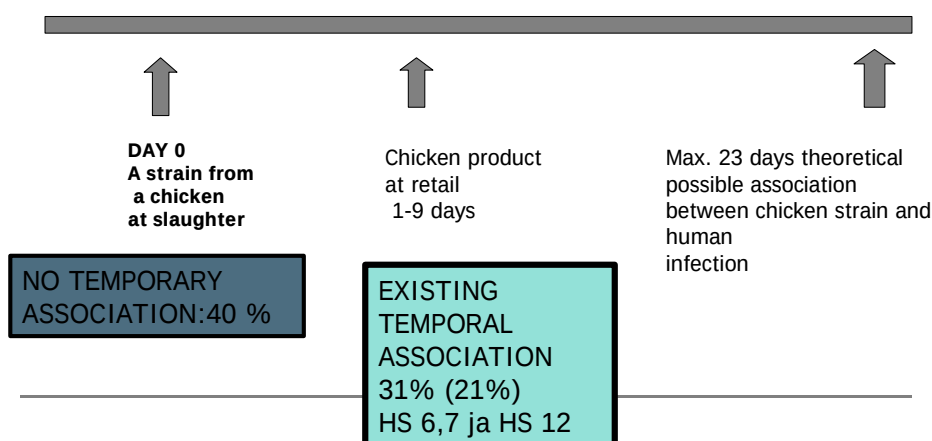
MLST typing (www.campylobacter.mlst.net)

- MLST types of > 2000 *C. jejuni* strains
- high diversity of types: appr. 800 ST types
- clonal complexes
- population genetic studies: population structure
- genotyping: not good for short term epidemiology (Sails et al. 2003)

MLST

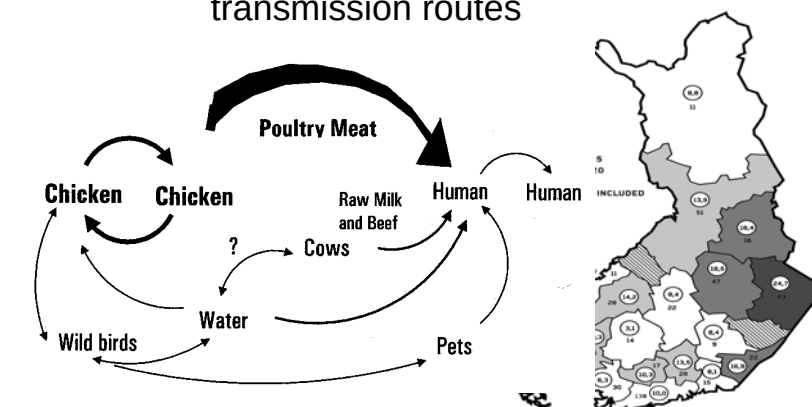
- Clonal complexes + single STs
- Clonal complexes ST 21 and ST 45 common and contain strains from human patients as well as from animal sources
- some STs seem to be source associated
- **A example (Dingle et al. 2002):**
 - - SIX clonal complexes covered 60% of human disease isolates (ST-21, ST-45, ST-206, ST-61, ST-48, ST-257) and these complexes were also common among food and environmental isolates

A model: spatial and temporary association of human and chicken strains in Finland in summer 1999



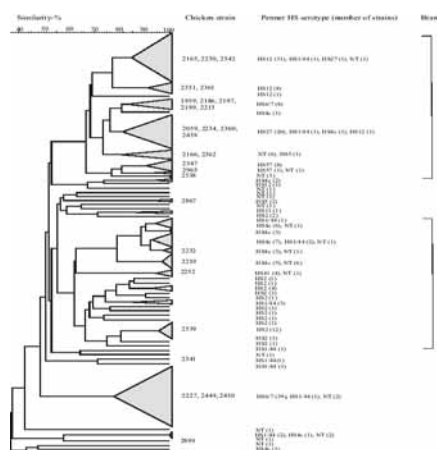
C. jejuni transmission – the 1999 model

- Circulation of different strains and many transmission routes



Comparison of chicken and human sero- and genotypes in Finland (Kärenlampi et al. 2003)

- 30 chicken and 208 human isolates
- 46% related sero- and genotypes
- Temporal association (2-23 days) reduced overlap to 31%
 - Human isolates prior to slaughter of positive flock
- Role of chicken as the source of human infection somewhat controversial



Conclusisons

- Evaluation of the experience of the use of molecular typing
- Continuation typing with selected method for strains with well-caharacterized backbround in order to find a perspective from a longer period of time on genotypes occurring in a country

Ongoing Danish research activities on interventions to control Campylobacter

Hanne Rosenquist, Vinni Mona Hansen, Tina Birk, Anja Bengtsson, and Bjarke Bak Christensen

At the Danish Institute for Food and Veterinary Research several research activities concern intervention techniques to control *Campylobacter* in the broiler chicken production. In the primary production, insect control, phage therapy and feeding strategies are areas of focus. During processing and post-processing the effect of steam-ultrasound treatment of carcasses, the gas composition in MA-packed products, and marinating ingredients such as organic acids, salt, and herbs are being examined. Further, projects aimed at supporting the intervention work are carried out. These include development of a NMKL method for quantification of *Campylobacter*, PCR based methods for rapid detection and enumeration of *Campylobacter*, and finally, model systems in which the reducing effect of interventions can be examined in small-scale.

Most of the research activities on interventions are still in their infancy, and no final conclusions have been reported as yet. However, preliminary results have indicated that phage therapy may be a potential intervention strategy based on in vitro studies, and that addition of lactic and acetic acid to chicken meat has shown potential as *Campylobacter* reducing ingredients.

With regard to the supporting projects, both traditional and PCR based methods have been developed for detection and enumeration of *Campylobacter*, and food model systems comprising a chicken juice model, a chicken skin-, and a chicken meat model have been set up. Further, a green fluorescent gfp-mutant has been constructed to visualize the contamination of *Campylobacter* on chicken skin and chicken meat.

The research activities mentioned as well as new intervention studies will be integrated in a newly funded project (2005-2008), which aims at identifying optimal reduction strategies for the control of *Campylobacter* in the Danish broiler chicken production. Options to control *Campylobacter* from rearing of broilers to consumption of chicken meat will be given in consideration of reducing ability, technical feasibility, costs, public perception of *Campylobacter* risk, the public acceptance of suggested reduction methods, the consumer's willingness to pay for low-risk products, and the saved socio-economic costs due to the expected lower number of human infections.

We hope that the outcome of the research activities in a few years will lead to a marked decrease in the incidence of human campylobacteriosis.



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Ongoing Danish research activities on interventions to control *Campylobacter*

- at the Danish Institute for Food and Veterinary Research

Hanne Rosenquist, senior scientist

Focus will be on activities at the Dep. of Microbiological Food Safety carried out by

Research assistant, Vinni Mona Hansen

Research assistant, Tina Birk

Bachelor student, Anja Bengtson



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Overview of ongoing research activities 1

Primary production

- Intervention studies
 - Feeding strategies
 - Insect control (flies)
 - Phage therapy
- Supporting projects
 - Development of PCR methods for rapid detection and enumeration of *Campylobacter*



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Overview of ongoing research activities 2

Processing / post processing

- Intervention studies
 - Steam-ultrasound treatment of carcasses
 - Gas composition of MA-packed products
 - Marinating ingredients (organic acids, salt, herbs)
- Supporting projects
 - Development and validation of a new NMKL method for quantification of *Campylobacter*
 - Development and evaluation of food models



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Phage therapy

Research assistant, Vinni Mona Hansen

Partners: Danish Institute for Food and Veterinary Research
Copenhagen University
Danish Poultry Association

Funding: DFFE



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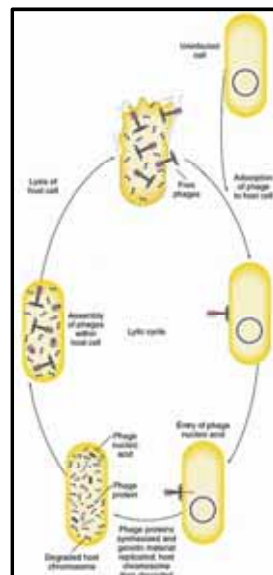
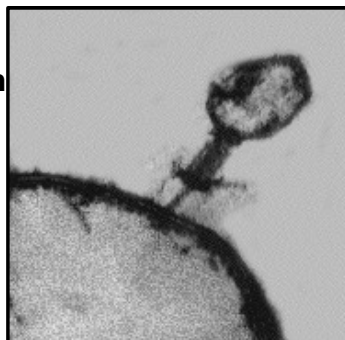
Main objective

To develop a product, that can be added to drinking water or feed and cause a reduction of the numbers of *Campylobacter* in the intestines of broilers.



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The phage reproduction cycle (lytic)





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Outcome of the project 1

- 308 samples have been collected from chicken intestines (228) and process wastewater (80)



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Outcome of the project 2

- 14 phages from intestines and 9 phages from wastewater were isolated using a plaque-assay
- Campylobacter strains with the serotypes most often isolated from patients and broilers (1,44; 2 and 4c) were sensitive towards several of the isolated phages





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Conclusion

The results obtained so far indicate that phage therapy may be potential intervention strategy, but more research in live animals is necessary to confirm this.



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Marinating ingredients –organic acids

Bachelor student, Anja Bengtsson

Objective:

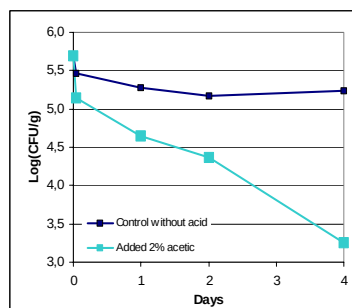
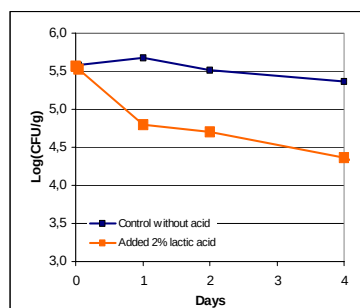
- to examine the reducing effect of organic acids on the number of *Campylobacter* on chicken breast filets



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Organic acids –preliminary results

Campylobacter counts in chicken breast meat artificially inoculated and following added 2% lactic or acetic acid



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Development and evaluation of food models

Research assistant, Tina Birk

Partners: Danish Institute for Food and Veterinary Research
The Royal Veterinary and Agricultural University

Funding: DFFE

Objective:

- to develop food models to study interventions in small scale



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Models that have been set up and examined

