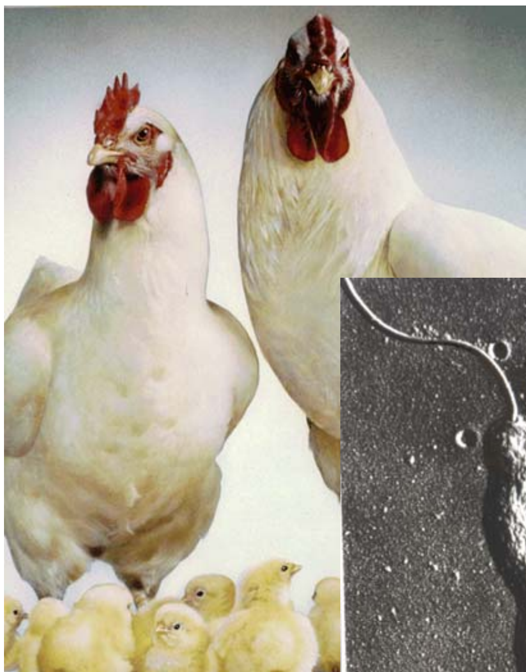
Fluoroquinlone resistant *Campylobacter*

Epidemiology and risk assessment

Frank M. Aarestrup, Prof., Head
DVM, PhD, Dr. Med. Vet.
fmaa@food.dtu.dk

Campylobacter jejuni/coli



- Primary foodborne pathogen in most developed and developing countries
