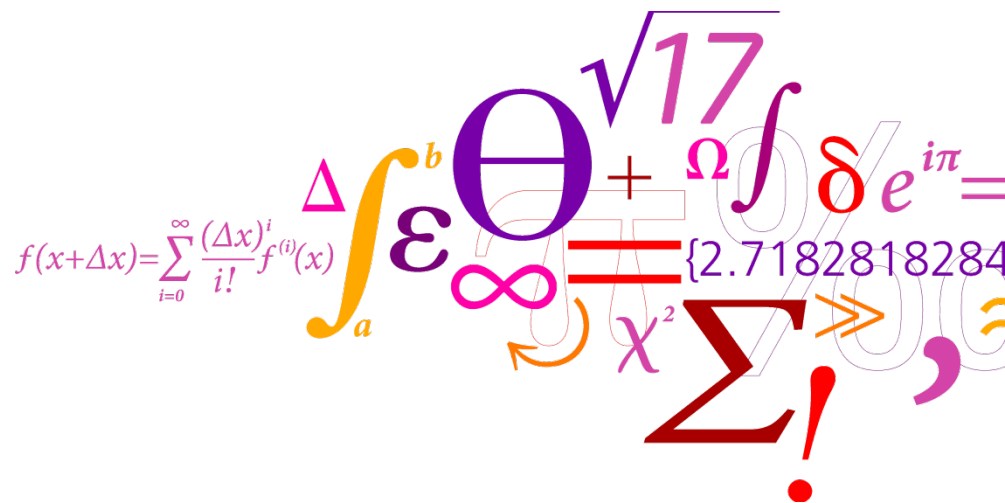




# Occurrence of ESBL-producing *E. coli* in Danish production animals and raw meat

Yvonne Agersø



# ESBL- one of the fastest emerging resistance problems

Definition:

- DANMAP 2010 “ESBL” describes the clinically important acquired beta-lactamases with activity against extended-spectrum cephalosporins; including the classical class A ESBLs (CTX-M, SHV, TEM), the plasmid-mediated AmpC (eg. CMY-2) and OXA-ESBLs. [Giske *et al.* *JAC* 63: 1-4].

# Consumption of 3. and 4. generation cephalosporins

## 2009

- 99 Kg active compound was used for pigs.

- Primary in breeding herds for sows/piglets (85%).

- 792 breeding herds used 3. or 4. gen. cephalosporins in 2009.

- Of the breeding herds 392 also used cephalosporins in another age group.