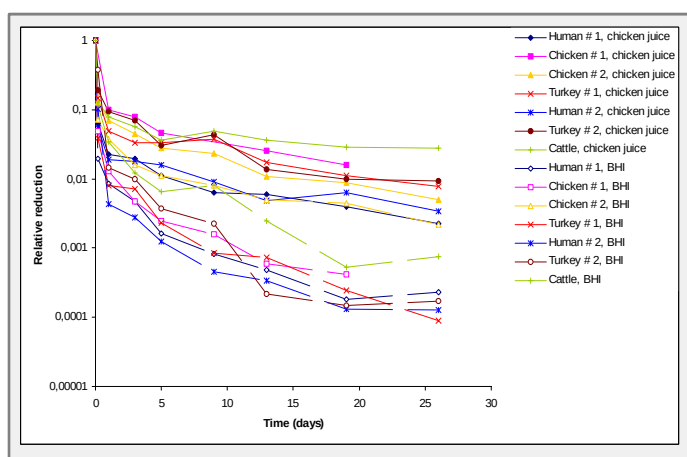
- A chicken juice model
 - Sterilized chicken drip fluid obtained during thawing of frozen chickens
- A chicken skin model
 - Small skin pieces
- A chicken meat model
 - Small meat pieces



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Food models – preliminary results

Relative reduction of different *Campylobacter jejuni* strains at freezing temperature (-18°C) in the chicken juice model system and BHI





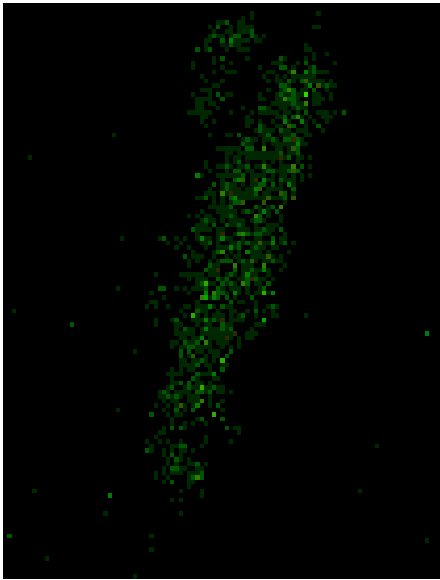
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Visualization of *Campylobacter* contamination

Objective: To identify where *Campylobacter* organisms are located on the meat/skin

Preliminary results:
A green fluorescent *gfp*-mutant has been constructed





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New funding

Title: Risk perception and cost benefit analysis of interventions to control *Campylobacter*

Funding: 10 mill. DKKr in 2005-2008

Partners: Danish Institute for Food and Veterinary Research
The Royal Veterinary and Agricultural University
Danpo A/S



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Objectives 1

1. to test the reducing effect of different interventions in the broiler chicken production by screening a wide range of intervention techniques in model systems
2. to examine the applicability and the effectiveness of the most promising interventions in large scale production
3. to identify public perception of food risk reduction strategies and the effect of these on consumer behaviour and their willingness to pay



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Objectives 2

4. to identify the most cost-effective reduction strategy by answering; what are the effective reduction strategies and what are the costs of implementing them
5. to make specific recommendations for future risk management by evaluating the sociological, economic, and microbiological aspects of reduction strategies



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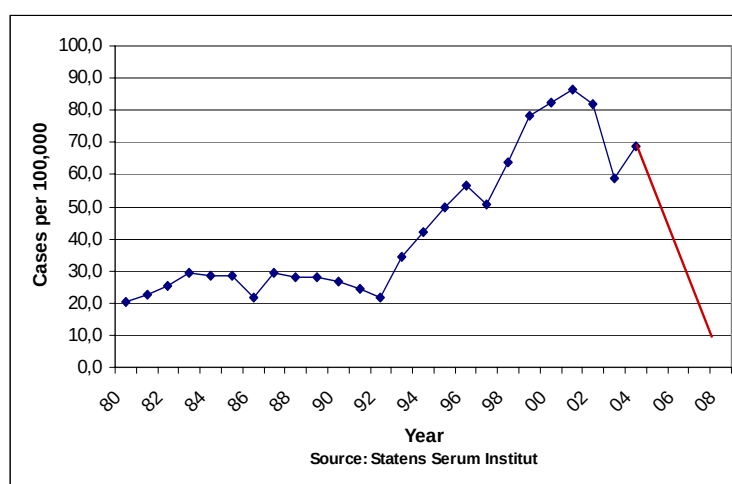
"New" interventions to be studied

Process	Interventions	Model systems ^a	Detection and enumeration of Campylobacter
Primary production	Organic acids, ethereal oils, etc. added to the feed or water. Prevention of insects from entering the broiler houses	Laboratory chickens, Investigations in broiler houses	Recently proposed NMKL-method Quantitative RT-PCR based rapid method ^c
Processing	Improved washing procedures including cold or hot water, treatment with steam, chemicals, freezing and chilling ^b	Chicken skin model Chicken meat model	
Post processing	Compounds such as salt, acid, alcohols. CO ₂ /oxygen ratio	Chicken juice model	



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Expected outcome of the intervention studies



Designing challenge trials with Campylobacter in chickens

Magne Kaldhusdal

Based on a literature study (see References) and general considerations, various ways of performing challenge trials with *Campylobacter* in chickens are presented and discussed.

Housing conditions may determine whether the study is based on individually housed birds or not, which may influence the type of outcome variable and statistical analysis. Interior design and ventilation system may influence the risk of inadvertent transmission by air of bacteria between birds and treatment groups. Housing conditions used in reviewed papers were very variable.

Feed, unused litter material and day-old chicks are considered unlikely to be sources of infection, which means that commercial suppliers of these items can be used in challenge trials. The literature included in this overview does not clarify whether *Campylobacter* colonization is affected by the presence of in-feed antibiotics, including ionophores. The significance of maternal antibodies is another unsettled matter.

Drinking water equipment may harbour *Campylobacter*, and should therefore be cleaned and disinfected before placement of the birds. The drinking water source must be free of *Campylobacter*.

Examinations for environmental *Campylobacter* should include experimental premises, drinking water equipment and pre-inoculation samples from birds.

Cross-contamination between housing units and treatments should be reduced as much as possible by means of strict hygienic procedures regarding staff activities, as well as combating of insects.

Age of birds at challenge varies between day-old and day 38, but most birds were challenged after day 9. Field birds appear resistant to colonization up to at least day 10.

Challenge strains used in examined references were mostly isolated from chickens, but other isolates were also used with success. Number of strains used per bird varied from 1 to 10. Colonization potential varies with strain, and may be enhanced through in-vivo passages.

Challenge dose varied between one single and 100 millions colony-forming units (CFU) per bird, but a lower limit of successful experimental colonization of 40 CFU per bird is suggested.

Design of reviewed studies varies much. Number of birds per housing unit varied from 1 to 12, and number of housing units per treatment varied from 1 to 10. Non-inoculated control groups were not always included.

Outcome variables used were indicators of enteric colonization. Most experiments used a binary outcome (infected or not), but quantitative outcomes were also commonly used.

Specimen types used for outcome evaluation included cloacal swabs, caecal contents, caecal tissue and blood sera. Caecal contents and cloacal swabs were used to enumerate *Campylobacter*.

Based on these findings and considerations, a pilot study is planned. We want to compare the results of examining cloacal swabs, faecal specimens and gut contents for *Campylobacter jejuni*. In this way we hope to learn more about the relationships between specimen type, outcome variable, design and statistical analysis.

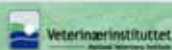
Experimental *Campylobacter jejuni* infection in chickens: **How is it carried out ?**

Magne Kaldhusdal, National Veterinary Institute Oslo, Norway



Contents of the presentation

- Housing
- Feed
- Birds
- Hygienic precautions
- Challenge strains
- Inoculum and inoculation
- Outcome variables
- A preliminary study protocol



Literature

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- Cawthraw, S. et al. "Isotype, specificity, and kinetics of systemic and mucosal antibodies to Campylobacter jejuni antigens, including flagellin, during experimental oral infections of chickens." *Avian Dis.* 38.2 (1994): 341-49.
- Chaveerach, P. et al. "Effect of organic acids in drinking water for young broilers on Campylobacter infection, volatile fatty acid production, gut microflora and histological cell changes." *Poult.Sci.* 83.3 (2004): 330-34.
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- Ringoir, D. D. and V. Korolik. "Colonisation phenotype and colonisation potential differences in Campylobacter jejuni strains in chickens before and after passage in vivo." *Vet.Microbiol.* 92.3 (2003): 225-35.
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- Sahin, O. et al. "Prevalence, antigenic specificity, and bactericidal activity of poultry anti-Campylobacter maternal antibodies." *Appl.Environ.Microbiol.* 67.9 (2001): 3951-57.
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- Wassenaar, T. M. et al. "Colonization of chicks by motility mutants of Campylobacter jejuni demonstrates the importance of flagellin A expression." *J.Gen.Microbiol.* 139 Pt 6 (1993): 1171-75.

Is housing important?

- Floor type
- Number of birds per m²
- **Number of birds per group** (design and outcome)
- **Ventilation and air flow** (aerosols)
- **Design and localization of housing units** (cross contamination)

Feed and feed additives

- No likely source of infection (Berndtson, E. et al." Preventive Veterinary Medicine 26 (1996): 167-85.)
- Effect of antibiotic growth promoters?
- Effect of anticoccidials?
 - ionophores

Birds

- Newly hatched chicks free ? (Newell, D. G. and C. Fearnley
Appl. Environ. Microbiol. 69.8 (2003): 4343-51)
- Chicks resistant to colonization up to day 10 ? (Ringoir, D.
D. and V. Korolik. Vet. Microbiol. 92.3 (2003): 225-35.)
- Protective effect of maternal antibodies ?

Hygienic precautions

- Feed, litter and birds: no problem?
- Water?
- The experimental facility
- Human activity
- Insects
- Aerosols

Challenge strains

- Origin
- One strain or several strains?
- Colonization potential
 - *In vitro* passages
 - *In vivo* passages

Outcome variables

- Unit of concern
 - Group
 - Individual
 - identity known?
- Specimen type
 - gut contents
 - cloacal swab
 - faeces
 - small intestinal
 - caecal
- Quantitative or qualitative?

A preliminary study protocol (1)

- Housing
 - Cages
 - Litter floor
 - Open front, otherwise closed
- Feed
 - Commercial starter with ionophores
 - Commercial finisher during the last 5 days
- Water
 - Municipal chlorinated tap water
- Litter material
 - Commercial type intended for experimental animals
- Birds
 - Commercial day-old broiler chickens

A preliminary study protocol (2)

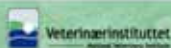
- Examinations for environmental Campylobacters
 - Premises
 - Water tank and pipes
 - Humid places
 - Water
 - Birds
 - Litter material?
- Hygienic precautions
 - Personnel
 - Feeding
 - Insects

A preliminary study protocol (3)

- Inoculation
 - Inoculate
 - Dosage
 - Bird age at inoculation: day 14
- Post-challenge sampling
 - Days 1, 4, 10 and 15 post inoculation
 - Cloacal swabs, faeces or gut contents?