- Few specific control strategies available
- Antimicrobial resistance a growing concern

Zoonoses in Denmark

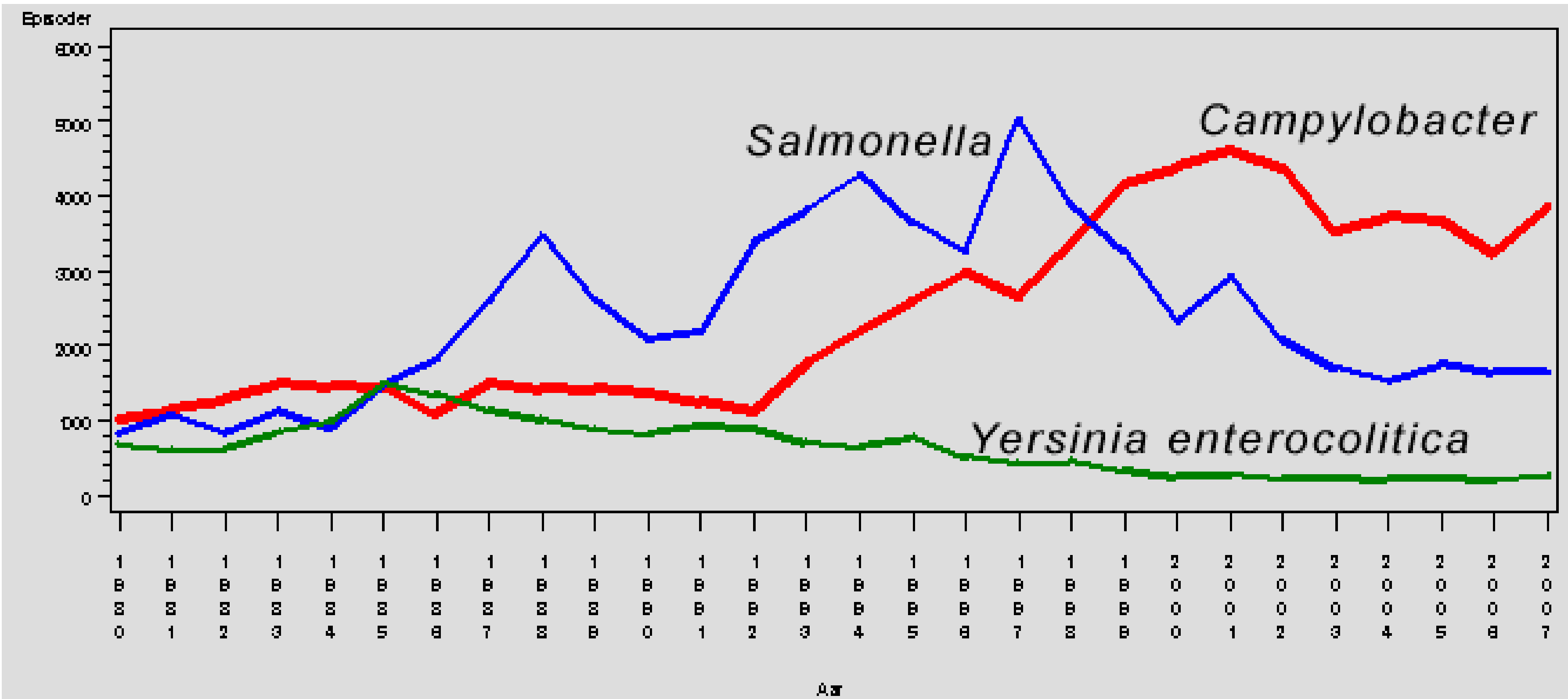
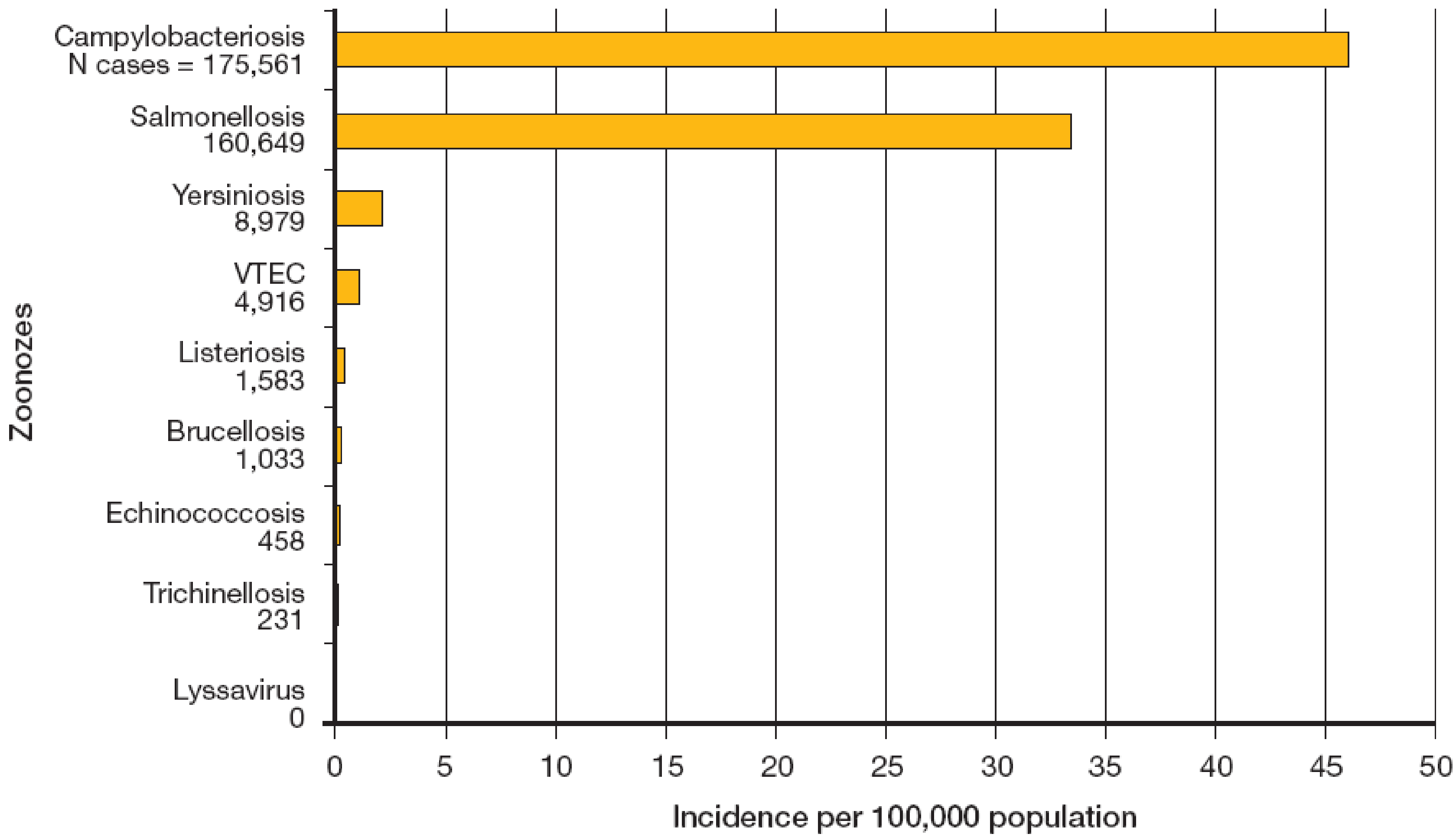
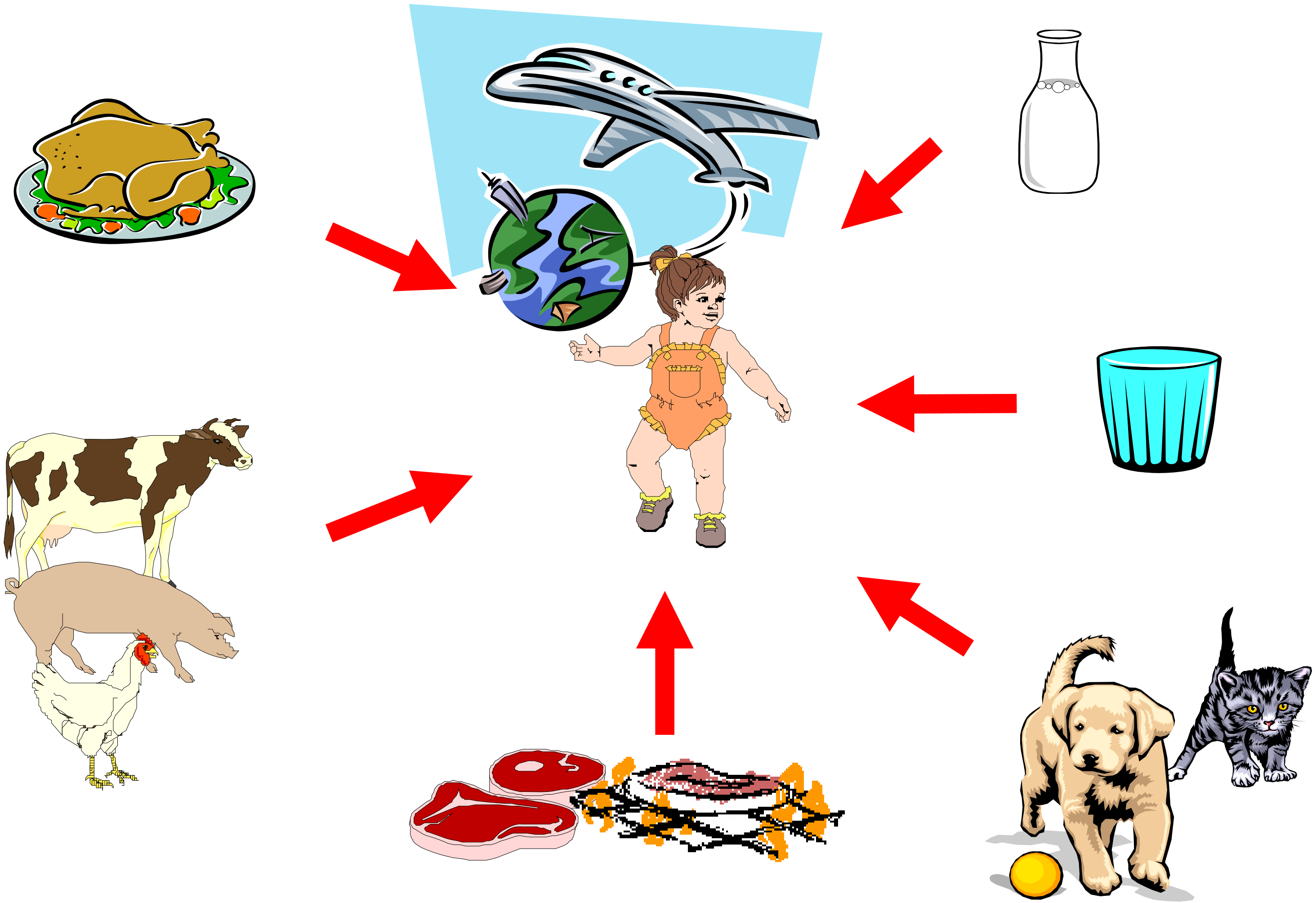


