BUNDESINSTITUT FÜR RISIKOBEWERTUNG

BfR

Risiken erkennen – Gesundheit schützen

# Bewertung des Virulenzpotentials von Shigatoxin 2e (Stx2e) bildenden *E. coli*-Stämmen

***Gladys Krause & Lothar Beutin***

# Shiga Toxin producing *E. coli* (STEC)

More than 200 serotypes...

Some of these can cause (bloody) diarrhea and HUS in humans

Shiga toxin family, action similar to plant toxin ricin

28s RNA-N-glycosidase, cytotoxic activity,

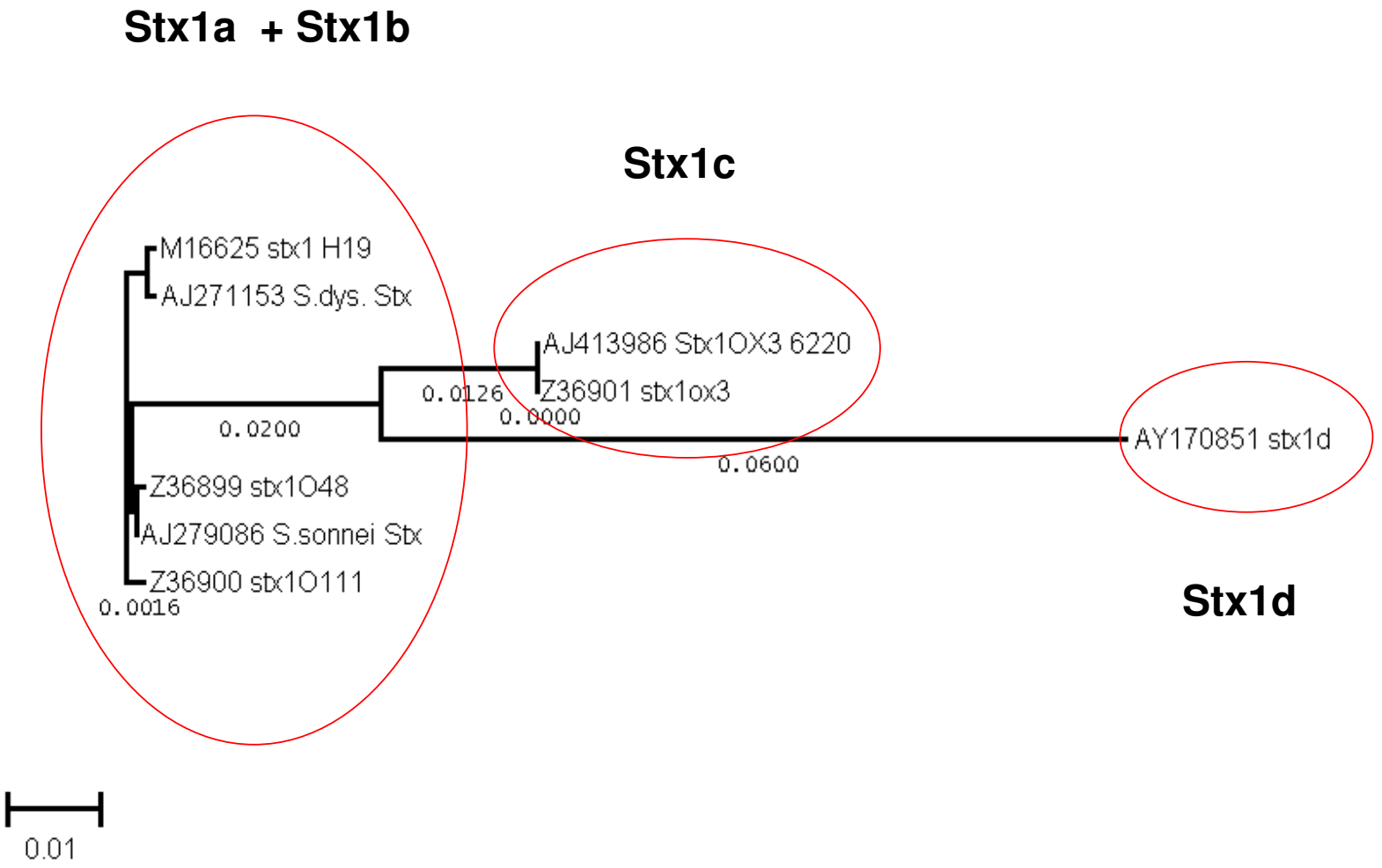
inhibition of protein biosynthesis, cell death and organ damage

# Stx1 & Stx2 Families

| Stx1 family | | Stx2 family | | |
|---|---|---|---|---|
| **stx-gene** | **gene bank access. no** | **stx-gene** | | **gene bank access. no** |
| $stx_1$ | M16625 | $stx_2$ | ← | X07865 |
| $stx_{1\text{-}CB168}$ | Z36900 | $stx_{2c}$ ($stx_{2v\text{-}ha}$) | ← | AJ605767 |
| $stx_{1\text{-}O48}$ | Z36899 | $stx_{2d\text{-}ount}$ | | AF043627 |
| $stx_{1\text{-}OX3}$ ($stx_{1c}$) | Z36901 | $stx_{2\text{-}Ox3:H21}$ | | X65949 |
| $stx_{1d}$ | AY170851 | $stx_{2\text{-}OX392}$ | | L11079 |
| $stx_{1d\text{-}like}$ | AY986980-82 | $stx_{2e}$ | | M21534 |
| | | $stx_{2f}$ | | AJ010730 |
| | | $stx_{2ev}$ ($stx_{2f\text{-}like}$) | | M29153 |
| | | $stx_{2g}$ | | AY286000 |
| | | $stx_{2\text{-}NV206}$ | | AF329817 |
| | | $stx_2$ & $stx_{2c}$ | ← | M59432 & M76738 |
| | | $stx_{2d1}$ & $stx_{2d2}$ ($stx_{2d\ mucus\ activatible}$) | ← ← | AF479828 + AF479829 |