A preliminary study protocol (4)

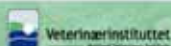
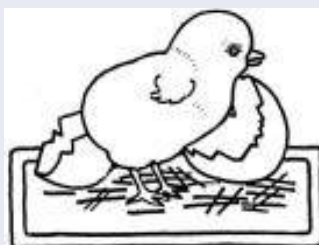
- Design
 - unit of concern: the individual chicken
 - number of birds per cage: 4
 - number of cages per treatment: at least 2
 - number of birds per treatment: at least 8
 - unchallenged control birds
 - outcome variable: quantitative (?)
- Statistical analysis
 - dependent on outcome variable



THANK YOU FOR YOUR ATTENTION

Acknowledgment:

Anna Marie Bratberg, Kofitsyo Cudjoe,
Anne-Gerd Gjevre, Merete Hofshagen,
Gro S Johannessen, Knut Rudi



The Number of Campylobacter Subtypes on Individual Chicken Carcasses and Slaughter Groups

Hans Lindmark, Christine Diedrich, Linda Andersson, and Eva Olsson Engvall

How often, chicken slaughter groups and individual carcasses carry several subtypes of *Campylobacter* was studied by subtyping isolates from carcasses, neck skin, and cloacal swabs from 16 slaughter groups. Also subtypes ability to change during rearing and slaughter was investigated. The carcass isolates originated from an earlier base line study where carcasses had been sampled postchilling and analysed for *Campylobacter*, with and without an enrichment step. Five colonies from the direct streak method and one colony obtained by the enrichment method were saved, when possible. The isolates from 16 slaughter groups, with two or more positive carcasses were subjected to macro restriction profiling (MRP) using SmaI and pulsed-field gel electrophoresis (PFGE). The average number of subtypes on the 36 individual carcasses from where two to six colonies were saved was 1.5. Two carcasses carried four different subtypes. The 16 slaughter groups (40 carcasses and 200 isolates) carried an average of 1.6 subtypes. Five different subtypes were found on carcasses from one slaughter group while carcasses from the other slaughter groups carried one or two subtypes.

The same subtypes on carcasses from 11 of the 16 slaughter groups were also present in the cloacal samples of the chickens of the same slaughter group. This was determined by subtyping isolates from the Swedish *Campylobacter* monitoring program. A subtype present on carcasses from one slaughter group was traced to the previously slaughtered group but the origin of some of the subtypes in the remaining four slaughter groups could not be determined. In two cases, two subtypes from the same slaughter group were very similar, also after cleavage with KpnI. For these two cases, it can be speculated that a common ancestor for the strains was introduced into the flock and subsequently a recombination/mutation occurred that created the two subtypes displayed.

It was concluded that the *Campylobacter* subtypes contaminating the carcasses at slaughter in general originated from the intestine of the same slaughter group.



National Food Administration

The Number of *Campylobacter* Subtypes on Individual Carcasses and Slaughter Groups Postchilling



National Food Administration

- **How many subtypes are there on an individual carcass?**
- **Do carcasses from the same slaughter group carry the same subtypes?**



National Food Administration

Sampling

Arrival

Scalding

Defeathering

Evisceration

Washing

Chilling

→ Base line study (600 carcasses)

Slaughter group	Abattoir and date	No: of carcasses	No: of isolates
1	A 12/5	3	12
2	B 20/5	2	9
3	C 10/6	2	10
4	B 11/6	2	4
5	A 23/6	3	18
6	D 30/6	2	12
7	A 1/7	2	11
8	B 1/7	2	12
9	B 15/7	2	12
10	E 11/8	3	8
11	D 11/8	3	13
12	D 12/8	4	20
13	A 19/8	3	18
14	C 26/8	2	12
15	A 26/8	3	17
16	F 8/10	2	12
		Σ 40	Σ 200



National Food Administration

The number of subtypes found on each of the 36 carcasses.

	Number of carcasses	Percentage of total
Carcasses with one subtype	22	61%
Carcasses with two subtypes	11	31%
Carcasses with three subtypes	1	3%
Carcasses with four subtypes	2	6%
	Σ 36	Σ 100%



National Food Administration

The number of subtypes present in each of the 16 slaughter groups

	Number of slaughter groups	Percentage of total
Slaughter groups with one subtype	10	62%
Slaughter groups with two subtypes	5	32%
Slaughter groups with five subtypes	1	6%



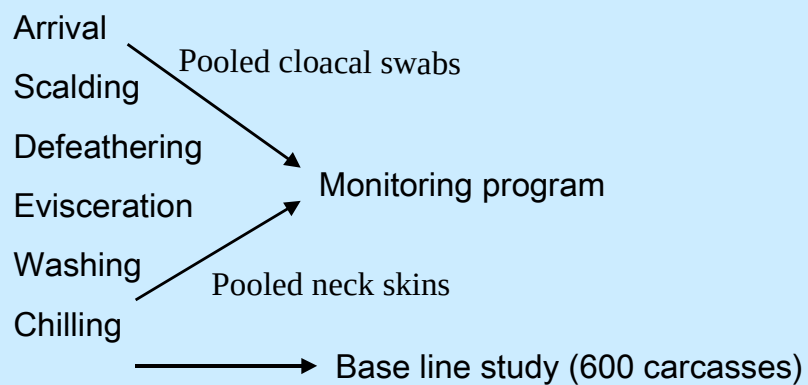
National Food Administration

- Have carcasses, neck skins and cloacal swabs from the same slaughter group the same subtypes?
- Do subtypes change during rearing and slaughter?



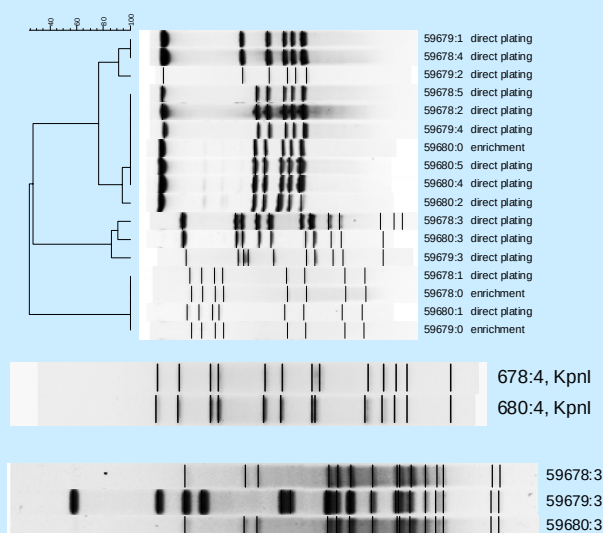
National Food Administration

Sampling



Comparison of subtypes from cloacal swab, neck skin and carcass

- For ten slaughter groups, the subtypes on cloacal swabs and neck skins/carcasses were the same.
- Five slaughter groups carried subtypes on neck skins/carcasses not found on cloacal swabs.
- One slaughter group carried a cloacal subtype that was not found on neck skins/carcasses.





National Food Administration

Conclusions

- The 36 carcasses carried on average 1.5 different subtypes
- The 16 slaughter groups carried on average 1.6 different subtypes
- Carcasses, neck skin and cloacal swabs from the same slaughter group carried in most cases the same pulsotypes.
- Pulsotypes did in most cases not change during rearing and slaughter

The work was done by:

National Food Administration

Christine Diedrich

Hans Lindmark

National Veterinary Institute

Linda Andersson

Eva Olsson Engvall

ABSTRACTS AND PRESENTATIONS

SESSION 3: RISK ASSESSMENT STUDIES

Risk Assessment Model of Campylobacter in Iceland

Jarle Reiersen

Human campylobacteriosis has been an increasing enteric disease during the last decade in Iceland and other countries. Many studies have pointed out broiler meat as a major source of human campylobacteriosis. The status of human and poultry campylobacteriosis and the location of Iceland have created an interest for researching the relationship between *Campylobacter* in environment, human and poultry campylobacteriosis. By applying the tools of routine microbiology of poultry, environmental and human, DNA-sequencing of isolates, routine epidemiological methodology, geospatial analysis and quantitative risk analysis the goal is to try to understand the risk factors of poultry and human campylobacteriosis. Further cost effective interventions will be developed to minimize the risk of human campylobacteriosis transmitted from poultry and poultry products. This study has been performed as a longitudinal total population study of the poultry production and the humans in Iceland. Sampling and data collection have been performed from April 2001 to end of September 2004.

The preliminary data shows that there is a relationship where density of ruminants is high and domestic human campylobacteriosis. In addition human domestic travels to certain areas turns out to be a risk factor. Risk factors for poultry campylobacteriosis are age at slaughter, flock size, vertical ventilation vs. horizontal ventilation, seasonality and biosecurity. There is no evidence for eggborne transmission from broiler breeders to day-old chicks, company/line, *Campylobacter* carried over from previous positive flock, time interval between flocks, use of floor eggs, density of birds or number of catch lots from each growing flock. Further analysis have to be performed to estimate and decide more details to better understand the myth of *Campylobacter*.

This project has been supported financially by all participating institutes in Iceland, USA, Canada and Sweden and by all broiler producers in Iceland. The major grant originates from the CSREES research program of USDA and ARS, USDA, in Athens GA, USA.



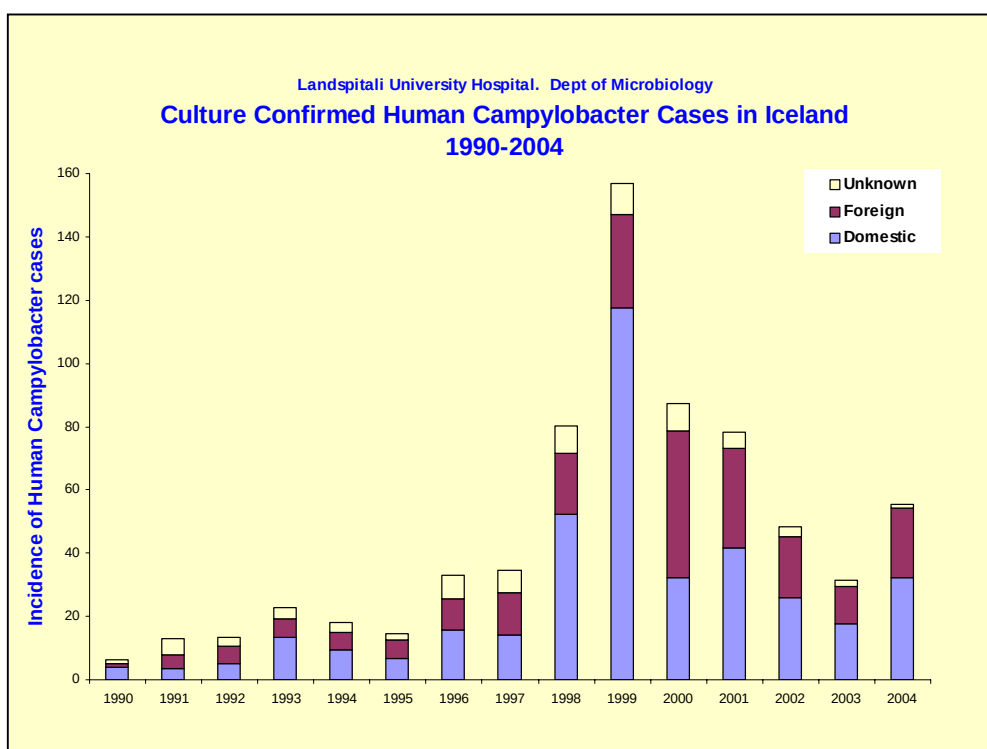
Risk assessment model of Campylobacter in Iceland