Figure SU1. The reported incidences of the zoonoses in humans, 2006

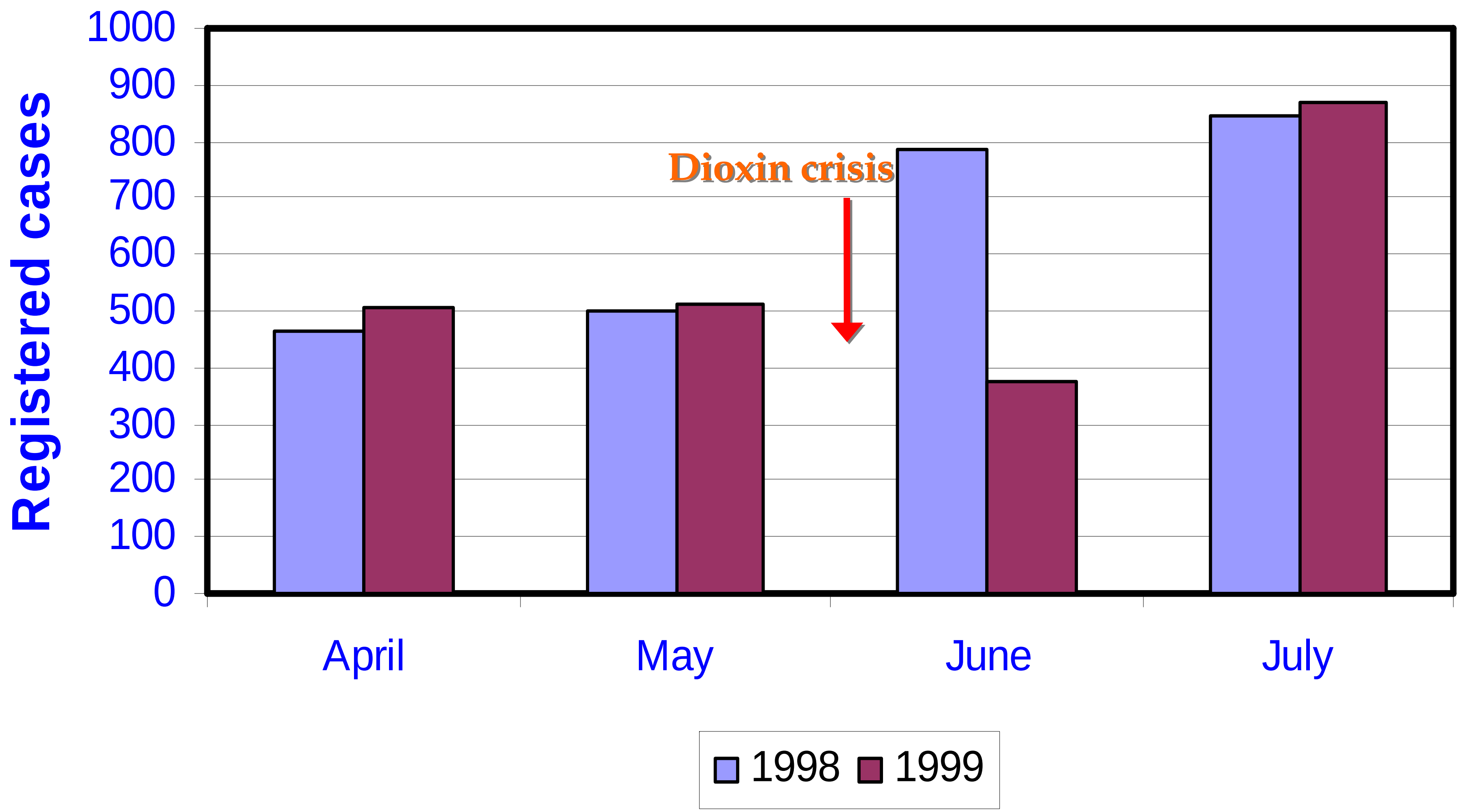


EFSA zoonoses report 2007

Sources of campylobacteriosis



Campylobacteriosis incidence in Belgium

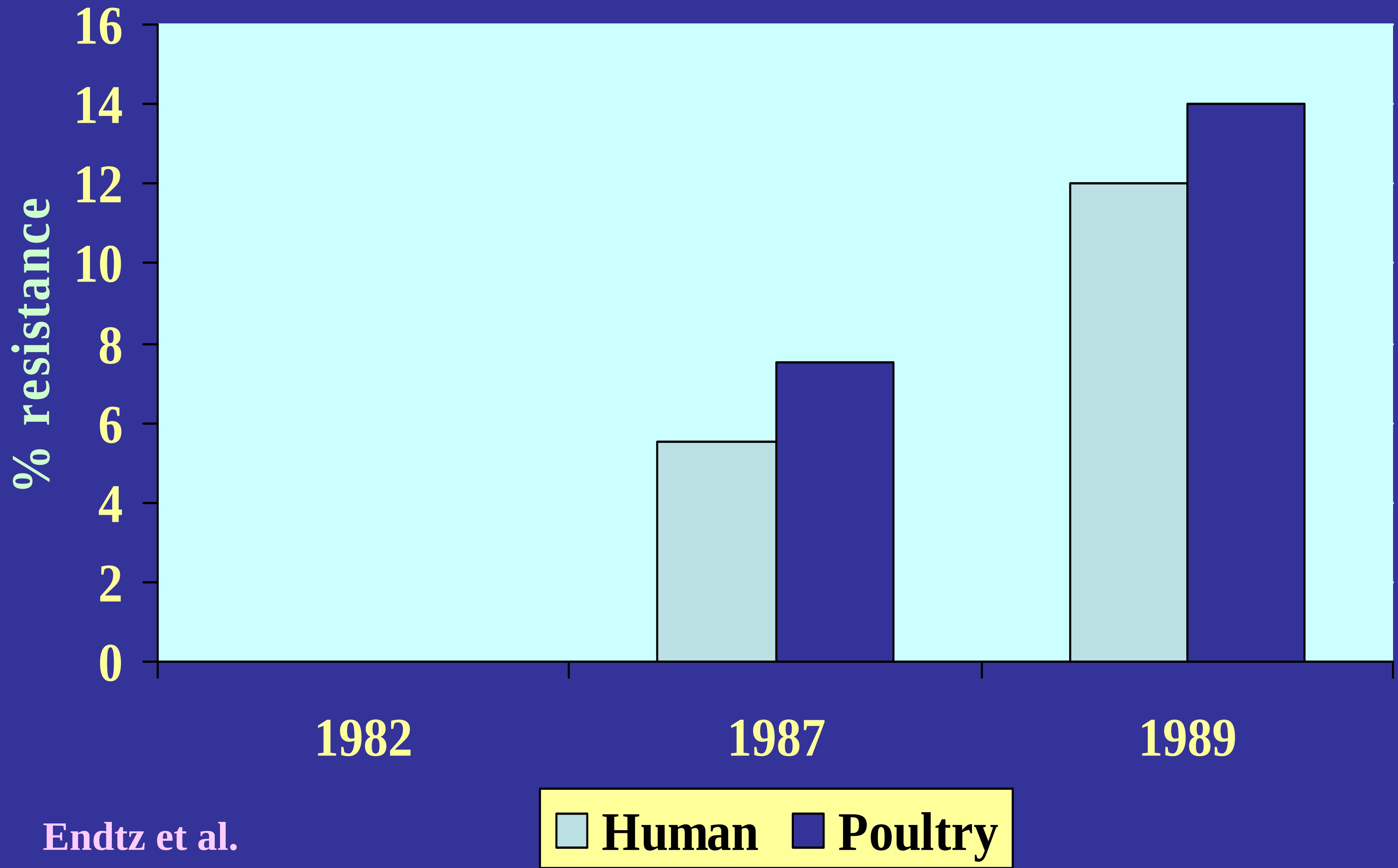


Data: Dr. Frank van Loock

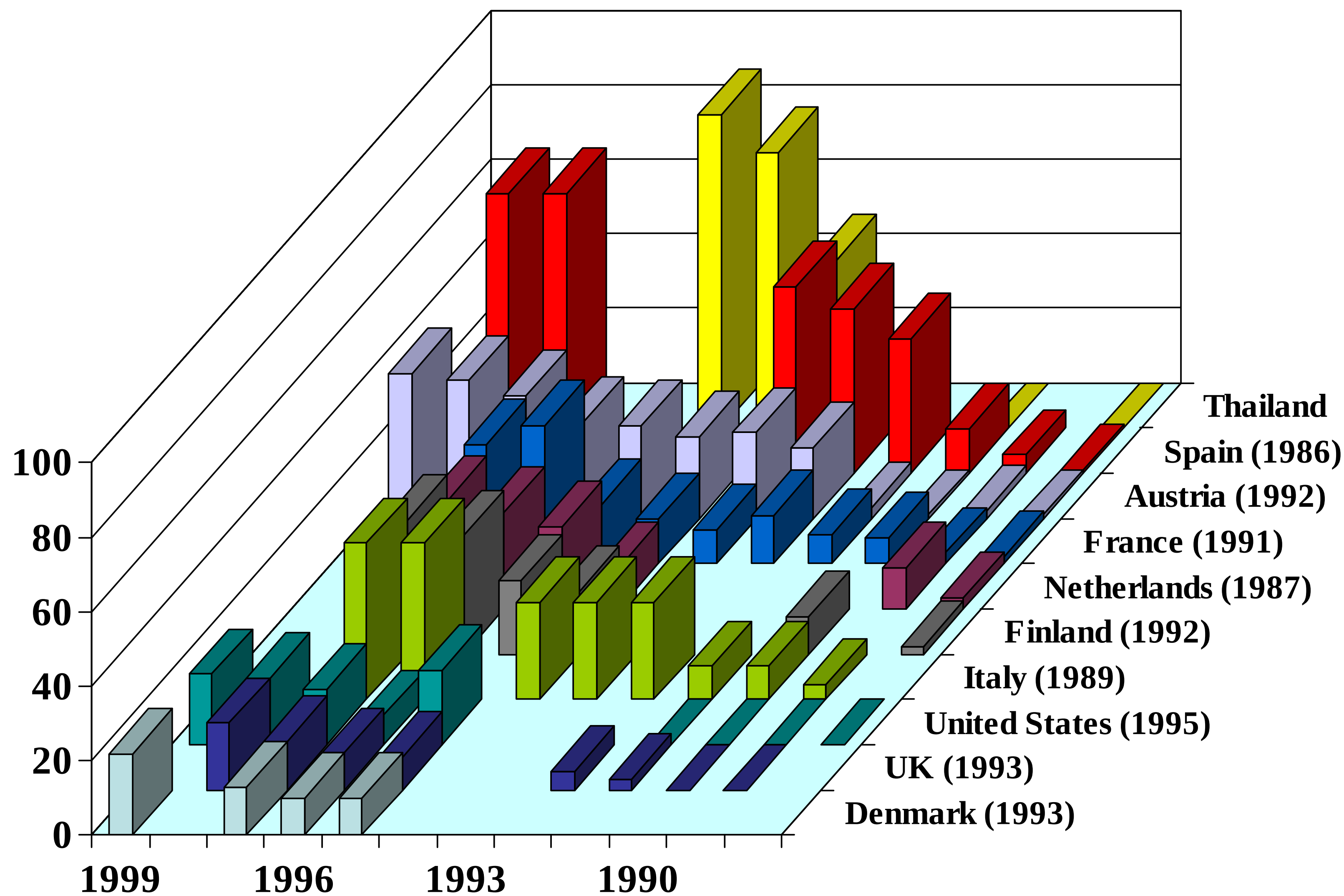
The basics

- Most or second most common zoonoses
- Most studies have indicated poultry as the main reservoir
- Resistance mediated by mutations in gyrA
  - Ala-70 to Thr
  - Thr-86 to Ile, Lys, Ala, Val
  - Asp-90 to Ala, Asn, Tyr
- Thus, spread of resistance is with the clone
- Large clonal instability – difficult to determine the spread