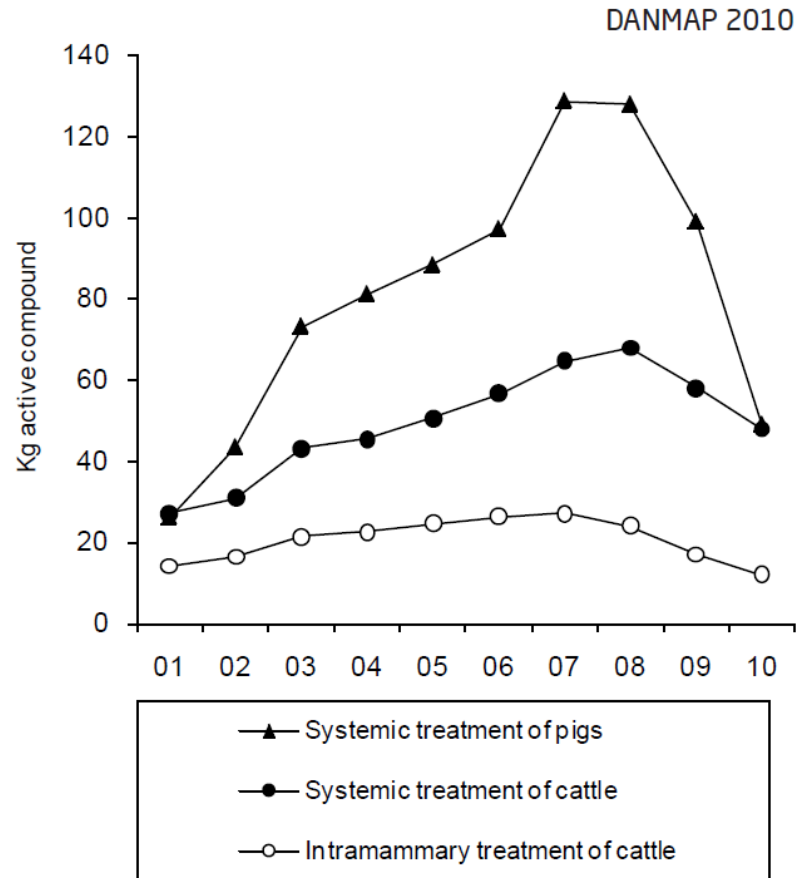
## 2010

**July voluntary stop for usage of cephalosporins in pigs**

Used for cattle

**Cephalosporins have not been used in the Danish broiler production for more than ten years.**

**Figure 4.3. Consumption of 3rd and 4th generation cephalosporins in pigs and cattle, Denmark**



# Background ESBL in Danish production animals



- **First detected in August 2003**, ESBL producing *E. coli* and *Salmonella* from production animals in Denmark (Aarestrup *et al.* 2004).
- The first cases were all associated with imported meat (products) or imported animals (Jensen *et al.*, 2006).
- In 2005/6 the first ESBL producing *E. coli* and *Salmonella* from pigs and *E. coli* from cattle reported (Aarestrup *et al.*, 2006).
- Increase in ESC in diagnostic submissions.
- 2009 first screening of ESBL in Danish pigs at slaughter by use of selective enrichment. 11% positive (incl. chromosomal ampC (17%))
- Imported poultry meat is the most important source (highest in 2008 7.6% ceftiofur resistant indicator *E. coli* from imported broiler meat); 6.8% in 2010.

# Sampling strategy

2009

Pigs at slaughter  
800 samples

2010

Pigs at slaughter  
400 samples

Broilers  
200 pooled samples

Cattle at slaughter  
200 samples

Pig herds  
100 samples

2011

Pigs at slaughter  
800 samples

Cattle at slaughter  
60 samples

Pig herds  
100 samples

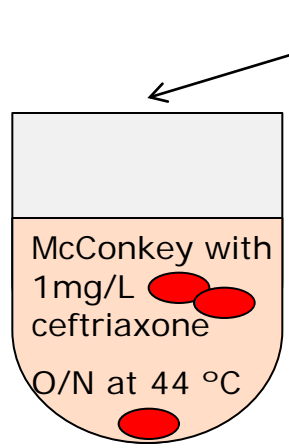
## Meat

Danish and imp.:  
pork, beef and  
broiler meat  
1000 prøver

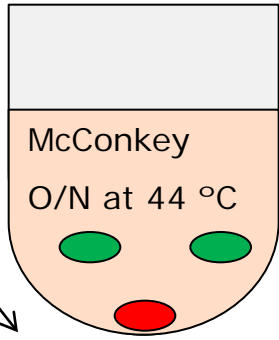
Danish and imp.:  
pork, beef and  
broiler meat  
1000 prøver

