Food Control and Research on *Campylobacter* spp. in Estonia

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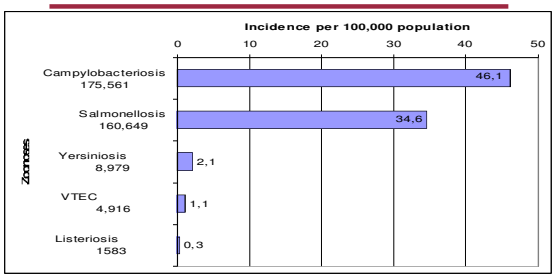
Actuality of the topic

- Campylobacteriosis represent an important public health problem with considerable socio-economic impact.
  - *Campylobacter* was the most commonly reported gastrointestinal bacterial pathogen in humans in the EU in 2005 and 2006 **(EFSA, Zoonoses Data Collection Reports).**
  - *C. jejuni* and *C. coli* are a major cause of human gastroenteritis worldwide - sporadic infections and outbreaks all over the year.
  - Antimicrobial resistance and simultaneous resistance to several antimicrobials within one strain (multiresistance) appears to be linked to the use of antimicrobials in human medicine and animal production.

The reported incidences of the zoonoses in humans, 2006 (EFSA Journal 2007)



Food Control in Estonia

- **Food control or governmental supervision shall be performed in Estonia by the:**
  - **Veterinary and Food Board since 01.07.2008 as the only food surveillance/monitoring institution in Estonia**
    - **before 01.07.08 additionally Health Protection Inspectorate (retail and catering)**
  - **Estonian Consumer Protection Board**
  - **Estonian Tax and Customs Board**

Mandatory to notify, state control

- *Campylobacter* is notifiable in *Gallus gallus* and in all animals in Estonia as well as in foods
  - *Campylobacter jejuni* was subjected to registration since 2000 in Estonia in accordance with the "List of Notifiable Diseases and Diseases subject to Registration" – Regulation No 34 Ministry of Agriculture

Previous studies in Estonia at state level, Health Protection Inspectorate

- 2004 – 56 samples taken and 26.8% were positive
- 2005 – 278 samples and 7.5% were positive
- 2006 – 80 samples and 6.3% were positive
  - In the year 2006 three outbreaks were not linked to any kind of food and one outbreak was linked to consumption of pork.

State monitoring of *Campylobacter*

- Monitoring at Veterinary and Food Board level of *Campylobacter* spp. in Estonia started in 2006. Sampling distributed evenly throughout the year at retail, slaughterhouse and meat processing plant level.
  - within frame of self-control studies in 2004 in Tallegg company (producing 100% of Estonian broiler chicken meat)
- In 2006 there were no *Campylobacter* isolated from poultry of domestic origin (n=165)
- In 2007 only 47 caeca samples and 48 neck skin samples were taken and analyzed
  - caeca samples – no positive samples were found
  - neck skin – one positive sample was found within state monitoring studies

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Commission Decision 2007/516/EC

- EU **baseline study** *Campylobacter* spp. in broiler flocks and their antimicrobial resistance in 2008
  - in Estonia at least 96 slaughter batches (in fact 110) shall be sampled
    - per slaughter batch 10 birds caeca (in laboratory will be pooled to one composite sample) and additionally one bird carcass should be sampled
    - for antimicrobial resistance monitoring if a lower number of isolates than the target sample size is available (in fact in Estonia), all isolates shall be included per *Campylobacter* species

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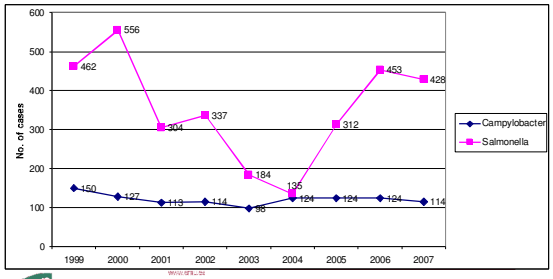
Laboratory-confirmed *Campylobacter* and *Salmonella* infections in Estonia during 1999-2007

http://www.tervisekaitse.ee/ Estonian Health Protection Inspectorate



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Intestinal infections in Estonia in 2006 and 2007

Estonian Health Protection Inspectorate

Enteric infections	2006		2007	
	Total no. of cases	Per 100 000 inhabitant	Total no. of cases	Per 100 000 inhabitant
Salmonellosis	453	33.8	428	31.9
Campylobacter enteritis	124	**9.2**	114	**8.5**
Shigellosis	53	3.9	114	8.5
Yersinia enterocolitica enteritis	42	3.2	76	5.7
Non-specified intestinal infections	**829**	**61.8**	**1515**	**112.9**