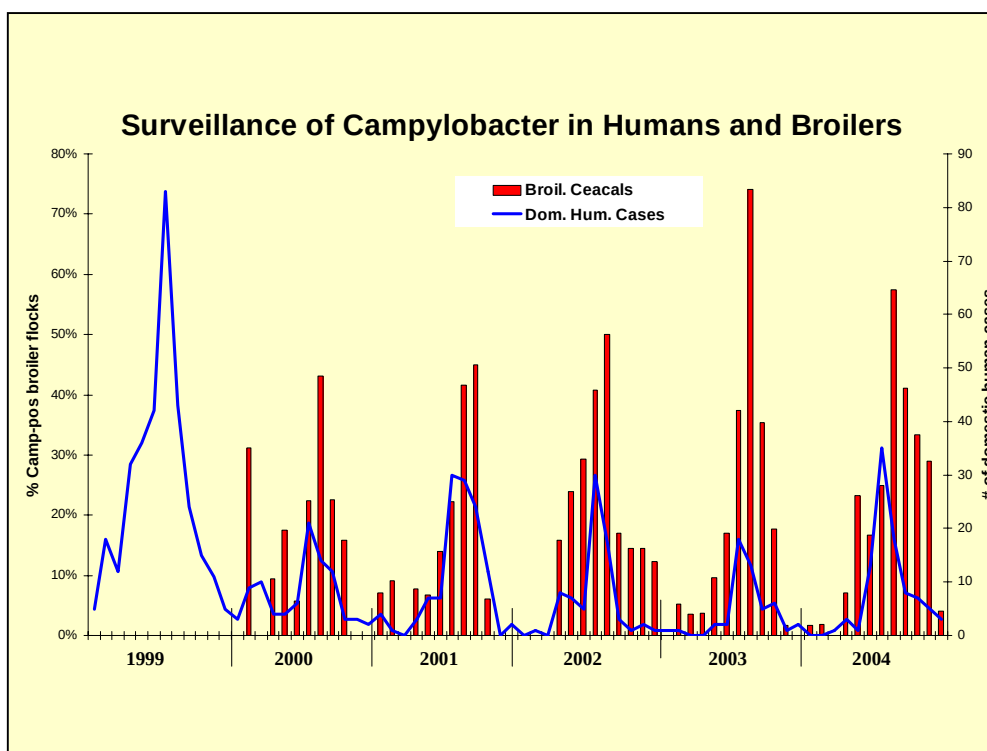
Jarle Reiersen, DVM

Veterinary Officer for Poultry Diseases

Icelandic Veterinary Services

Nordic Meeting on Advances in the Identification of Risk Factors for Campylobacter
Pre-harvest Infection and Post-harvest Contamination of Chicken Broilers
Uppsala, 12.-13.04.2005





Campylobacter in humans and broilers

Campylobacter – project “Campy on Ice”:

N. Stern, R. Lowman, K. Hiett, J. Reiersen et al:

Sources and Risk Factors for Campylobacter in Poultry and Impact of Human Disease in a Closed System

Started 01.04.2001 (preliminary projects 1999 and 2000)

End of study: 30.09.2004

Goal: Try to understand risk factors of Campylobacter transmission in poultry production and importance to human disease



“Campy on Ice”: Objectives

- Determine **sources** for transmission of Campy in broilers
- Estimate **risk factors** for sources of transmission of Campy in broilers
- Identify **points in production** with greatest potential for mitigation of risk
- Clarify **role of poultry** in human campylobacteriosis
- Investigate **seasonality** of Campy

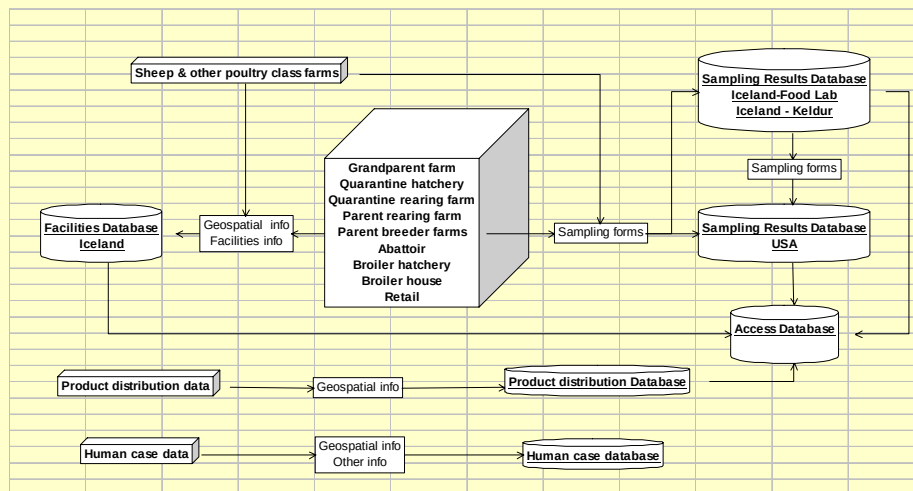


“Campy on Ice”: Focus of Investigation

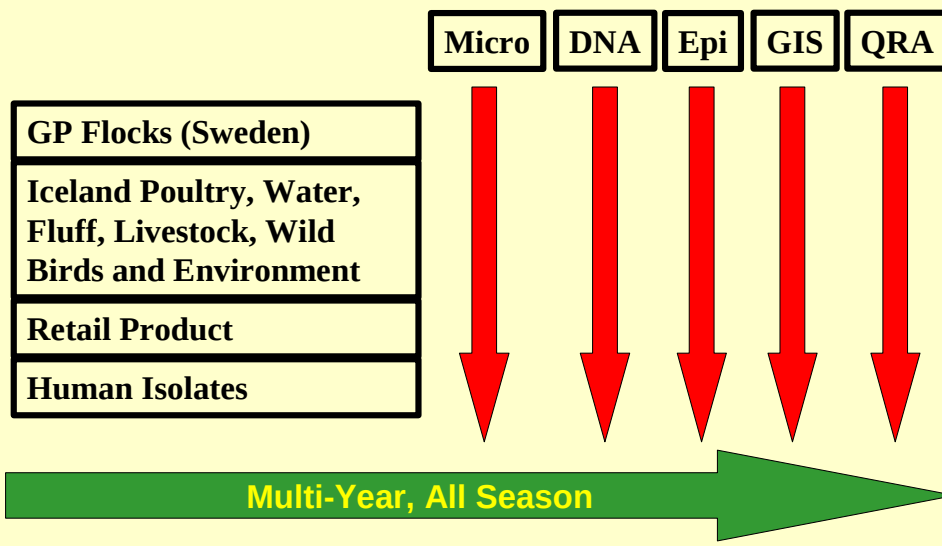
Sources of Campylobacter to Broiler Flocks

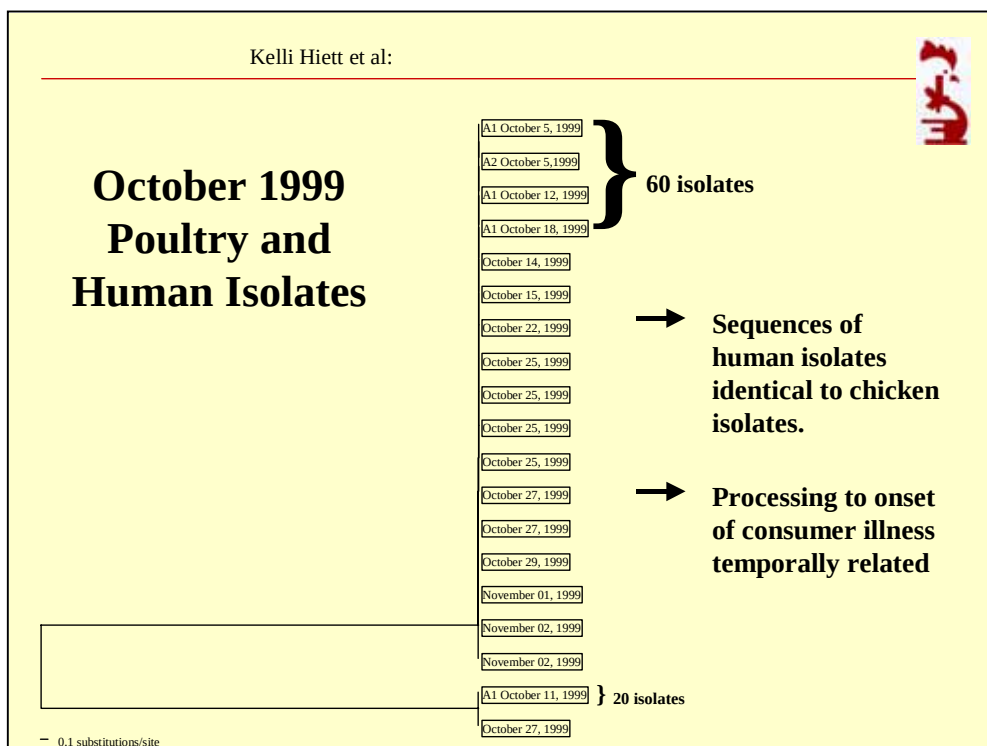
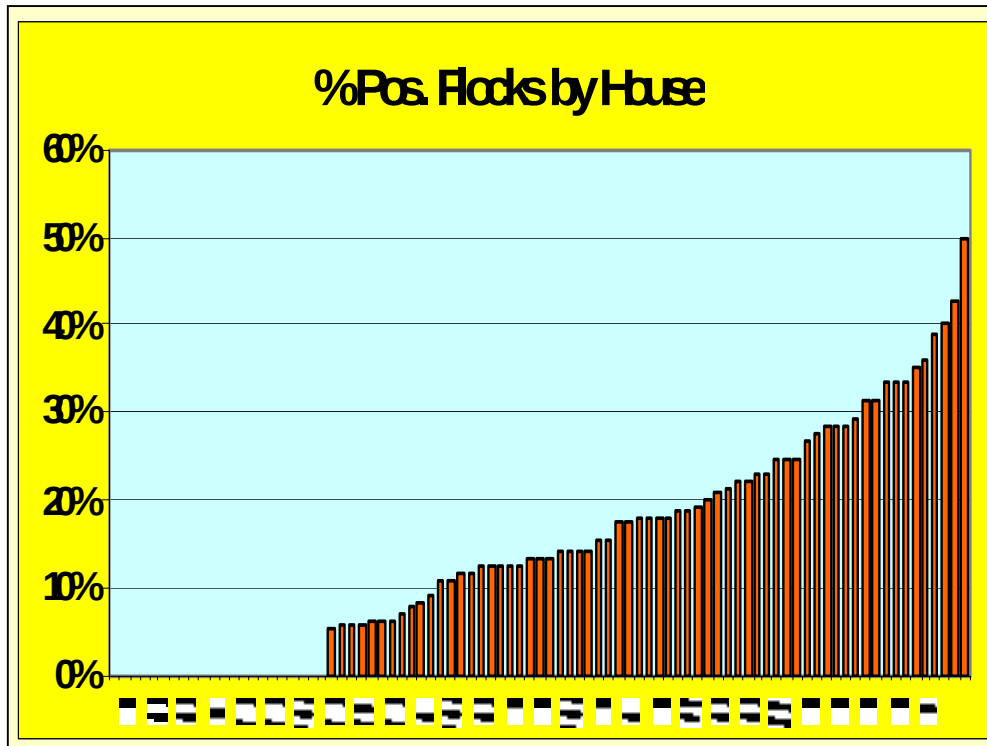
- Parent breeders: Egg borne
- Water
- Environmental:
 - Carryover from previous flock;
 - Migratory & resident wild birds;
 - Insects;
 - Farm animals: Sheep, cattle, etc.
 - Others: Mice, etc.