Danish and imp.:  
pork, beef and  
broiler meat  
1000 prøver

# Selective enrichment method compared with indicator *E. coli*



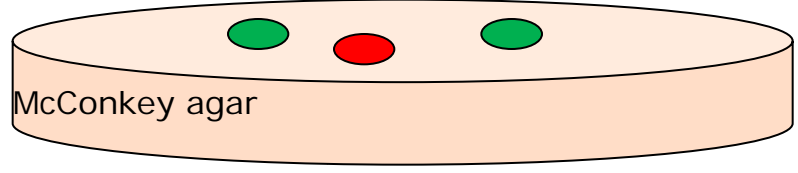
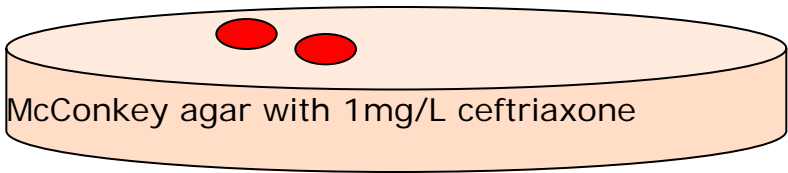
Growth of *cef<sup>R</sup> E. coli*



Growth of *E. coli*

↓ Transfer of 10 μL, O/N 44 °C

↓ Transfer of 10 μL, O/N 44 °C

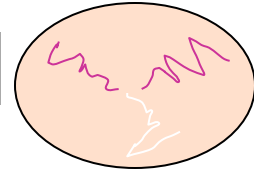


+/- (very sensitive method)

Resistance testning

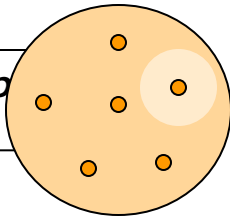
# Screening of ESBL genes

**Verification** of ID on Chrome Agar

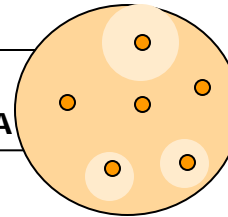


**Phenotypic** Disc diffusion test  
(FOX (cefoxitin), FEP, CAZ (ceftazidime), [CAZ + CLA], CTX (cefotaxime), [CTX + CLA])