Campylobacter enteritis in Finland: 4107 (**78** per 100 000 inhabitant) in 2007
Salmonellosis in Finland: 2735 (52 per 100 000) in 2007

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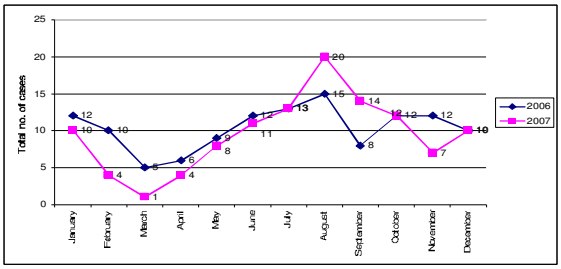
Campylobacter enteritis in Estonia in 2006 and 2007, Seasonality

http://www.tervisekaitse.ee/ Estonian Health Protection Inspectorate



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The aims of the present study were:

1. To determine *Campylobacter* spp. in raw poultry meat in Estonia in oder to provide data for understanding the significance of poultry as a potential source of human *Campylobacter* infection in Estonia.
2. To serotype and PFGE genotype *Campylobacter* isolates originating from raw retail poultry meat to understand the distribution and diversity of serotypes and PFGE genotypes in Estonia.
3. To determine the antimicrobial susceptibility of the isolated *Campylobacter* strains in oder to compare it to respective levels in other EU countries and to understand the problem severity in Estonia.

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Campylobacter isolates

- Poultry meat (from 2000 to 2006)
  - 128 *Campylobacter* positive samples from a total of 1040 randomly selected raw poultry meat samples (826 Estonian origin and 214 imported) were obtained. One isolate per positive sample was collected for further studies.
- Fecal and cecal material (2005 and 2006)
  - 105 *Campylobacter* positive samples were obtained from a total of 1518 fresh fecal and cecal samples at an Estonian chicken farm and at slaughterhouse level. One isolate per positive sample was collected for further studies.

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Methods (1)

- Nordic Committee on Food Analysis (NMKL) method, vol. 119 *Campylobacter jejuni/coli* detection in foods, 2nd edition.
- Serotyping of *C. jejuni* by heat-stable antigen (Denka Seiken, Japan). According to the manufacturer instructions.
- PFGE protocol ("CAMPYNET"). Application for the analysis of *Campylobacter jejuni/coli*. The DNA was digested with *Sma*I and *Kpn*I. The computer software BioNumerics 3.5 was used for numerical analysis.

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Methods (2)

In 2002 and 2003 all *Campylobacter* isolates were tested by **disc diffusion method** (Oxoid), and by the **E-test** (AB biodisc, Solna, Sweden) in the Department of Food and Environmental Hygiene, University of Helsinki.

In 2005 and 2006 all *C. jejuni* isolates were tested for minimal inhibitory concentration (MIC) by a **broth microdilution method** (VetMIC™Camp method, National Veterinary Institute, Uppsala, Sweden) in the laboratory of the Department of Food Science and Hygiene, Estonian University of Life Sciences.

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Results, the prevalence

Year or study period	Sampling site	No. of positive samples/total no. of samples	Positive %
2000	Fresh chicken meat	32/90	35.6
2002-2003	Raw poultry meat	70/610	11.5
2005-2006	Fresh chicken meat	26/340	7.6
Total poultry meat		**128/1040**	12.3
2005-2006	Fresh fecal and cecal samples	**105/1518**	6.9

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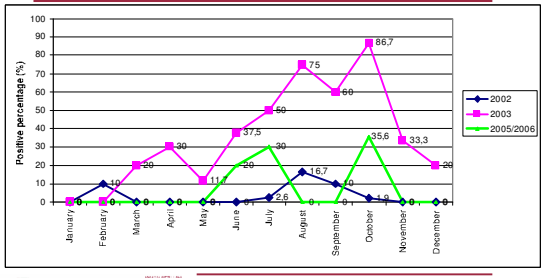
Proportion of *Campylobacter* positive poultry meat samples at retail level in Estonia during 2002, 2003 and 2005/2006, Seasonality



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Results, serotypes and genotypes

- The most common serotypes were O:1,44; O:21 and O:55, accounting for 28%, 13% and 13% of the isolates.
- The serotype O: 1,44 was most common both in domestic and imported poultry products and this serotype seems to have global distribution.
- 22% of *C. jejuni* isolates were nonserotypeable.
- PFGE genotyping of 70 *Campylobacter* isolates yielded 29 *Sma*I PFGE types and *Kpn*I was more discriminatory enzyme, yelding 34 PFGE types.
- The genotypes of the isolates from the poultry products of different countries were not overlapping.