Campy on Ice - Project design

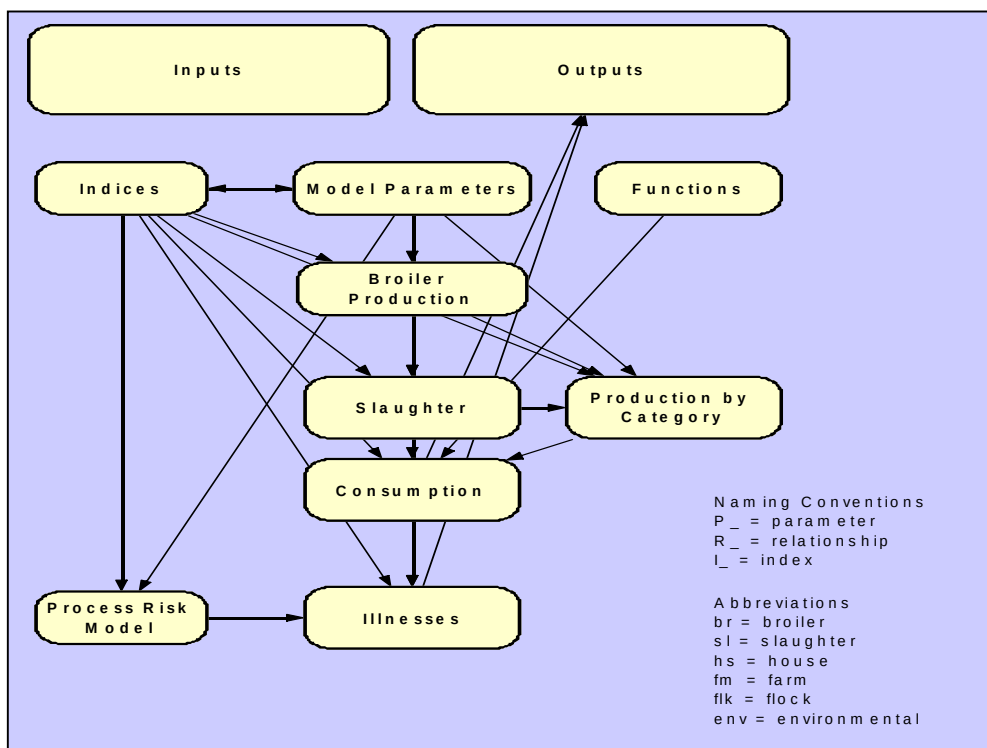
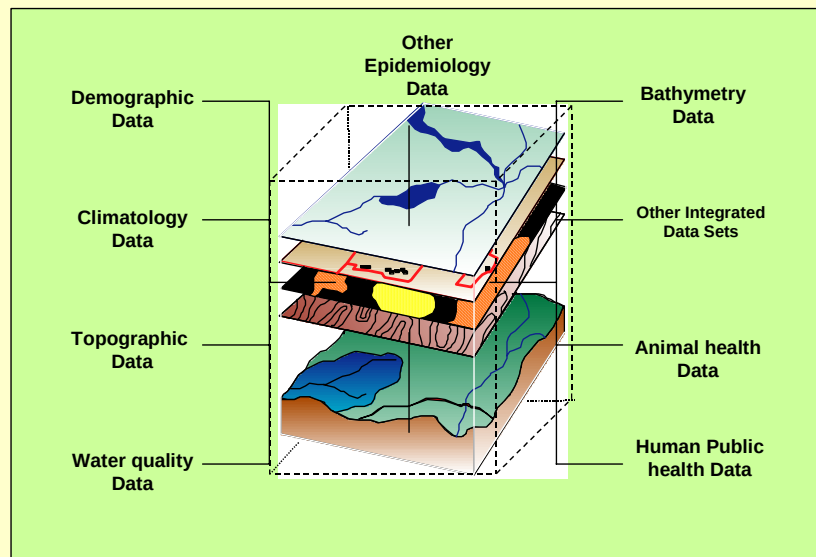


Total Population Study: Longitudinal - Vertical - Multidisciplinary

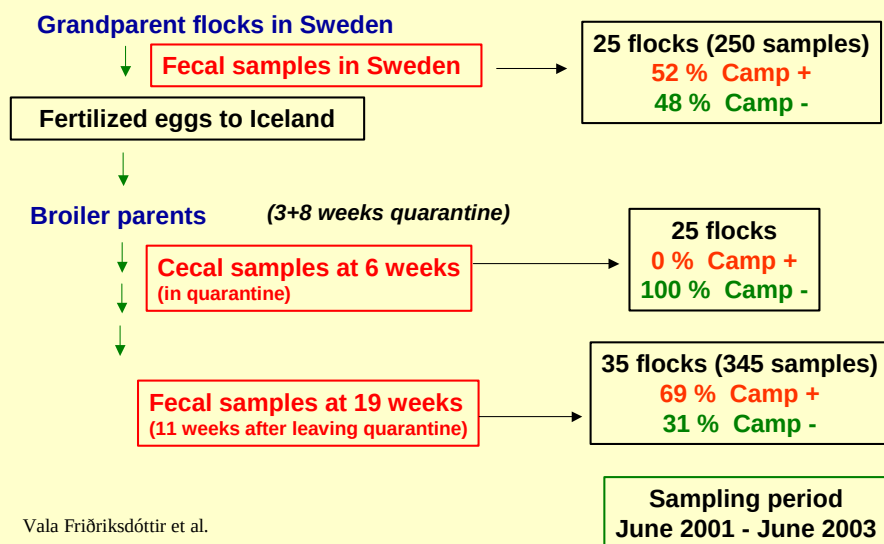




The data overlay concept in Geomatics



Vertical transmission study - results

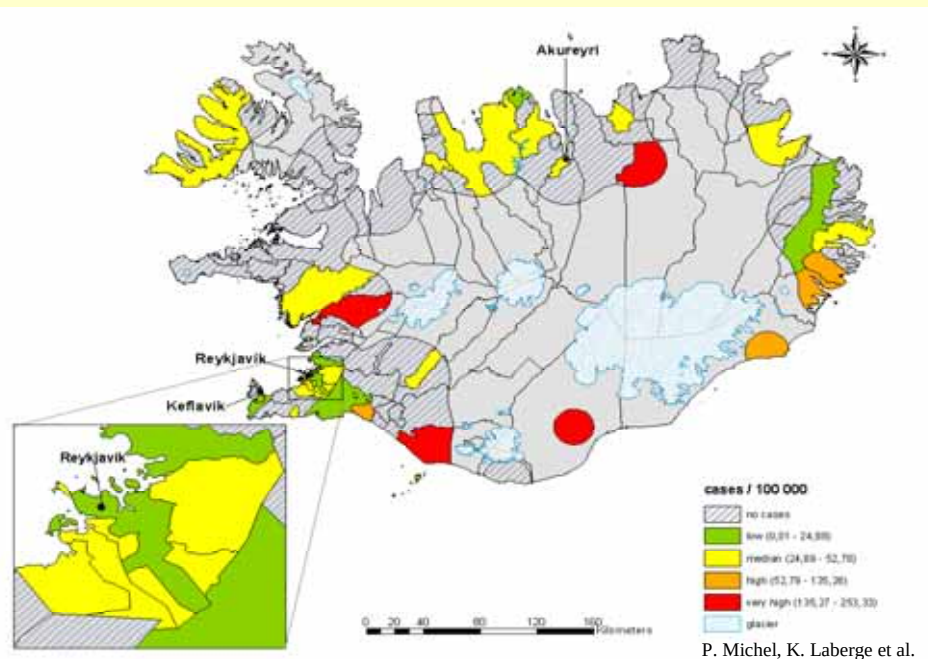


Is vertical transmission taking place from hen to broiler chicken?

- Our results – **no (not found Campylobacter in the material tested)**
 - Grandparent flocks; **Camp+**
 - Progeny at the age of 6 weeks; **Camp–**
 - Progeny at the age of 19 weeks; **Camp+**
- Co-worker Ken Callicott – results in agreement
 - Compared the sequence of the flaA short variable region (SVR) from isolates from grandparent flocks and broiler parent flocks
 - Did **not** observe any Campylobacter flaA SVR alleles shared between grandparent and offspring flocks

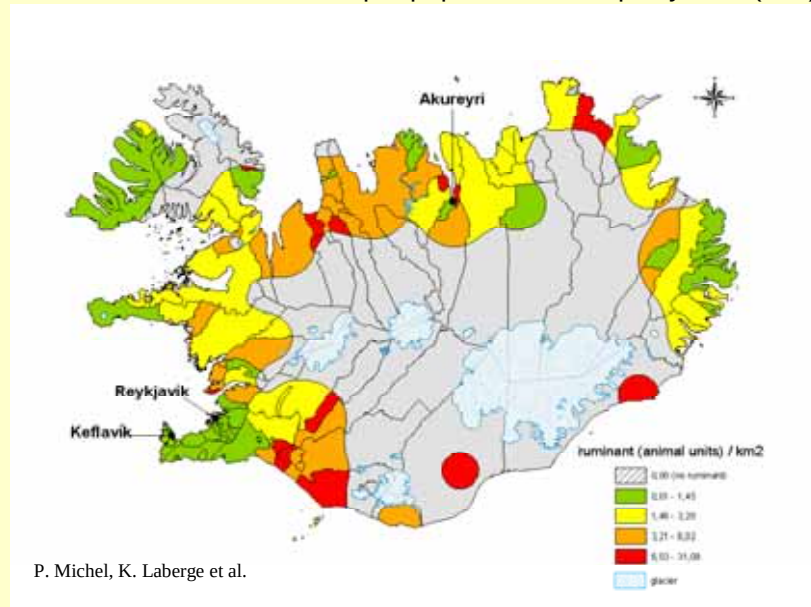
Vala Friðriksdóttir et al.