Fluoroquinolone resistance in Campylobacter

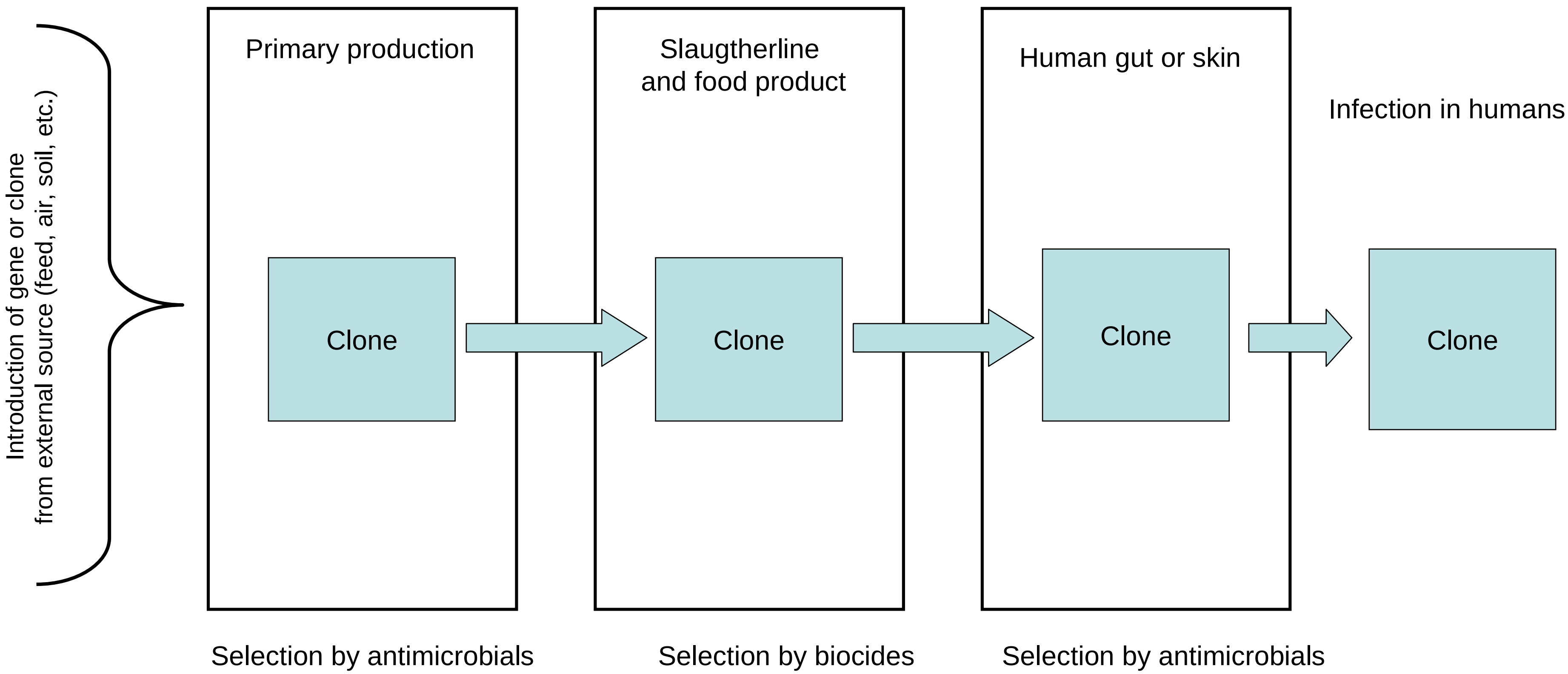


Endtz et al.
1991



100
80
60
40
20
0
1999
1996
1993
1990
Thailand
Spain (1986)
Austria (1992)
France (1991)
Netherlands (1987)
Finland (1992)
Italy (1989)
United States (1995)
UK (1993)
Denmark (1993)

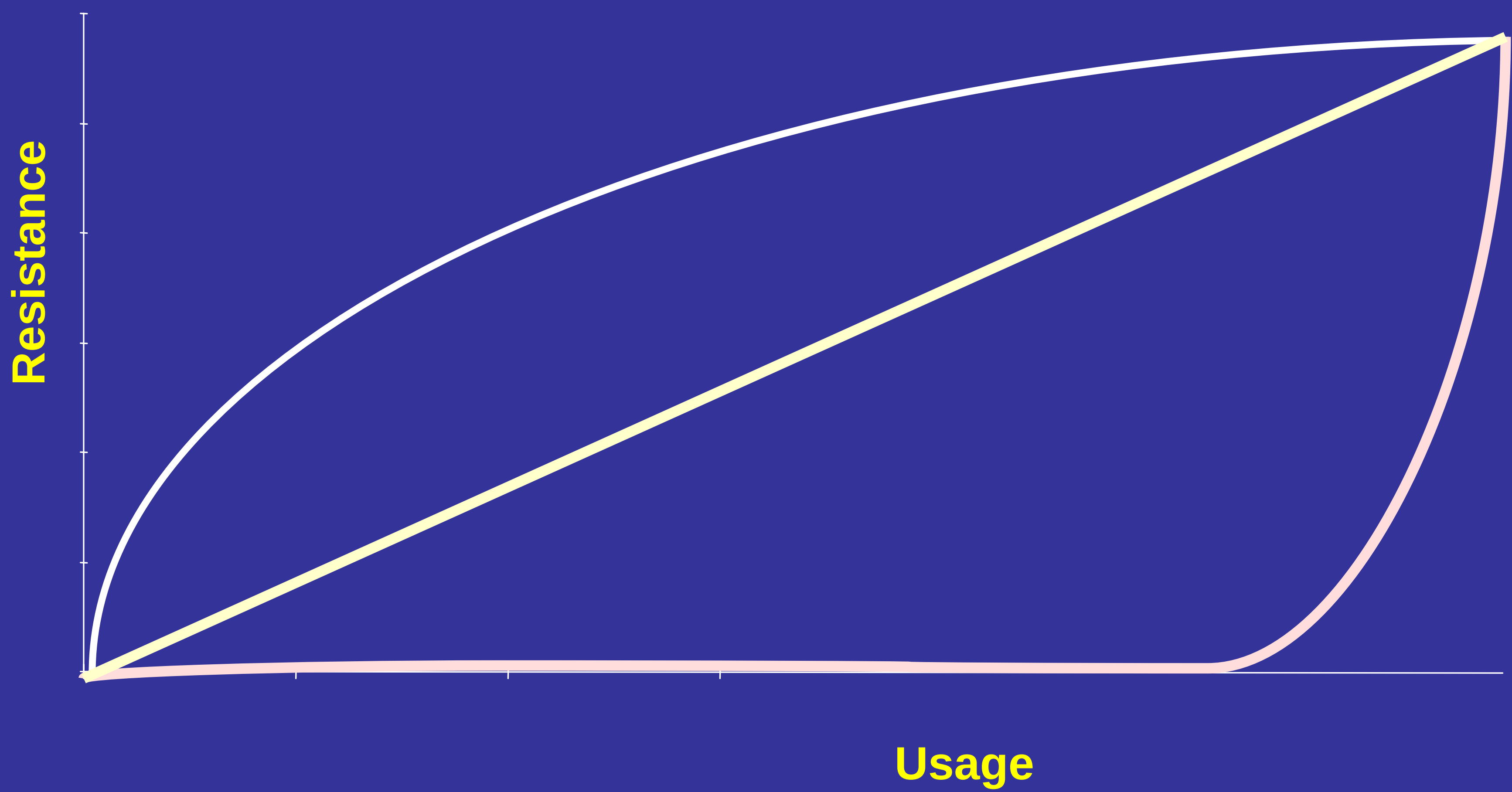
Campylobacter FQ - resistance epidemiology



Experimental studies on the emergence of antimicrobial resistance in Campylobacter following treatment.

Author and reference	Animal species	Number of experiments and number of animals in each group	Treatment	Outcome
Takahashi et al. (112)	Chickens	**Study 1.** Two groups of 15 chickens inoculated with 10^6 *C. jejuni* ATCC 33560 at day 17. **Study 2.** Two groups of 15 chickens inoculated with 10^7 and 10^8 C. jejuni ATCC 33560, at day 18 and 23, respectively.	50 ppm enrofloxacin in drinking water for three days to one group	**Study 1.** *C. jejuni* were isolated from approximately 50% of the control group and disappeared from the administration group during treatment. No resistant isolates were found. **Study 2.** Isolation of *C. jejuni* from most chickens and a 100% resistance from the administration group.
Van Boven et al. (11)	Chickens	Sixteen individually housed chickens colonized with FQ-susceptible *C. jejuni* at day 8, from day 21 eight chosen for further study.	50 ppm in drinking water from day 21 to 30 to 6 of the 8 chickens	In 5 chickens of the treatment group an emergence of FQ-resistant isolates were observed.
McDermott et al. (64)	Broiler chickens	**Study 1.** Two groups (treatment and control) of 25 chickens each. Colonized with a mixture of 5 *C. jejuni* strains. **Study 2.** Two groups (treatment and control) of 50 chickens each. Colonized with a mixture of 5 *C. jejuni* strains.	**Study 1.** Enrofloxacin at 40 ppm in drinking water for five days. **Study 2.** Sarafloxacin at 40 ppm in drinking water for five days.	Rapid and persistent emergence of ciprofloxacin resistance in *C. jejuni*.
Delsol et al. (23)	Piglets	Two groups of six piglets	One group given 15 mg enrofloxacin /pig/day for five days.	FQ-resistant *C. coli* found at levels of 40-80% in the treated group.
Lin et al. (55)	Broiler chickens	**Study 1.** Three experiments with 10-15 chickens in each group. A control and a treatment group. Experiment A was inoculated with a mixture of two *C. jejuni* strains. Experiment B with a mixture of two C. coli strains and experiment c with a C. jejuni strain. **Study 2.** Two experiments with 9-11 chickens in each group all inoculated with an C. jejuni strain.	**Study 1.** Tylosin at 0.53 g/L in drinking water for three days for experiment A and B. Three times treatment with tylosin at 0.53 g/L in drinking water for three days. **Study 2.** Tylosin at 50 mg/Kg feed for 41 days.	**Study 1**. No emergence of erythromycin resistant isolates. **Study 2.** Erythromycin resistance emerged at 17 and 31 days after inoculation, respectively.