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Results, antimicrobial susceptibility 2002 and 2003 (n = 70)

- Isolates were resistant to:
  - ciprofloxacin, 66%
  - nalidixic acid, 66%
  - tetracycline, 44%
  - ampicillin, 34%
  - erythromycin, 14%
- Resistance occured in 81% of *Campylobacter* isolates to at least one of the tested antimicrobials.

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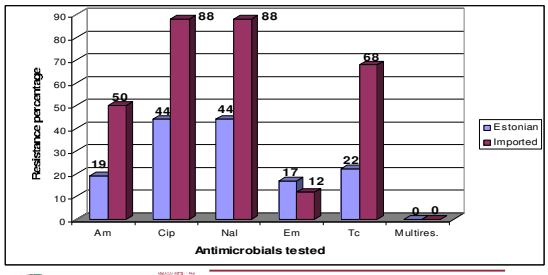
Antimicrobial susceptibility of *Campylobacter* isolates (n = 70) in 2002-2003



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Results, antimicrobial susceptibility 2002 and 2003 (n = 70)

- We found no simultaneous resistance to three or more unrelated antmicrobials
- Resistance of isolates to two unrelated antimicrobials was mainly to a combination of ciprofloxacin/nalidixic acid and tetracycline
- Results of disk dffusion method and the E-test were similar
  - all isolates resistant or susceptible by the disk diffusion method showed the same results by E-test

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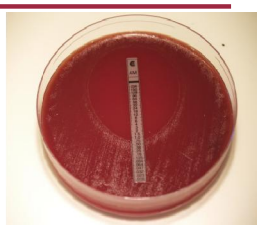
Results, antimicrobial susceptibility 2002 and 2003, E-test®

Resistant strain



Susceptible strain

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Results, antimicrobial susceptibility, VetMIC™Camp 2005 and 2006 (n = 131)

The highest frequency of resistance was to nalidixic acid and enrofloxacin, 75.6% and 73.3%, respectively and followed by:

oxytetracycline 32.1%

erythromycin 19.8%

gentamicin 19.1%

ampicillin 7.6%

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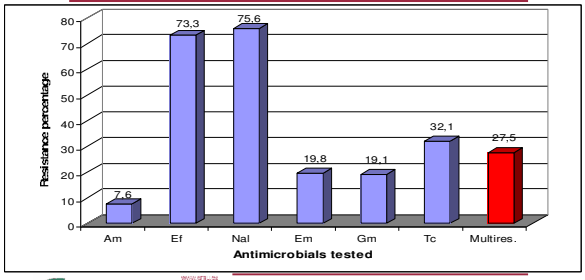
Antimicrobial susceptibility of *C. jejuni* isolates (n = 131) in 2005-2006



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Results, antimicrobial susceptibility VetMIC™Camp, 2005 and 2006 (n = 131)

- In 2005 and 2006 an important finding was **the high percentage** (79.4%) of antimicrobial-resistant *Campylobacter jejuni* isolates, of which 27.5% exhibited **multiresistance** (resistance to three or more unrelated antimicrobials). Resistance was especially high to enrofloxacin (80 isolates MIC ≥ 4 µg/ml).

Antimicrobial resistance of *Campylobacter jejuni* isolates in 2005 and 2006 (n = 131)

Antimicrobial resistance phenotype		Number of strains	Proportion (%)
Am/Ef/Em/Gm/Nal/Te	MULTIRESISTANCE	3	2.3
Am/Ef/Em/Nal/Te		1	0.8
Am/Ef/Em/Gm/Nal		1	0.8
Ef/Em/Gm/Nal/Te		11	8.4
Ef/Em/Nal/Te		10	7.6
Ef/Gm/Nal/Te		6	4.6
Am/Ef/Gm/Nal		2	1.5
Am/Ef/Nal/Te		1	0.8
Ef/Gm/Te		1	3.8
Am/Ef/Nal		1	0.8
Ef/Nal/Te		5	0.8
Ef/Nal		54	41.2
Gm/Te		1	0.8
Nal/Te		1	0.8
Am		1	0.8
Nal		3	2.3
Te		2	1.5
Resistant to one or more antimicrob.		**104**	**79.4**