**Phenotype Amp<sup>C</sup>**  
(FOX<sup>R</sup> og FEP<sup>S</sup>)



**Phenotype classical ESBL**  
(FOX<sup>S</sup> and FEP<sup>R</sup>) synergy with CLA



**Unknown**  
(Unclear interpretation)

**Sequencing of Amp<sup>C</sup> (pigs)**  
and PCR for CMY-2 (meat, broiler)

**PCR for CTX-M,**  
SHV or TEM

**Clondiag Array**

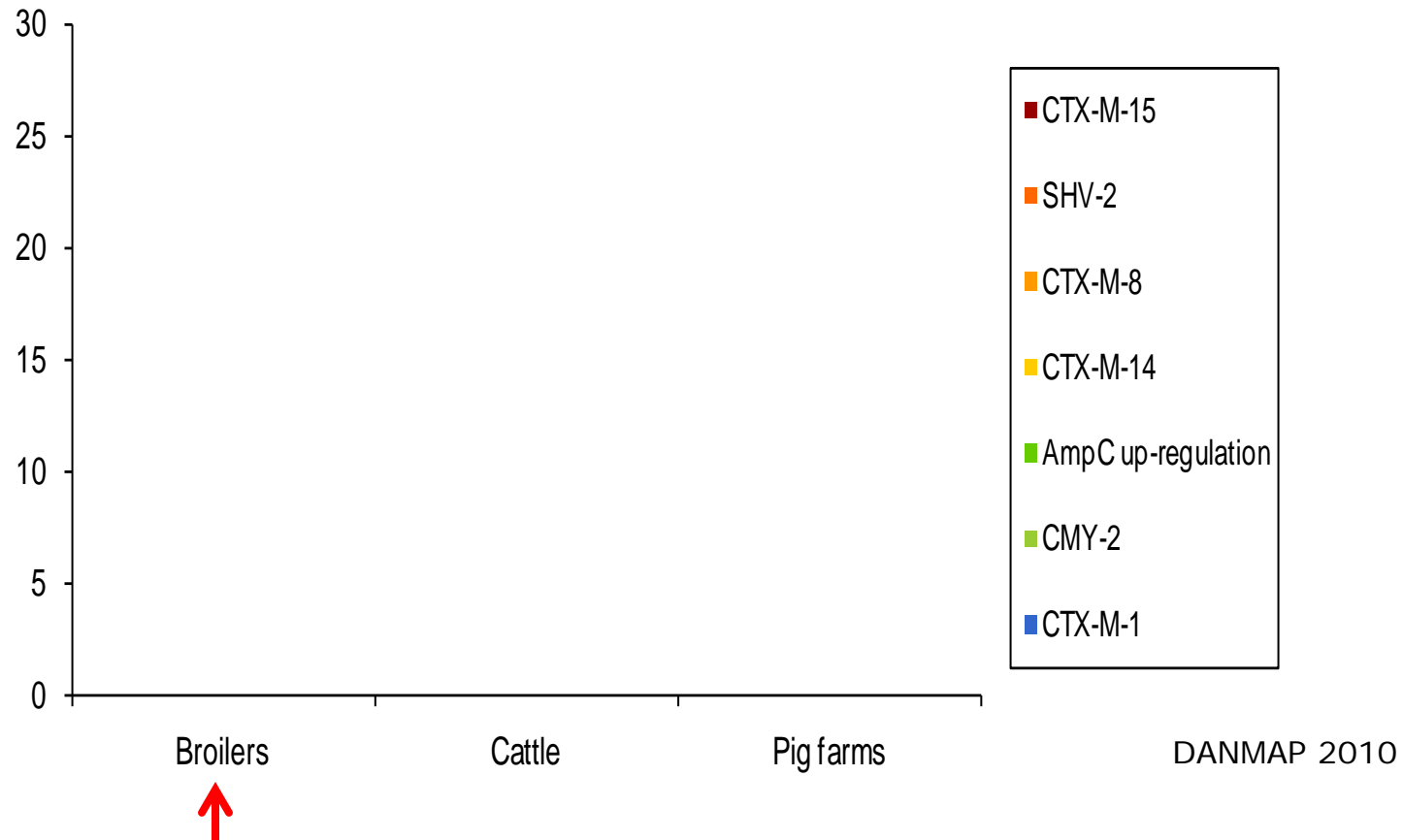
**If positive**  
sequencing of  
PCR product