Usage – resistance relationships



Exposure

Table 1. Presence of thermotolerant *Campylobacter* at the poultry slaughterhouse level

Product	Stage of process	Sample type	n[a]	% Positive[b]	Enumeration (log CFU)	Country	Year	Reference
Chicken feathers	Before scalding	1.5 g	18	nd[c,d]	5.4/g	USA	1999	Berrang et al. (2000)
Chicken breast skin	Before scalding	6.5 g	18	nd[d]	3.8/g	USA	1999	Berrang et al. (2000)
Chicken crop	Before scalding	5.1 g	18	100[d]	4.7/g	USA	1999	Berrang et al. (2000)
Chicken ceca	Before scalding	7.8 g	18	100[d]	7.3/g	USA	1999	Berrang et al. (2000)
Chicken colon	Before scalding	3.1 g	18	nd[d]	7.2/g	USA	1999	Berrang et al. (2000)
Chicken carcasses	After scalding	Rinse	125	92	nd	USA	2004	Son et al. (2007)
Chicken carcasses	After plucking	Rinse	15	53.3	6.5/sample	Germany	2006	Klein et al. (2007)
Chicken carcasses	Before evisceration	Rinse	800	74.5[d]	2.7/ml	USA	2005	Berrang et al. (2007)
Chicken intestine	Before evisceration	Swabs	202	94[d]	nd	USA	2001	Jeffrey et al. (2001)
Chicken skin	Before evisceration	Swabs	202	78[d]	nd	USA	2001	Jeffrey et al. (2001)
Chicken crop	Before evisceration	Swabs	202	48[d]	nd	USA	2001	Jeffrey et al. (2001)
Chicken carcasses	After evisceration	Rinse	15	66.7	6.0/sample	Germany	2006	Klein et al. (2007)
Chicken offal	After evisceration	10 g	21	19	nd	France	1999	Denis et al. (2001)
Chicken neck skin	Before chilling	10 g	16	100	nd	France	1999	Denis et al. (2001)
Chicken carcasses	Before chilling	Rinse	75	100	nd	USA	2004	Son et al. (2007)
Chicken neck skin	After chilling	10 g	16	100	nd	France	1999	Denis et al. (2001)
Chicken carcasses	After chilling	Rinse	800	34.9[d]	0.43/ml	USA	2005	Berrang et al. (2007)
Chicken carcasses	After chilling	Rinse	15	40	5.4/sample	Germany	2006	Klein et al. (2007)
Chicken carcasses	After chilling	Rinse	213	100[d]	5.2/carcass	Italy	2003–2004	Manfreda et al. (2006)
Chicken carcasses	After chilling	Rinse	636	16	nd	Sweden	2002–2003	Lindmark et al. (2006)
Chicken carcasses	After chilling	Rinse	125	52	nd	USA	2004	Son et al. (2007)
Chicken wings, legs, fillet	Meat cutting	10 g	12	33	nd	France	1999	Denis et al. (2001)
Chicken breasts with skin	Meat cutting	Rinse	15	33.3	4.4/sample	Germany	2006	Klein et al. (2007)
Chicken breasts without skin	Meat cutting	Rinse	15	26.7	4.1/sample	Germany	2006	Klein et al. (2007)
Chicken skin, liver, neck	Not specified	Not specified	111	45.9	nd	Germany	1995–1997	Atanassova and Ring (1999)
Turkey skin	After killing	Swabs	43	76[e]	nd	Germany	2002	Alter et al. (2005)
Turkey skin	After scalding	Swabs	43	37.2[e]	nd	Germany	2002	Alter et al. (2005)
Turkey skin	After plucking	Swabs	43	58.1[e]	nd	Germany	2002	Alter et al. (2005)
Turkey skin	After evisceration	Swabs	43	72.1[e]	nd	Germany	2002	Alter et al. (2005)
Turkey carcasses	Before chilling	Rinse	59	36.9	nd	Canada	2005	Arsenault et al. (2007)
Turkey skin	Chilling 20 min	Swabs	43	67.4[e]	nd	Germany	2002	Alter et al. (2005)
Turkey skin	Chilling 24 hours	Swabs	43	25.6[e]	nd	Germany	2002	Alter et al. (2005)
Turkey breasts	Meat cutting	Not specified	22	4.2	1.9/g	Germany	2005	Atanassova et al. (2007)
Turkey wings	Meat cutting	Not specified	22	5.6	2.3/g	Germany	2005	Atanassova et al. (2007)
Turkey thighs and drumsticks	Meat cutting	Not specified	28	6.9	2.0/g	Germany	2005	Atanassova et al. (2007)
Turkey stock	Meat cutting	Not specified	20	2.8	2.3/g	Germany	2005	Atanassova et al. (2007)
Turkey offal	Meat cutting	Not specified	52	9.7	2.5/g	Germany	2005	Atanassova et al. (2007)
Wild pheasants, various	Not specified	Not specified	52	25.9	nd	Germany	1995–1997	Atanassova and Ring (1999)

[a] Total number of samples examined.
[b] Detection of *Campylobacter*, based on enrichment culture unless otherwise stated.
[c] Not determined.
[d] Detection of *Campylobacter*, based on direct culture.
[e] Detection of *C. jejuni* by PCR.

Exposure and dose-response just as for all other *Campylobacter*

Consequences

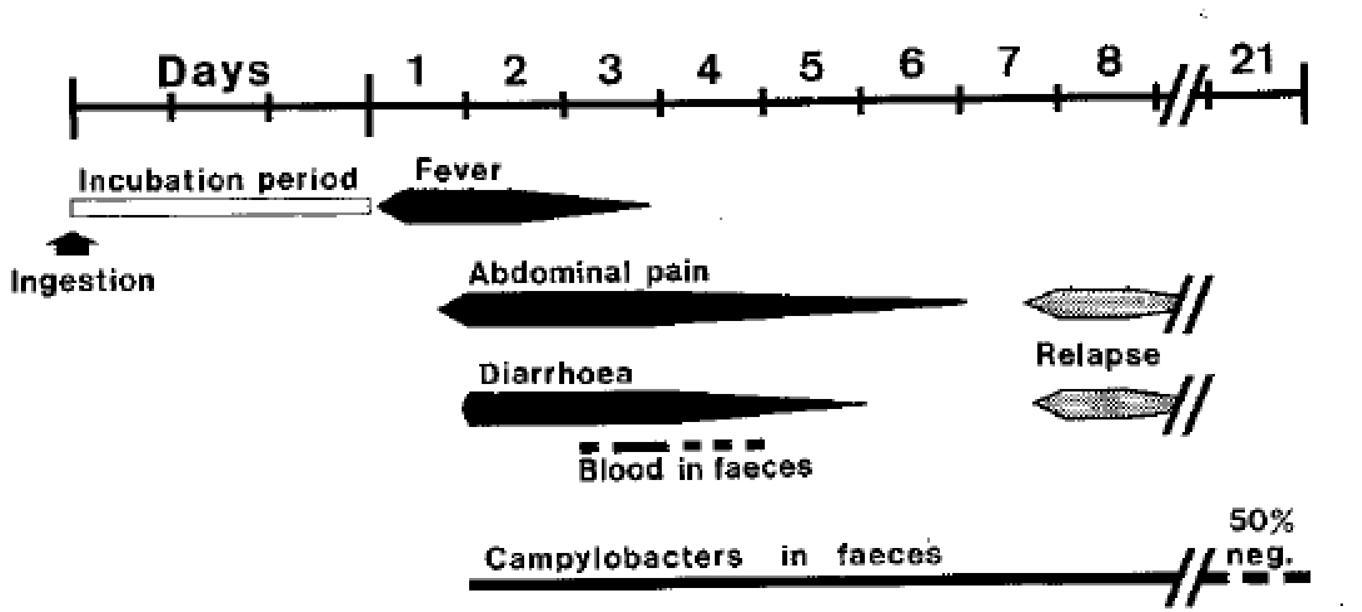


Figure 2. Diagram illustrating the typical course of *Campylobacter* enteritis. Reprinted from D. Greenwood, R. Slack, and J. Peutherer (ed.), *Medical Microbiology*, 15th ed. (Churchill Livingstone, Edinburgh, 1997).