Domestic Campylobacteriosis in Iceland: Yearly age-standardized incidence rates per 100.000 population, 2001-2003 (n = 239, average = 27.82/100 000)



Ruminant Density in Iceland (2002) :

Total number of animal units per populated municipality area (km²)

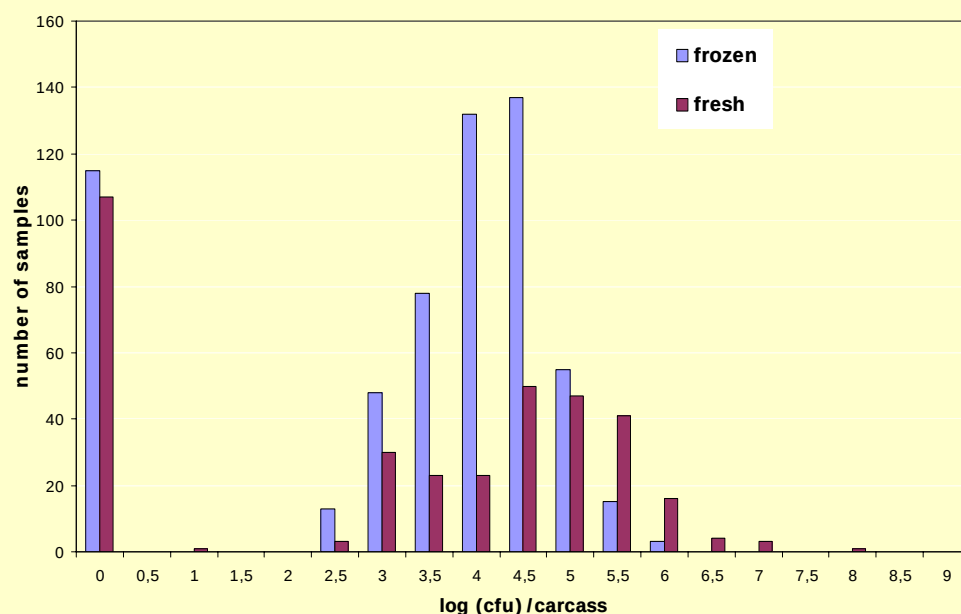


Main regression model for the annual mean incidence of campylobacteriosis

Variables	Coefficients	Standard error	P-values
Rural	0,096	0,294	0,001
Ruminant density	1,282	0,404	0,001
Presence of poultry	-0,660	0,220	0,003
Intercept	-8,803	0,259	0,000

P. Michel, K. Laberge et al.

Levels of *Campylobacter* contamination among Iceland Broiler Carcasses--Distribution by log (cfu) /carcass; 1,019 carcass rinse samples enumerated in 2003





“Campy on Ice”

From Pablo Romero Barrios:

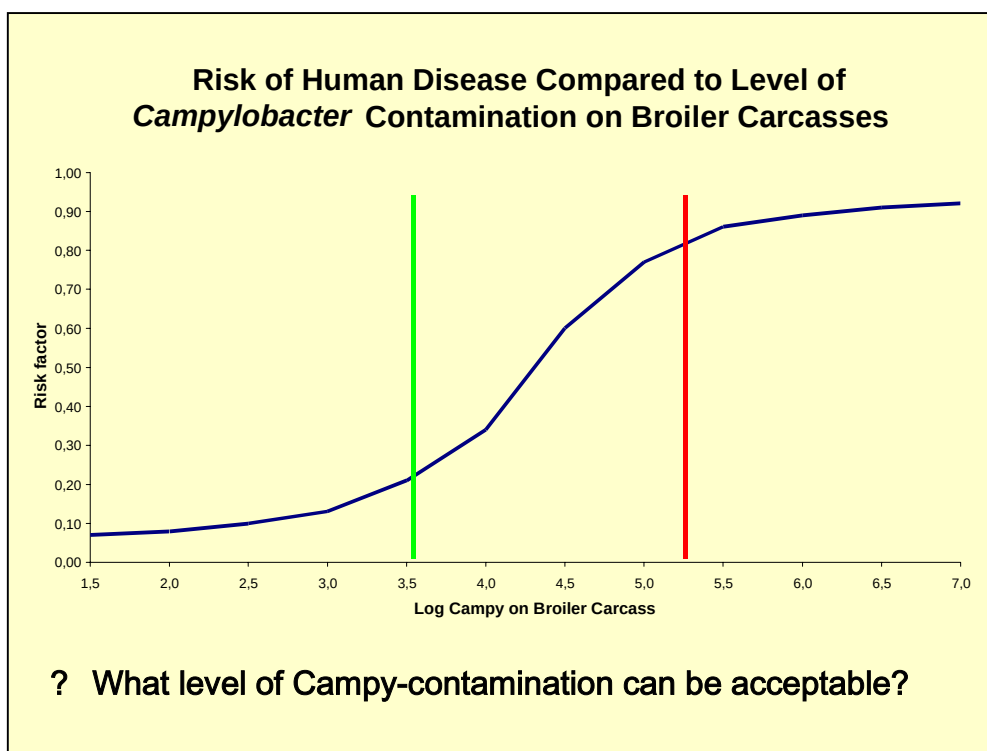
- Increased proportion of Campy-positive flocks with increased age and flock size
- Increased proportion of Campy-positive flocks with vertical ventilation compared to horizontal ventilation
- No evidence of effect of year, company/line, Campy carried-over from previous flock, time interval between flocks, use of floor eggs, density of birds, number of catch lots from each growing flock.



“Campy on Ice”

Results - summary:

- The Icelandic 1998-2000 human Campylobacteriosis epidemic traced to consumption of fresh broiler meat
- Detection of “hotspots” with high human prevalences
- No evidences for egg borne transmission of Campy
- Differences between broiler farms and broiler houses regarding prevalences of Campys in broiler products
- Higher counts of Campys on carcasses originated from positive flocks prior to slaughter



“Campy on Ice” Consortium

- **Iceland:**
 - Haraldur Briem, Vala Fridriksdóttir, Eggert Gunnarsson, Franklín Georgsson, Hjördís Harðardóttir, Karl Kristinsson, Guðrún Sigmundsdóttir, Jarle Reiersen
 - Directorate of Health, Institute of Experimental Pathology, The Environmental and Food Agency of Iceland, National University Hospital, Icelandic Veterinary Services
- **Sweden:**
 - Eva Berndtson
 - Swe-Chick
- **Canada:**
 - Ruff Lowman, Jean-Robert Bisailon, Victoria Edge, Pascal Michel, Greg Paoli, Aamir Fazil
 - Canadian Food Inspection Agency, Public Health Agency of Canada, Decisionalysis Risk Consultants, University of Guelph
- **USA:**
 - Norman Stern, Ken Callicott, Kelli Hiett
 - USDA-Agricultural Research Service



Evaluation of options at the producer and consumer stages for reducing the risk of *Campylobacter jejuni* illness from consumption of chicken

Roland Lindqvist and Mats Lindblad

Campylobacteriosis is a bacterial infectious disease of great concern in the Nordic countries. Control programs of varying scope and ambition exist with the focus of controlling this disease agent in chicken. The purpose of the present study was to use data from a national baseline study of *Campylobacter* in Swedish chicken in order to evaluate some risk management options at the producer and consumer levels. More specifically, at the producer level the effect of reducing the prevalence and concentration on chicken carcasses, respectively and the effect of channelling the chicken from high *Campylobacter* prevalence producers to frozen chicken were evaluated. In addition, it was attempted to assess the extent of cross contamination and under-cooking at the consumer level under different assumptions of the annual number of *Campylobacter* cases due to consumption of chicken in Sweden. Further, the implications of these estimates for information campaigns at the consumer were discussed.

A probabilistic model addressing variability but not uncertainty was developed and the modelling unit was the probability of illness per serving if the chicken was handled in a way so that cross contamination or under cooking occurred. A number of assumptions were made based on published literature data in order to describe the cross contamination and undercooking processes, respectively, as well as consumption. Assumptions include the fraction of bacteria on the carcass being available for cross contamination, the transfer rate, and the fraction of bacteria being consumed, and the proportion of bacteria not being inactivated during heat treatment, and the time and temperature conditions experienced by the protected bacteria.



Livsmedelsverket

Evaluation of options at the producer and consumer stages for reducing the risk of *Campylobacter jejuni* illness from consumption of chicken

Roland Lindqvist and Mats Lindblad



Nordic Meeting on Advances in the Identification of Risk Factors for Campylobacter Pre-harvest Infection and Post-harvest Contamination of Chicken Broilers, Uppsala 12-13 April, 2005



Livsmedelsverket

Objectives:

Producer stage: To evaluate the effect of

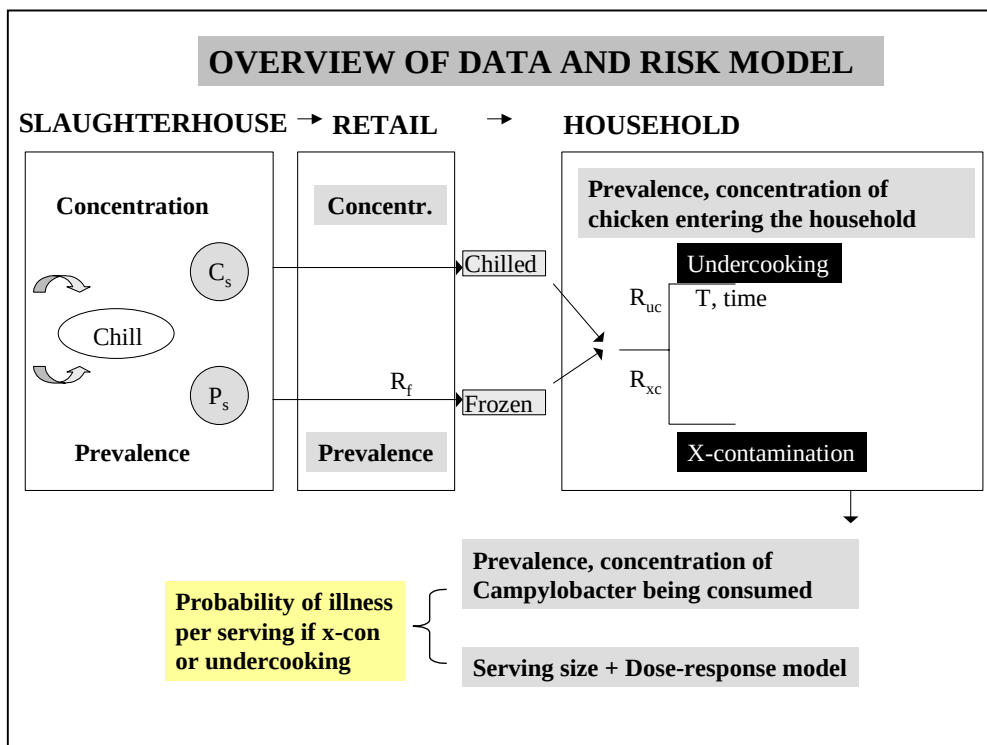
- **Reduced prevalence**
- **Reduced concentrations**
- **Channeling (High prevalence producers)**

Consumer stage: To

- **Frame the extent of cross contamination / undercooking**
- **Discuss the potential for information**

Approach:

- Probabilistic model describing variability (not uncertainty).
- In each iteration the probability of illness after handling and consuming a serving of chicken is calculated
- Model iterated 20 000 times. The sum of the iterations gives the number of cases per 20 000 (“fatefully”) mishandled servings of chicken





Livsmedelsverket

Key assumptions

- Log Reduction freezing Uniform(3 - 100 times)
- Reduction chilling Negligible

X-Contamination:

- Fraction of carcass contributing to x-contamination Uniform(0,01- 0,7)
- Log transfer rate Uniform(0.25-0.0001)
- Fraction of transferred bacteria consumed Uniform(0.01-1.0)
- Prevalence in chicken 0.14, baseline study



Livsmedelsverket

Assumptions

Undercooking:

- Cooking temperature Triang(55;60;65)
- Cooking time Triang(0,5;1;1,5)
- D-value $10^{((-0,1575 \cdot T) + 9,004)}$
- Proportion of bacteria in protected sites Triang(0,1;0,15;0,2)

Dose-response:

Beta-Poisson model from WHO/FAO risk assessment



Livsmedelsverket

Results – estimated # of cases

Cases per 20 000
mishandled servings

Ratio cases chilled/ frozen

Frozen x-con 31

Chilled x-con 104

X-contamination: 3

Frozen undercooked 15

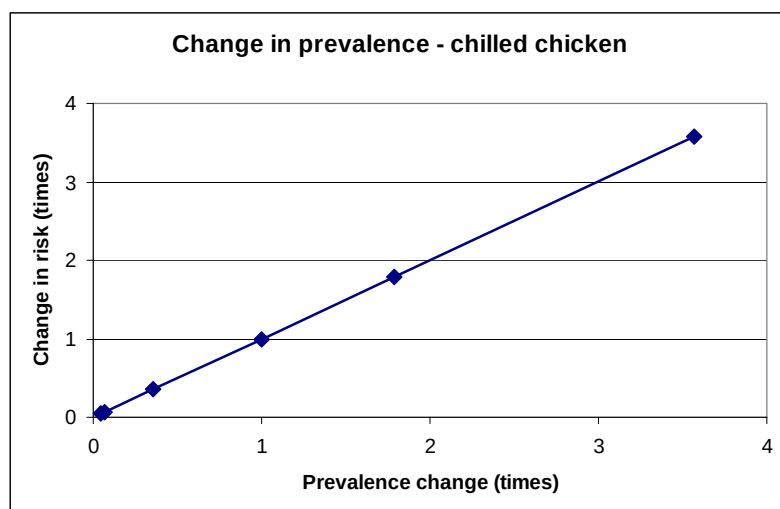
Chilled undercooked 55

Undercooked: 4

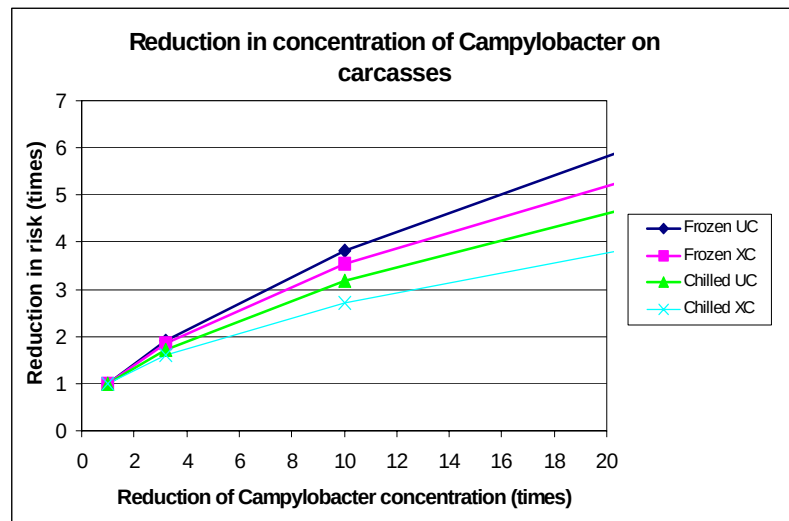


Livsmedelsverket

Results – Prevalence



Results – Concentrations



Framing the extent of mishandling

$$P_{\text{ill}} * \text{Servings} * X_{\text{eff}} = \text{"Chicken Cases"}$$

$$X_{\text{eff}}(\text{X-con}) = \text{"Chicken Cases"} / P_{\text{ill}} * \text{Servings}$$

$$X_{\text{eff}}(\text{uc}) = \text{"Chicken Cases"} / P_{\text{ill}} * \text{Servings}$$

Assumptions:

- 9 millions consumers eat Swedish chicken once a week
- 70/30 frozen/chilled
- Age group 20-29, 1.7 times higher incidence
- No underreporting or 10 times underreporting



Livsmedelsverket

Framing the extent of mishandling

Assumed # cases from chicken	X _{eff} ("fateful" mishandling)		
	X-con	Undercook	
➤ 50 % of reported	1 in 1 000	1 in 500	} < 0.2 %
➤ 10 % of reported	1 in 5 000	1 in 2500	
➤ 1 % of reported	1 in 50 000	1 in 25 000	
➤ 10 % of reported, age group 20-29 years	1 in 3000	1 in 1500	
➤ 10 % of 10 x reported	1 in 500	1 in 250	0.4 %

Mishandling of chicken 20 –30 %?
Effect of information?



Livsmedelsverket

Results – Chilled /frozen

No of cases due to frozen or chilled chicken (70/30)

Proportion of cases chicken	X-con		UC	
	frozen	chilled	frozen	chilled
50 %	506	729	475	760
10 %	101	146	95	152
1 %	10	15	10	15

Ratio chilled/frozen: Assumption; rates the same
X-con: 1.4 for chilled and frozen chickens
UC: 1.6



Livsmedelsverket

Results – channeling

- “High prevalence” producers produce 40% of Campy chickens
- Diverting chickens from producers with high prevalence to frozen chicken only reduces cases by 1/5
- If production of frozen/chilled is 50/50 the estimated improvement is about 1/4
- The overall reduction is an effect of a decrease in concentration due to freezing (although prevalence in frozen chicken increases) and a decrease in prevalence in chilled chicken

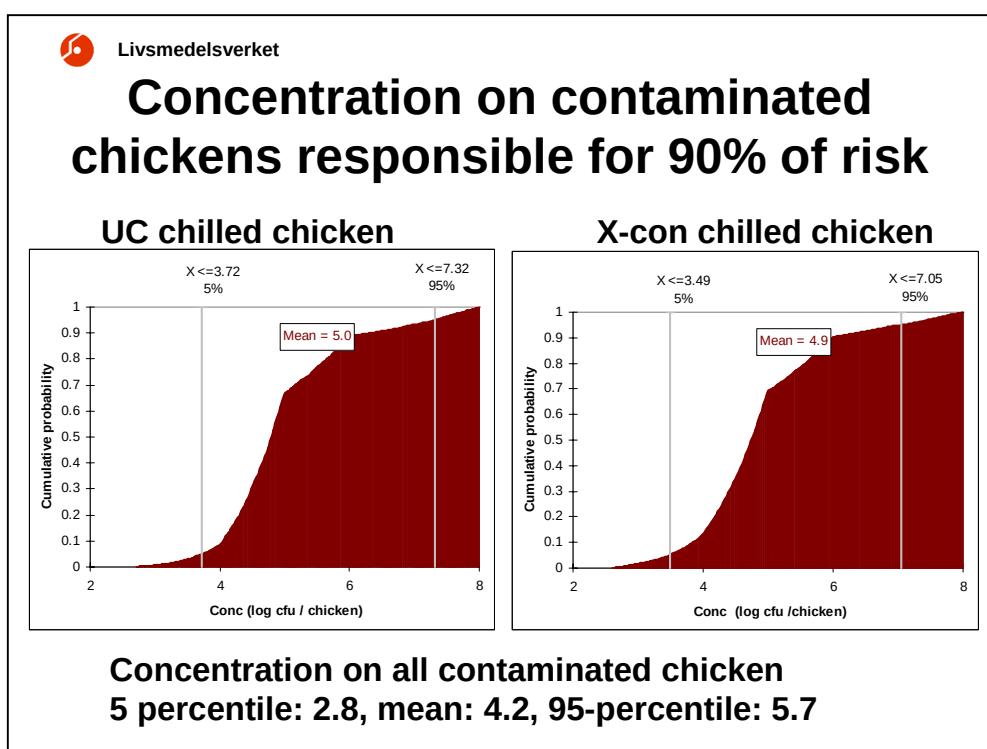
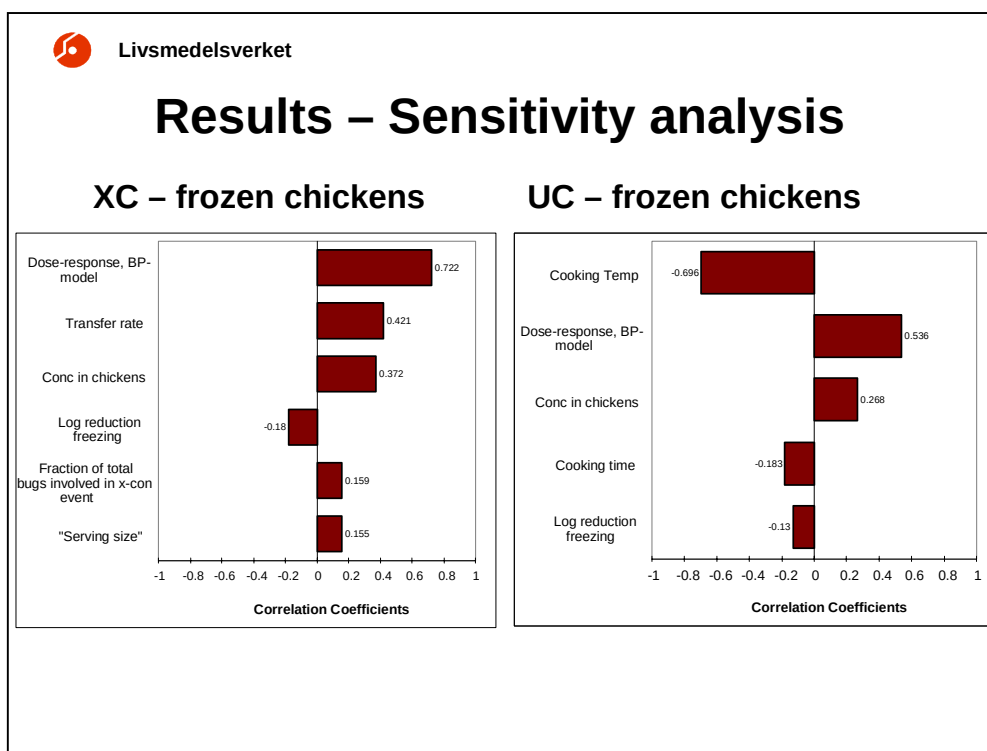


Livsmedelsverket

Results – channeling

Possible to detect improvement?