**Or ClondiagArray**

**PCR and sequencing**

**Genotype**

## ESBL *E. coli* in Broilers, pig herds and cattle in 2010



No registered Cephalosporins usage for broilers in DK in at least ten years



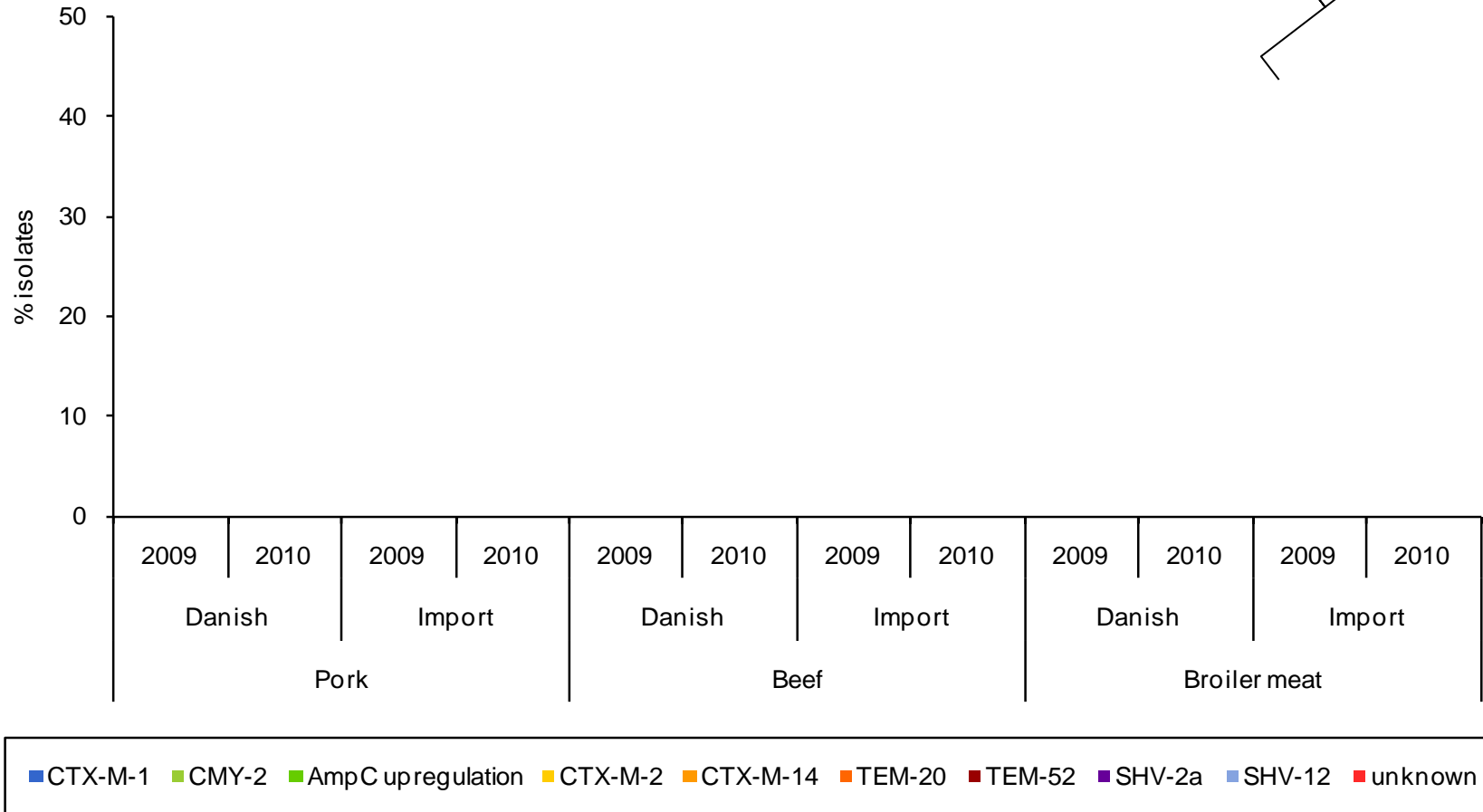
# results

- **Cattle at slaughter** primary young bulls. Two types of consumption of cephalosporins (systemic and intramammaria).
  - The importance of usage on the prevalence of ESBL should be further investigated.
- **Broilers**
  - Focus on the breeding pyramid
- **Pig herds**
  - Occurrence as in 2009 in pigs at slaughter with CTX-M-1 as the dominating genotype.
  - Effect of the voluntary ban