Table 5. Studies evaluating the duration of illness in patients infected with quinolone-resistant or quinolone-susceptible *Campylobacter* strains[a]

Reference	Resistant		Sensitive		*P* value
	No. of patients	Duration of diarrhea (days)	No. of patients	Duration of diarrhea (days)	
Smith et al. (1999)	69	10	115	7	0.03
Neimann et al. (2001)[b]	5	14	31	9	0.13
The *Campylobacter* Sentinel Surveillance Scheme Collaborators (2002)					
Domestically acquired infection	—	12.7	—	13.5	0.56
Travel-related infection	—	11.8	—	11.2	0.66
Engberg et al. (2004)[c]	86	13.2	381	10.3	0.001
Nelson et al. (2004)[d]					
Model A	26	9	264	7	0.04
Model B	7	12	56	6	0.04
Model C	9	8	76	6	0.2

[a] Reprinted from Engberg et al., 2005.
[b] Stratified by treatment, but not on antimicrobial agent used for treatment.
[c] Analysis not stratified by treatment.
[d] Model A, analysis of 290 persons who did not receive antidiarrheal medications; model B, analysis of 63 persons who did not receive antimicrobial agents

Approx. 2-3 days additional illness

Part conclusion

- Use of FQ selects for resistance
- Exposure and infectivity as for other Campylobacter (NB patients in ciprofloxacin treatment)
- Consequences 2-3 days additional illness

FDA Fluoroquinolone-Resistant Campylobacter Risk Assessment

- To determine the feasibility of estimating risk to human health
- Possible regulatory tool for assessing future risks
- Possible tool for establishing regulatory “triggers” based on surveillance

http://www.fda.gov/cvm/default.htm

Fluoroquinolone resistant Campylobacter in poultry

FDA-CVM / Vose2000

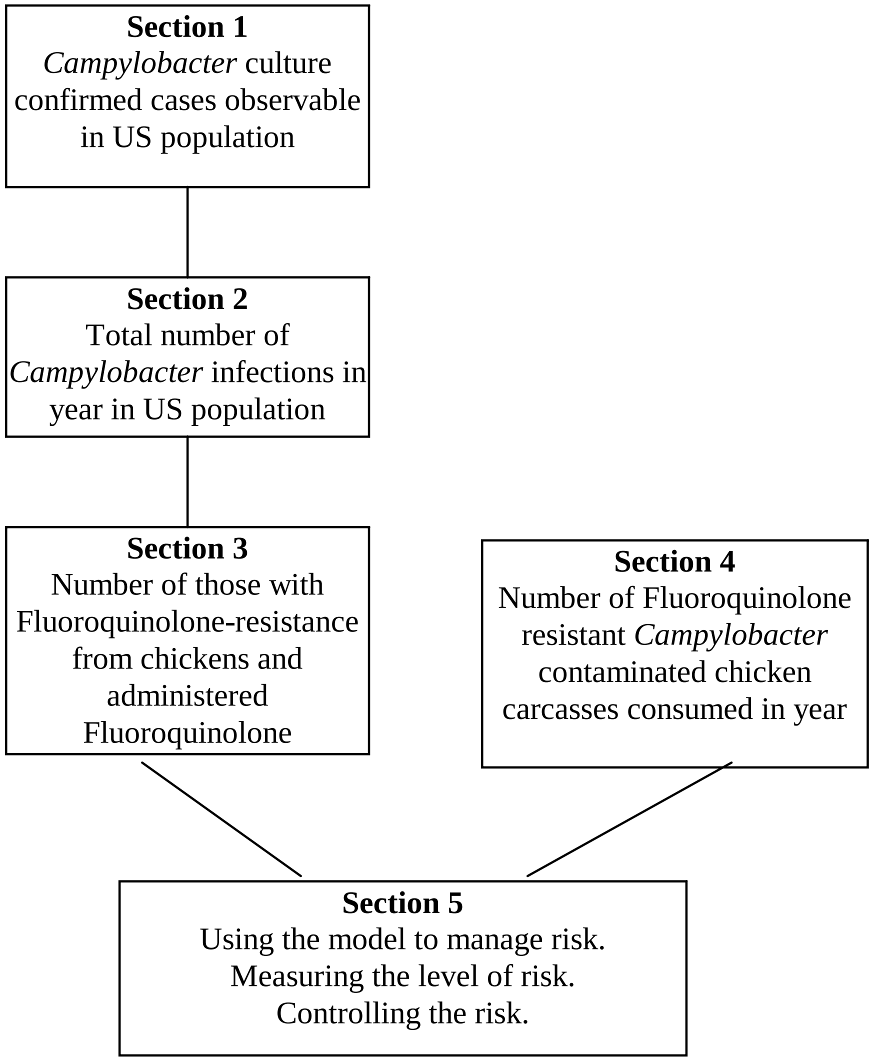
This model relates a number of contaminated carcasses N *consumed domestically to the number of illnesses* I *that resulted.*

It then predicts that for a future number of contaminated carcasses n, *there will be* i *infections where:*

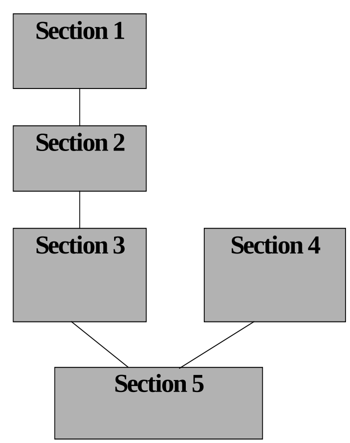
i=n*(I/N)

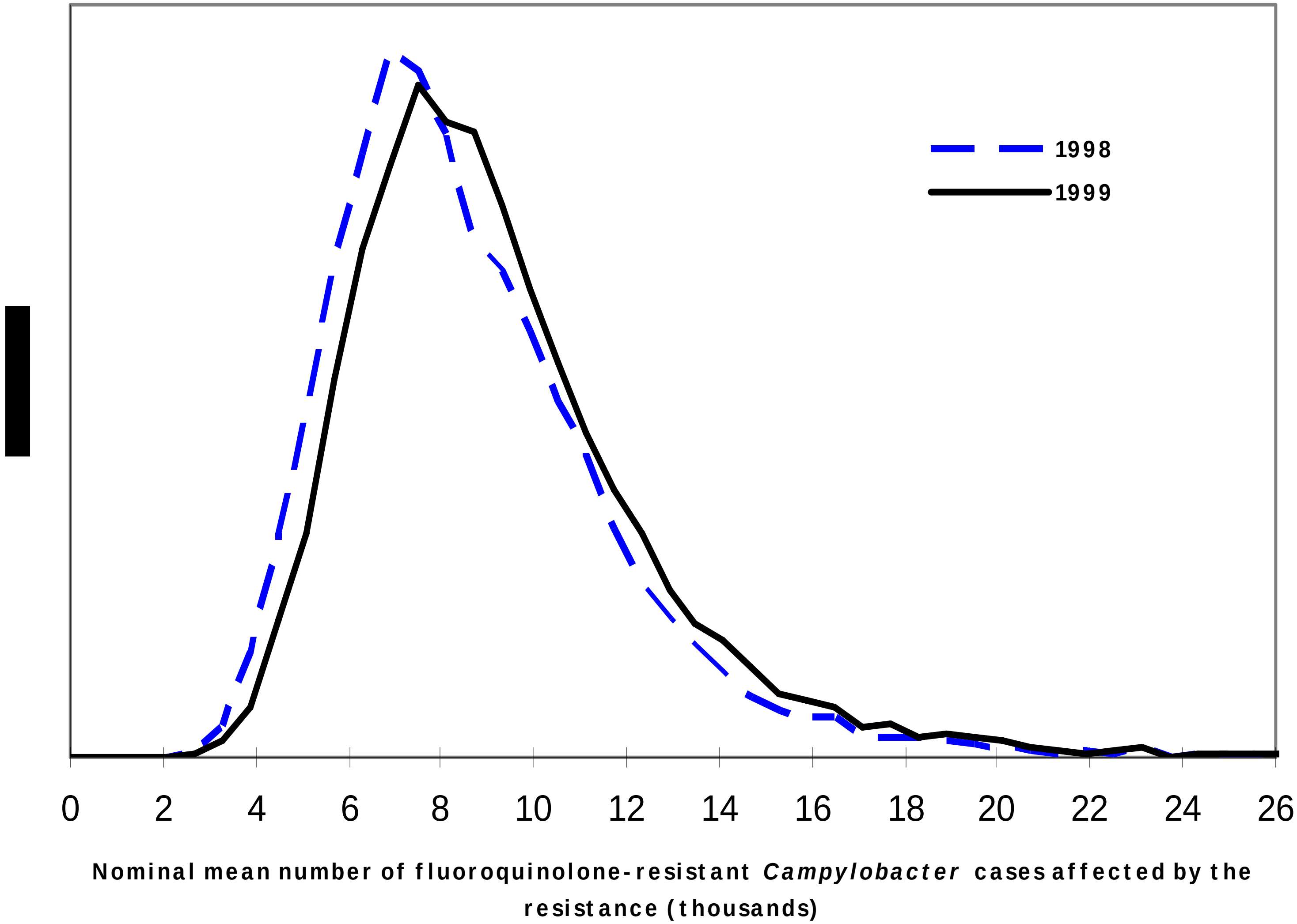
Model assumes that practices after production remain the same, but has some ability to make corrections

Model ends up with exactly the same behaviour as the Danish model!