Antimicrobials registrated for poultry treatment in Estonia

- Enrofloxacin and flumequine (fluoroquinolones) – possibly explaining the high level of resistance detected among Estonian isolates;
- Doxycycline (tetracycline antibiotics group, tetracycline and oxytetracycline in our resistance study);
- Amoxicillin (belonging to the penicillin group of beta-lactam antibiotics, ampicillin resistance in our study);
- Tylosin (antibiotic of the "macrolide" class - same class as erythromycin)

Antimicrobials registrated for poultry treatment in Estonia

- Tiaphenicol (wide spectrum antibiotic, amphenicol group);
- Florfenicol (wide spectrum, like chloramphenicol);
- Sulfadiasine + Trimetoprim (sulfonylamide + trim);
- Sulfachloropyridasine + Trimetoprim (sulfonylamide + trim);
- Lincomycine + Spectinomycine (lincosamides, wide spectrum)
  - Generally, these veterinary medicinal agents are allowed to use for treatment of food animals/birds in Estonia to which the maximum residue limits in foodstuffs of animal origin are established in the Council Regulation (EEC) No. 2377/90.

Antibiotics as feed additives

- Since January 2006 the use of antimicrobial feed additives has been banned within the EU
  - Regulation No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition
    - Article 5, section 4 constitutes that antibiotics, other than coccidiostats or histomonostats, shall not be authorized as feed additives

Directions in European monitoring EFSA Journal (2007), 96,1-46
Task Force on Zoonoses Data collection

- For harmonized monitoring of antimicrobial resistance in *Campylobacter jejuni* and *C. coli* in EU
  - disc diffusion is not advocated for European monitoring because different methodologies are used with different criteria
  - **only quantitative data on MIC will be accepted**
  - for *Campylobacter* spp. dilution methods shall be performed according to the methods described in Clinical and Laboratory Standards Insitute (CLSI) guidelines M31-A3 – Third Edition
  - Antimicrobials to be included: erythromycin, ciprofloxacin, tetracycline, streptomycin, gentamicin

Directions in European monitoring EFSA Journal (2008), 155:1-49

- The quantitative detection of *Campylobacter* spp. shall be done according to EN ISO/TS 10272-2:2006 Part 2: Colony-Count Technique
  - detection method can be excluded from the programme
- PCR method is the preferred method for *Campylobacter* speciation as phenotypical methods bear a certain risk of giving intermediate or incorrect test results.

Conclusions (1)

- Fresh chicken meat of the small-scale company were significantly ($P < 0.001$) more prevalent for *Campylobacter* than on those originated from the large-scale company 35.6% and 6.3%, respectively (2000 and 2002).
- Proportion of *Campylobacter* positive samples on fresh chicken products of Estonian origin was 9.1% compared to 15.9% obtained from imported frozen raw poultry products in 2002 and 2003.
- Compared to raw poultry products collected in Tallinn retail outlets, more commonly *Campylobacter* spp. positive samples were obtained from products collected from Tartu retail markets in 2002 and 2003
- Seasonal peak of *Campylobacter* on chicken meat was from June to October.

Conclusions (2)

- Our studies showed high serotype and genotype diversity among *Campylobacter* isolates from raw poultry meat in Estonia.
- Compare to *Sma*I the *Kpn*I was more discriminatory enzyme in PFGE typing.
- PFGE had good typeability and it was a useful tool in molecular typing of isolates from food.
- In our study the majority of the isolates sharing a similar PFGE genotype originated from one country.

Conclusions (3)

- Multidrug resistance in 2005 and 2006 was significantly associated with enrofloxacin and nalidixic acid resistance (correlation coefficient 0.372 and 0.310, $p<0.01$).
- Multidrug resistance in Estonian broiler chicken isolates was one of the highest reported in studies of broiler chicken *Campylobacter* isolates.

Co-operation

- Estonian University of Life Sciences
  - Prof. Ari Hörman
  - Kadrin Juhkam, MSc
  - Terje Tamme, DVM, MSc
- State Veterinary and Food Laboratory
  - Liidia Häkkinen, DVM
  - Toomas Kramarenko
  - Piret Põltsama

Co-operation

- University of Helsinki
  - Prof. Marja-Liisa Hänninen
  - Prof. Hannu Korkeala
  - Kristi Praakle-Amin, DVM
- Broiler industry in Estonia

Thank you for your attention!

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