# Resultat: Prevalence (%) of ESBL in raw meat in 2009 and 2010 –selective enrichment

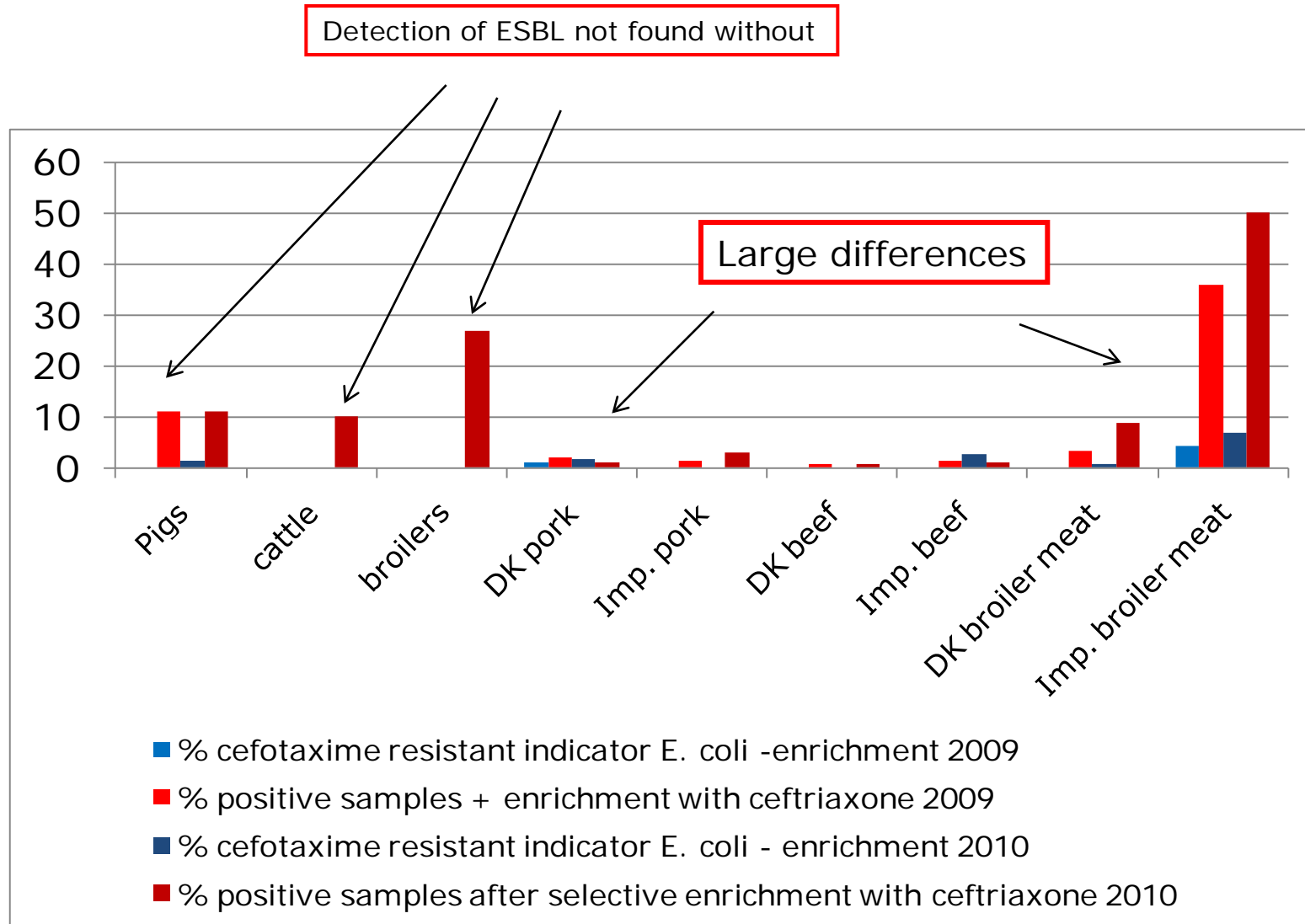


Significant increase

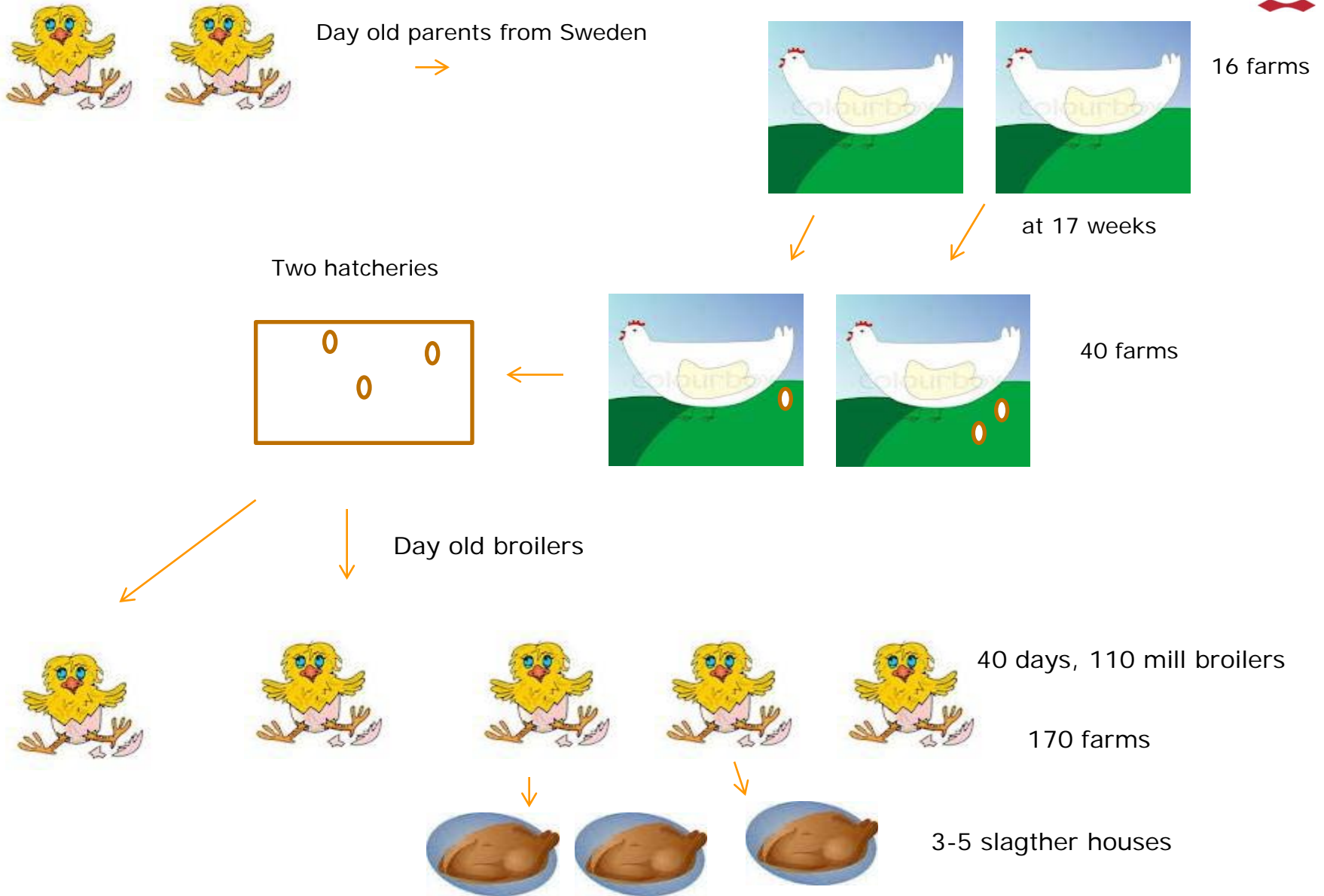


DANMAP 2010

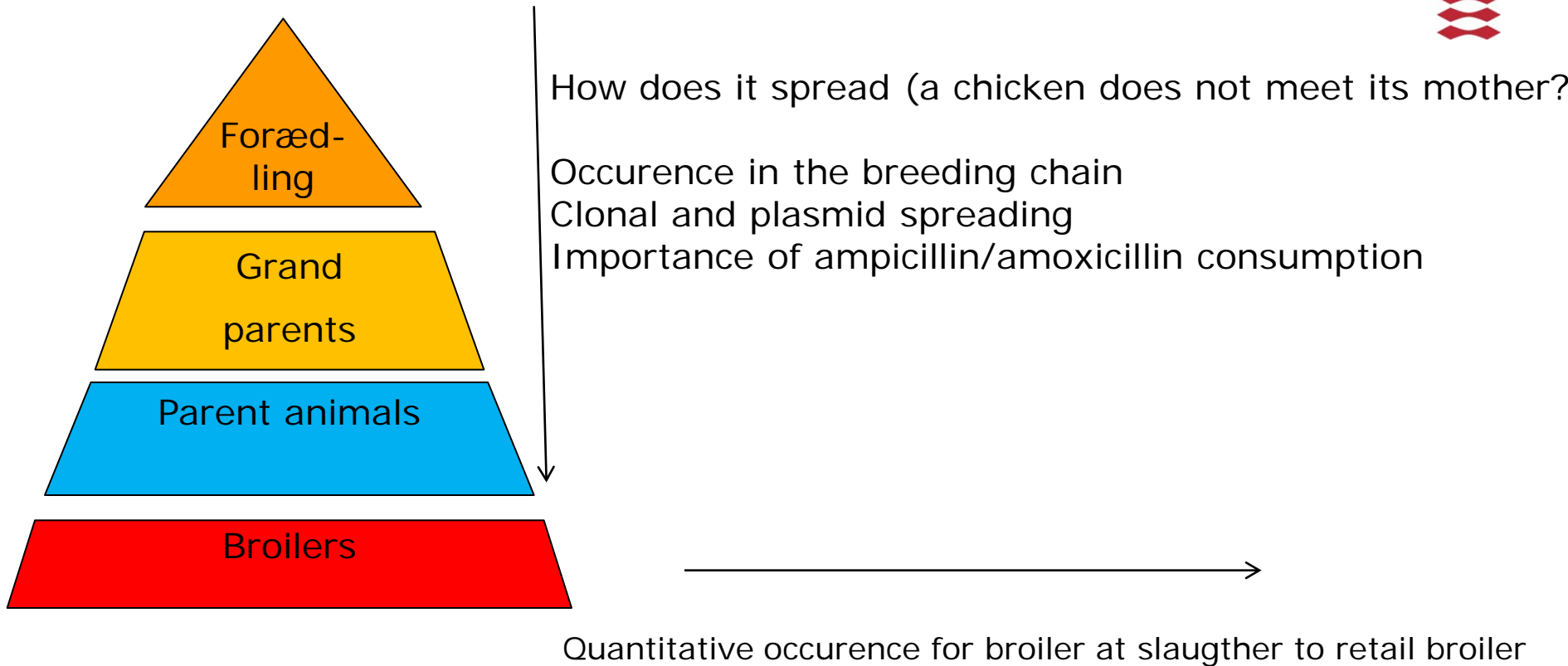
# Results: Comparison of methods



# Danish broiler production (conventional)



## Fokus på ESBL kyllingeproduktionen



Findings. Samme genes and clones in parents, broilers and danish broiler meat.  
Poly clonal and some are dominating

# Discussion/Conclusion



- Use of selective methods for detection of ESBL,
  - Detects ESBL in animals/meats where not previously found.
  - More detailed picture.
- The high occurrence in broiler meat is surprising and are probably due to spread via the breeding.
- The most important source is imported broiler meat. Probably due to spread via breeding and consumption of cephalosporins and other antibiotics abroad.
- Certain genotypes are dominant in *E. coli* from certain sources and can be useful for source attribution.
- Some are genotypes, found in bacteria causing human infection. Should be further investigated.

# Future studies

- The presence of ESBL *E. coli* in slaughter pig herds and cattle at slaughter are investigated in 2011.
- The presence of ESBL *E. coli* in pigs at slaughter in 2011 and 2012.
- The prevalence of ESBL in case-by-case samples of broilers and turkeys.
- The presence of ESBL *E. coli* in meat in 2011 and 2012
- The CFU of ESBL in broilers at slaughter and after slaughter I 2012.

- **Involved in the study**

- The regional laboratories administrated by the Danish Food Agency.
- Anne Mette Seyfarth og Karl Pedersen og Zoonose lab., National Food institute.
- Hanne Nørgård Nielsen, Christina Aaby Svendsen, Jacob D. Jensen og Yvonne Agersø Unit of antimicrobial resistance and mol. Typing, National Food Institute.
- Peter Johansen, Danhatch.
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