Symbol	Description	Formula
Section 1	**Expected nominal number of observable confirmed cases**	
n_{US}	US population	Data
n_{FN}	FoodNet catchment population	Data
o_i	FoodNet observed invasive cases of Campylobacter	Data
o_e	FoodNet observed enteric cases of Campylobacter	Data
λ_i	Expected observed FoodNet invasive cases of Campylobacter	$=\text{Gamma}(o_i,1)$
λ_e	Expected observed FoodNet enteric cases of Campylobacter	$=\text{Gamma}(o_e,1)$
N_i (= $N1_i$)	Nominal observed mean invasive infections in population	$=\lambda_i * n_{US} / n_{FN}$
N_e	Nominal observed mean enteric infections in population	$=\lambda_e * n_{US} / n_{FN}$
p_b	Proportion of enteric infections with bloody diarrhea	Beta distribution based on data
$\mathbf{N1_{eb}}$	Nominal mean number of confirmed enteric infections in population with bloody diarrhea	$=N_e * p_b$
$\mathbf{N1_{en}}$	Nominal mean number of confirmed enteric infections in population with non-bloody diarrhea	$=N_e*(1- p_b)$

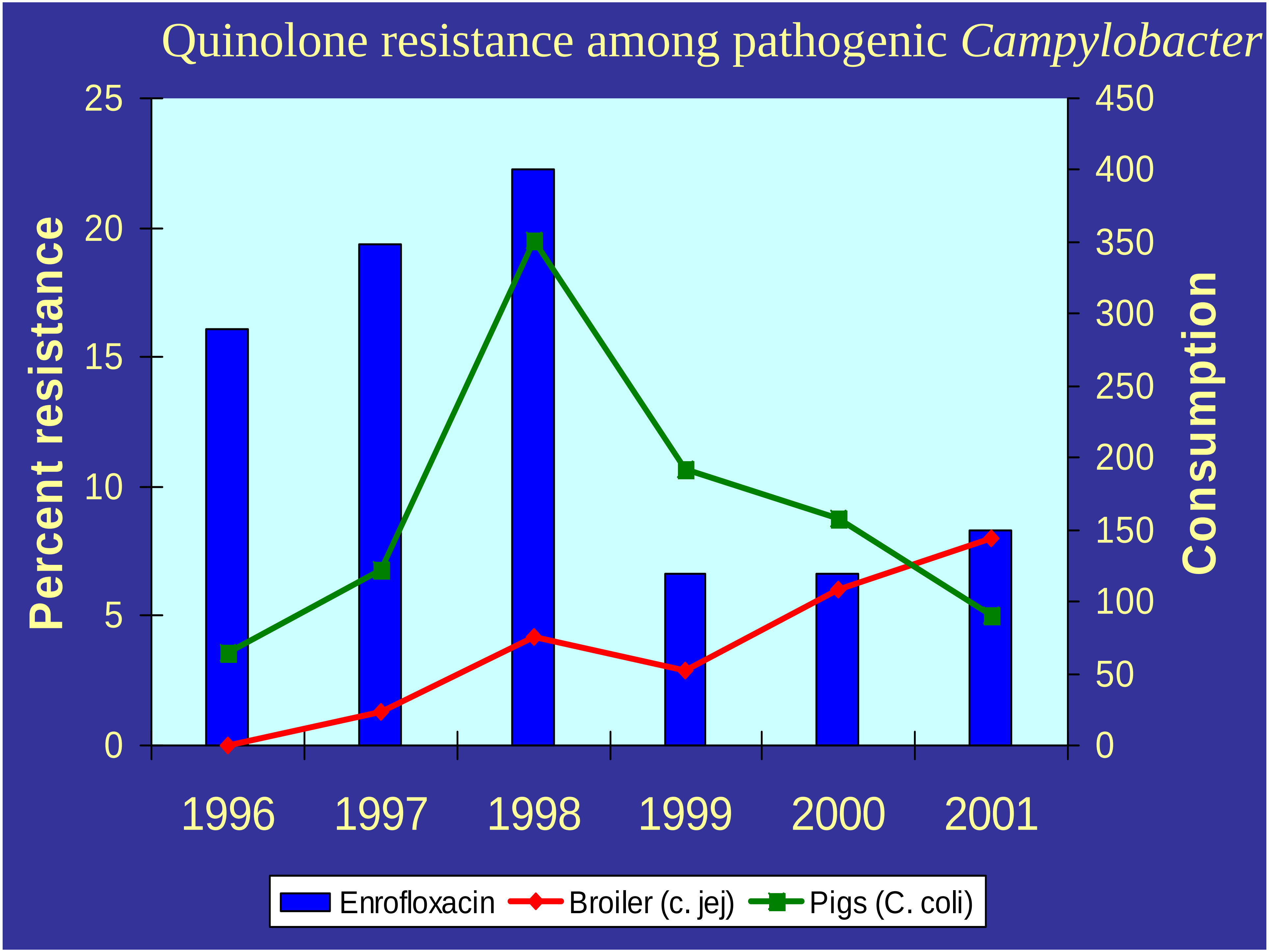




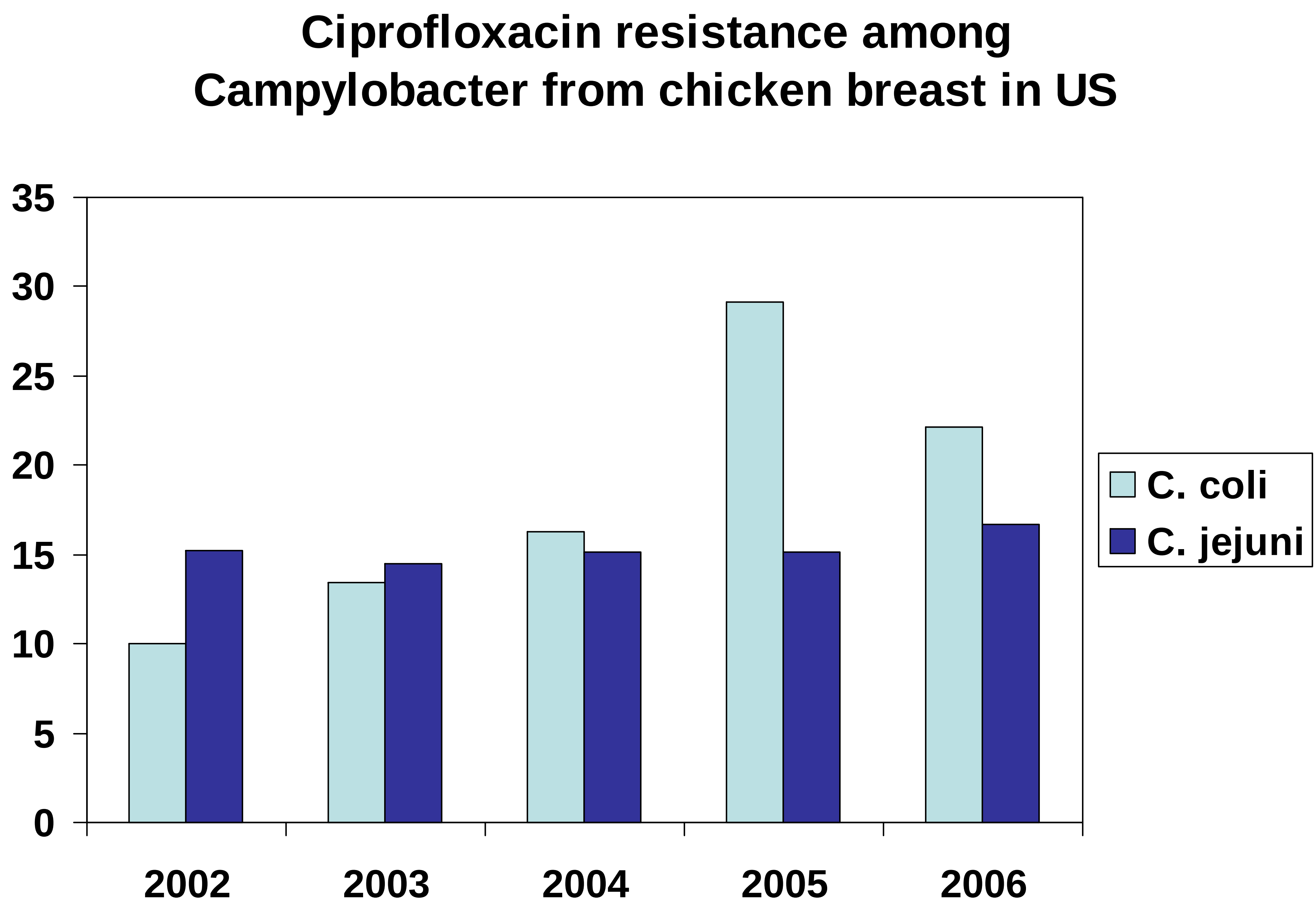
What is an acceptable level of risk?