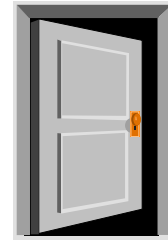
Proportion of chicken cases	Change in number of cases	
	X-con	UC
50 %	- 209 (83-292)	- 226
10 %	- 42	- 45
1 %	- 4	- 5
10%, age 20-29	- 9	- 9





Livsmedelsverket

Conclusions/Summary



- **Prevalence:** There is a 1:1 relation between risk and prevalence
- **Concentration:** A 100 times reduction reduces risk by a factor of 9 – 18 times for investigated transmission routes
- **Channeling:** Estimated to decrease the number of cases by 20% - the effect may be difficult to detect in reported cases
- **“Fateful” mishandling:** Appears this is only a small fraction. If information can reduce this fraction it will reduce illness to the same extent.

WORKSHOP DISCUSSIONS – SUMMARIES

Introduction

In order to give opportunities for interaction during the meeting time for discussion was prioritised in the workshop programme. Issues relating to the three sessions were discussed in connection with each presentation, in smaller groups during an evening session, and during a plenary discussion. The summaries of the group discussions held during the evening session and presented by the rapporteurs are presented below, together with a summary of the plenary discussion. A first version of the summary was drafted by the project group and distributed for comments to the members of the programme committee. The final version is presented in the report.

Group discussion- Campylobacter control/surveillance programs

Aim

To reduce human health problems.

Problem

Increased import makes domestic results less important.

Solution

Setting performance objectives in EU

- after processing, before marketing
- to fulfil a food safety objective (achieved in different ways)
 - less than ...CFU/g
 - no quinolone resistance?

Knowledge gaps

- What is the food safety objective?
- Other sources for human infections?
- Basic (current) level (prevalence/quant. results) in EU?
- What happens at retail (M.A.P.)?
- Effective interventions at farm level?
- Climate effects?
- “*Campylobacter*” account in humans?

Group discussion – Risk factors and molecular epidemiology

Revised Aims

- Identify and rank risk factors for *Campylobacter* in broilers
- Identify pros and cons of current methods for molecular epidemiological studies
- Important ways for transmission of *Campylobacter* to humans
- Suggest Nordic based research studies in this area

Factors of importance for bringing *Campylobacter* into primary production (of chicks)

- Environment
- Temperature, weather conditions
- Vectors
- Hygiene
- Management

Routes and vectors

- Flies
- Water
- Humans
- Ventilation systems

Suggested study

- Joint study of risk factors for Nordic countries
- Look at specific factors, e.g. ventilation systems

Molecular methods – for epidemiological studies

- Methods used in Nordic countries: PFGE, AFLP, MLST, flaA-typing, (serotyping)
- Limitations due to great genetic diversity
- Useful for small scale epidemiological investigations
- Focus on isolates with well defined and background information, e.g. geographical, age, time

Suggestion for studies

- Strain sets with connected background
- Comparison of Nordic strain sets
- Use standardised protocols
- New methods only if specific criteria are met (better, novel findings, etc)
- Comparison of data from specified isolation periods/sources

Important risk factors/risk behaviours for transmission to humans

- Data to be found in different presentations
- Travel
- Age
- Drinking water
- Outdoor activities (swimming, bicycling)
- Animal (pet) contact
- Poultry (consumption and handling)
- Flies?

Suggested studies

- Reporting and investigation following same procedures as for trace-back Salmonella cases (DK)
- Personal hygiene
- Food hygiene (street vendors)
- Estimation of hygiene measures
- Estimation infectious dose
- Animal models, challenges

Group discussion- Risk assessment

Risk assessment studies

Aim

To identify primary knowledge and data gaps.

Primary production

Data gaps

Within flock contamination/colonisation

Strain variability as regards

- colonisation of chicks
- survival during breeding and processing
- virulence to humans

Knowledge

Flock prevalence

The environment is important for flock colonisation

The age of the birds is important

Thinning

Etc.

Influence of in-house environment on the colonisation rate (i.e. breeding conditions, feed, health status)

Impact of contamination routes

Influence of surroundings, e.g. salt, temperature, humidity, pH (chalk)...

Generally

Questions from risk managers:

concentrate on the data gaps relevant for answering the questions!

Important data gaps:

- data for validation of risk models
- the “true” *Campylobacter* incidence
- the contribution of chicks to this incidence
- the “true” number of *Campylobacter* (better and more sensitive methods)
- dose-response models for different populations
- consumption patterns
- kitchen hygiene; number of events that allow cross-contamination
- number of organisms transferred

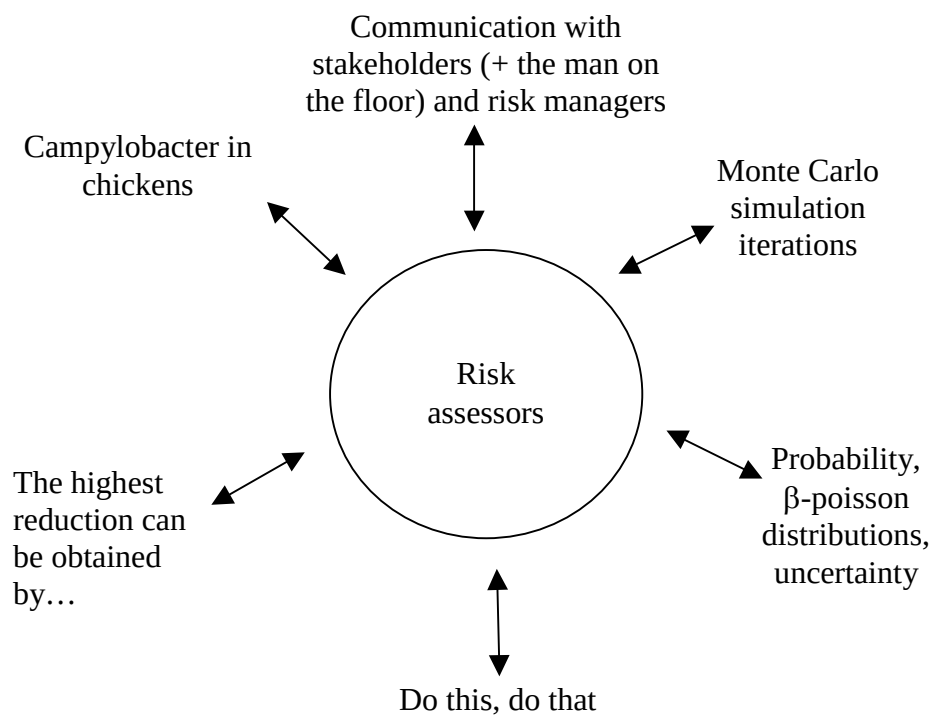
There is good data on:

- flock prevalence
- prevalence and concentration in chicken meat
- human incidence

Other available information is sporadic...

Monitoring should be carried out where needed in each case.

Another discussion point was:



LEVEL OF COMMUNICATION IS OF GREAT IMPORTANCE

Summary of the plenary discussion

Were any new knowledge presented during the workshop? Two opposing views were expressed. On one hand we still cannot rank the risk factors for colonization of chicken with *Campylobacter* or identify the food vehicles for *Campylobacteriosis*. On the other hand we do have much knowledge. For instance we know that the hygienic barriers are important to prevent the entry of *Campylobacter* into the flocks, that the timing of colonisation of the flock is important since the later the colonization the lower the concentrations on the chicken carcasses, that concentration may be more important than prevalence in terms of management of risk, that many flocks are contaminated during transport, and the importance of climatic and seasonal factors.

In view of this are we asking the correct questions? Several participants raised the problem during the discussion that risk managers along the food chain are not using the available knowledge. To some extent this may be a consequence of the fact that our knowledge about risk factors are qualitative and not quantitative. For instance, there appears to be a degree of unpredictability in terms of which risk factors explain why a breeder produces flocks with or without *Campylobacter*. This may indicate that some still unidentified risk factors are important. There is also a possibility that a ranking of risk factors may be different depending on the farm.

Several of the Nordic countries have succeeded in reducing the prevalence of *Campylobacter* in chicken flocks but there is no noticeable reduction in the number of human cases reported. The knowledge gained, however, was still considered useful, and the opinion that we should not get stuck in the number of reported cases was expressed. Public health may be improved despite the fact that it is not detected in the number of reported cases.

The consumption of chicken with origin from countries outside the Nordic countries is increasing. The implications of this change in terms of risk management of *Campylobacter* were discussed. It was emphasized that the efforts to reduce *Campylobacter* contamination in the domestic chicken must proceed since improvement of public health is the goal. However, in relations to the changes in consumption it is of vital importance to improve the contamination levels also on the chicken from outside the Nordic countries. This should be achieved by international efforts to develop and implement food safety objectives and performance objectives. In respect to the behaviour of consumers the industry representatives were pessimistic about the willingness of consumers to pay for the increased costs due to management actions to reduce *Campylobacter*. A conclusion of this reasoning is that any risk management actions must be, if not cheap, very cost effective.

Different methods of reducing the concentration of *Campylobacter* on the carcasses were discussed. Freezing is one treatment, which has been successfully applied in Iceland. Decontamination of carcasses was also discussed with many different opinions expressed depending on the specific method, e.g. hot water and rinsing with hypochlorite. Ethic concerns were raised and reference was also given to the current legislation. The efficacy of such treatments was also a concern, at least in isolation, and the importance of good husbandry was emphasised.

The possibility of actions in the primary production was discussed. The possibility of reducing or delaying the colonization of flocks with *Campylobacter* was one topic discussed. The Finnish example with “Nurmi soup” (competitive exclusion) to reduce colonisation of chickens with *Salmonella* was one cited example. In Denmark work is going on to reduce colonization by inoculation of chicken with bacteriophages.

Transport was identified as a point where the prevalence may be reduced. The concentration of *Campylobacter* on chicken contaminated during transport is often low. In addition, actions at the slaughterhouse were also discussed. The focus should be at reducing fecal contamination. It was suggested to replace the scalding tank with steam treatment. This would reduce cross-contamination and also to some extent inactivate bacteria. Further, rinsing of chickens would benefit from an optimisation according to the size of the individual carcass.

The occurrence and properties of chicken and human *Campylobacter* strains was discussed. Our knowledge about strains from other sources is more limited, as is the correlation between specific genes and survival and virulence characteristics.

During the discussion several ideas about future work and projects were identified and it was suggested that these ideas be communicated to the project group in the coming "ÄK-livsprojekt Center of Excellence in Campy Research". Examples include: A ranking of risk factors in the primary production. Studies of the ecology of *Campylobacter*, especially reservoirs and the effect of climate. Projects about functional genomics, especially the relation between genes and virulence and survival. The opinion was also that in addition to developing food safety and performance objectives more attention should be put on integrated solutions based on our knowledge.

ACKNOWLEDGEMENT

This workshop, as part of the project “Studie och workshop om faktorer som påverkar förekomst, halter och typer av Campylobacter i kyckling (no. 681048-40238)”, was funded through the Nordic Council of Ministers (Nordiska ministerrådet).