Example Assumptions - FDA Risk Assessment

- Fluoroquinolone resistance (after removal of travelers, those who took a fluoroquinolone prior to culture and those for whom the time of taking the fluoroquinolone was unknown) is attributed to chickens
- The incidence rates for culture-confirmed *Campylobacter* infections in the FoodNet catchment are representative of incidence rates for culture-confirmed *Campylobacter* infections in the United States.
- The CDC study estimate on number of stool samples taken at the doctors office as remembered by the patient (18%) was better than the estimate as remembered by the doctor (78%).



Quinolone resistance among pathogenic *Campylobacter*
Percent resistance
Consumption
25
20
15
10
5
0
450
400
350
300
250
200
150
100
50
0
1996
1997
1998
1999
2000
2001
Enrofloxacin
Broiler (c. jej)
Pigs (C. coli)


Ciprofloxacin resistance among
Campylobacter from chicken breast in US
35
30
25
20
15
10
5
0
2002
2003
2004
2005
2006
C. coli
C. jejuni

In conclusion

- FQ selects for resistance
  - The effect of different treatment regimes has not been determined
- It is possible to model the expected number of cases and additional effects
  - Requires a lot of data and money to generate those data
- The effect of withdrawal not well documented (however, continuing must be expected to be worse)

The solution

Ban all use of fluoroquinolones?

Risk Analysis, Vol. 26, No. 1, 2006 DOI: 10.1111/j.1539-6924.2006.00723.x

Quantifying Potential Human Health Impacts of Animal Antibiotic Use: Enrofloxacin and Macrolides in Chickens

Louis Anthony (Tony) Cox, Jr.[1]* and Douglas A. Popken[1]

Table II. Results for Human Health Impacts Model

	Meaning	Base Case	Sensitivity Analyses				
Input							
$[(P^{-})MN)] * Q_s$	Current illness-days per year from chicken	$8.9 \times 10^5 = 1.48 \times 10^5$ cases per yr. $*$ 6 days per case					
ΔF	Fractional increase in servings from ill flocks if ban is implemented	0.005				0.1	0.1
R	Ratio of risk per serving from ill versus well flocks	10	2			139	139
$(1 - s)$	Resistant fraction of human illnesses if no ban	Macrolides: 0.01 Fluoroquinolones: 0.064					
$f * r$	Adverse clinical outcome probability for resistant cases	0.5 (= probability of being prescribed the resisted antibiotic)				$0.5 * (1/39) = 0.0128$	0.0128
K	Consequence ratio (e.g., of illness-days) for resistant versus susceptible cases	1.002 ← Or 1.333		1.3	2	2	
Output							
BENEFIT (illness-days)	Illness-days per year *prevented* by continued use $= [\Delta F(R - 1)] * [(P^{-})MN)] * Q_s$	40,050					
RISK (illness-days)	Illness-days per year *caused* by continued use $= (1 - s) * [f * r * (K - 1)] * [(P^{-})MN] * Q_s$	57 (Or 19,000) for enrofloxacin, 9 for macrolides					
RATIO for enrofloxacin	BENEFIT/RISK	703 for enrofloxacin	78	4.7	1.4	1.7×10^4	8.4×10^6
RATIO for macrolides	BENEFIT/RISK	4,500 for macrolides	500	30	9	1.1×10^5	5.4×10^7

f and r already included in the estimate of K

PROCESSING AND PRODUCTS

The Effect of Airsacculitis on Bird Weights, Uniformity, Fecal Contamination, Processing Errors, and Populations of *Campylobacter* spp. and *Escherichia coli*

S. M. Russell[1]

Department of Poultry Science, Poultry Science Bldg., University of Georgia, Athens, Georgia 30602-2772

ABSTRACT A study was conducted to determine if the presence of airsacculitis in broiler chickens contributes to loss of saleable yield, lack of uniformity, fecal contamination, processing errors, and increases in populations of pathogenic and indicator bacteria. In a commercial processing facility, groups of carcasses from airsacculitis (AS)-positive (ASP) and airsacculitis-negative (ASN) flocks were selected from the line and weighed, evaluated for cut or torn areas on the digestive tracts, and assessed for *Campylobacter* and *Escherichia coli* counts. Additionally, fecal contamination was monitored and recorded. The presence of AS reduced ($P \leq 0.05$) carcass weight averages in two of five repetitions. Although not significantly different in repetitions 1, 4, and 5, the means were higher for ASN flocks. The net loss averaged over five repetitions was 84 g/carcass, equating to a loss of 14,686.9 k (32,379 lb) of chicken meat for one growout house per year as the result of AS infection. ASP carcasses had higher ($P \leq 0.05$) fecal contamination in four of five repetitions. The number of total digestive tract cuts or tears were much higher on ASP carcasses at 42, 49, 37, 60, and 59% as compared to 14, 12, 17, 24, and 16% for ASN carcasses in repetitions 1 to 5, respectively. In three of the five replications, the presence of AS in the flocks increased ($P \leq 0.05$) the number of *Campylobacter* recovered from broiler carcasses. Hence, there appears to be a relationship between the presence of AS and *Campylobacter*-positive carcasses. *Escherichia coli* counts for ASP flocks were significantly higher than ASN flocks in repetitions 1 and 3. In repetition 5, *E. coli* numbers were significantly lower for the AS flock. These data differ from previous unpublished data from two separate pilot studies that demonstrated that *E. coli* counts for ASP flocks are significantly higher than ASN flocks. This difference may be attributed to the fact that in the pilot studies visibly infected carcasses were sampled, and in this study healthy birds that had passed inspection were sampled within an ASP flock. Because flocks of chickens showing signs of AS have lower weights, more fecal contamination, more processing errors, and higher levels of *Campylobacter* spp., broiler companies should emphasize control of AS in the flocks as a means of preventing subsequent food-borne bacterial infection.

(*Key words*: airsacculitis, processing error, fecal contamination, *Campylobacter*, *Escherichia coli*)

2003 Poultry Science 82:1326–1331

RUSSELL

TABLE 5. The effect of airsacculitis on *Campylobacter* spp. counts

	Replicate 1 $\log_{10}$ cfu/mL	Replicate 2 $\log_{10}$ cfu/mL	Replicate 3 $\log_{10}$ cfu/mL	Replicate 4 $\log_{10}$ cfu/mL	Replicate 5 $\log_{10}$ cfu/mL
Airsacculitis positive	2.05[a] ↑	2.26[a] ↑	2.89 ↓	1.56[b] ↓	1.69[a] ↑
Airsacculitis negative	0.05[b]	0.00[b]	3.10	2.30[a]	0.00[b]
n	20	20	20	20	20

[a,b]Means within a column with different superscripts are significantly ($P \leq 0.05$) different. No superscript indicates no significant difference.

10.45 / 5 = 2.09

5.45 / 5 = 1.09

Diff. 10 x higher load of Campylobacter from AS positive flocks

Actual numbers:

Positive: 123 + 182 + 776 + 36 + 49 = 1166 / 5 = 233

Negative: 1 + 0 + 1259 + 200 + 0 = 1460 / 5 = 292

Diff. 1,25 x higher load from AS negative flocks

Table II. Results for Human Health Impacts Model

	Meaning	Base Case	Sensitivity Analyses				
Input							
$[(P^{-})MN)] * Q_s$	Current illness-days per year from chicken	$8.9 \times 10^5 = 1.48 \times 10^5$ cases per yr. $*$ 6 days per case					
ΔF	Fractional increase in servings from ill flocks if ban is implemented	0.005				0.1	0.1
R	Ratio of risk per serving from ill versus well flocks	10 ← Or -1,25	2			139	139
$(1 - s)$	Resistant fraction of human illnesses if no ban	Macrolides: 0.01 Fluoroquinolones: 0.064					
$f * r$	Adverse clinical outcome probability for resistant cases	0.5 (= probability of being prescribed the resisted antibiotic)				$0.5 * (1/39) = 0.0128$	0.0128
K	Consequence ratio (e.g., of illness-days) for resistant versus susceptible cases	1.002 ← Or 1.333		1.3	2	2	
Output							
BENEFIT (illness-days)	Illness-days per year *prevented* by continued use $= [\Delta F(R - 1)] * [(P^{-})MN)] * Q_s$	40,050 ← Or -5,006					
RISK (illness-days)	Illness-days per year *caused* by continued use $= (1 - s) * [f * r * (K - 1)] * [(P^{-})MN] * Q_s$	57 for enrofloxacin, 9 for macrolides ← Or 19,000					
RATIO for enrofloxacin	BENEFIT/RISK	703 for enrofloxacin	78	4.7	1.4	1.7×10^4	8.4×10^6
RATIO for macrolides	BENEFIT/RISK	4,500 for macrolides	500	30	9	1.1×10^5	5.4×10^7

f and r already included in the estimate of K

Conclusions Cox & Popkten

- Some mistaken factors and numbers
- No uncertainty estimates
- Numbers could be looked at differently

- Useful for pointing out that potential benefits might also arise from the use of antimicrobials to animals