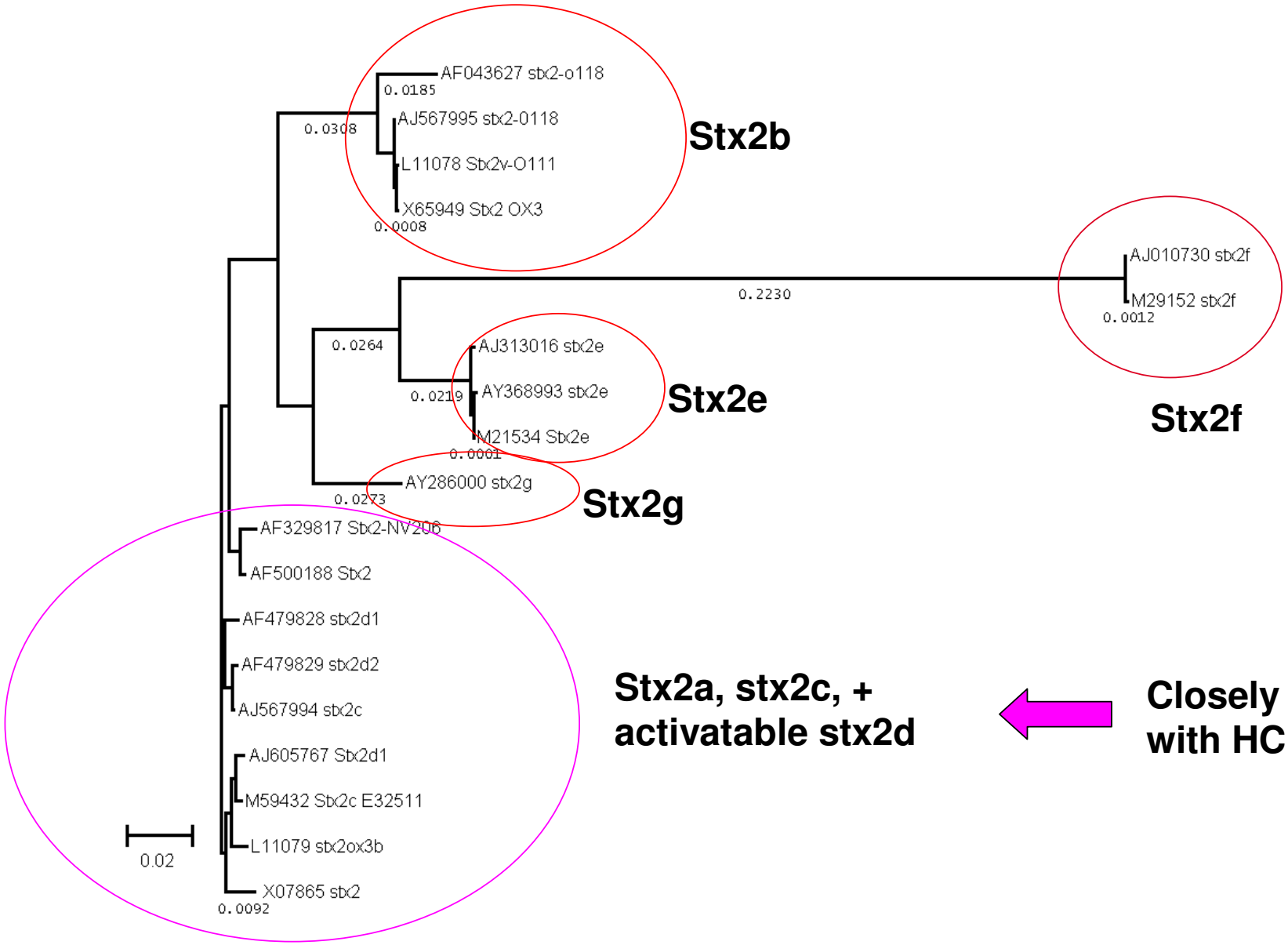
Stx-Variants with high virulence ←

# Stx1 family: three major subgroups




genetic distance

# Stx2 family: five major subgroups



# The major reservoir for STEC in nature are animals, often used for food production

**stx1b**

**stx2b**

**stx2c**

**stx1c**

**stx2b**

**Major variants of the Stx1 and the Stx2 family are associated with distinct animal host species**

# Shigatoxin 2e

**The P27 Phage carries the Stx2e-Gene**

# Possible role of Stx2e as human pathogens

**Stx2e**

**19% of STEC**

isolated from food in Germany*

**Beutin L, et al. AEM 2007 73:4769-75*

**Stx2e**

**0.9%**

of patients with STEC in Germany**

***Sonntag AK, et al. AEM 2005 71:8855-63*

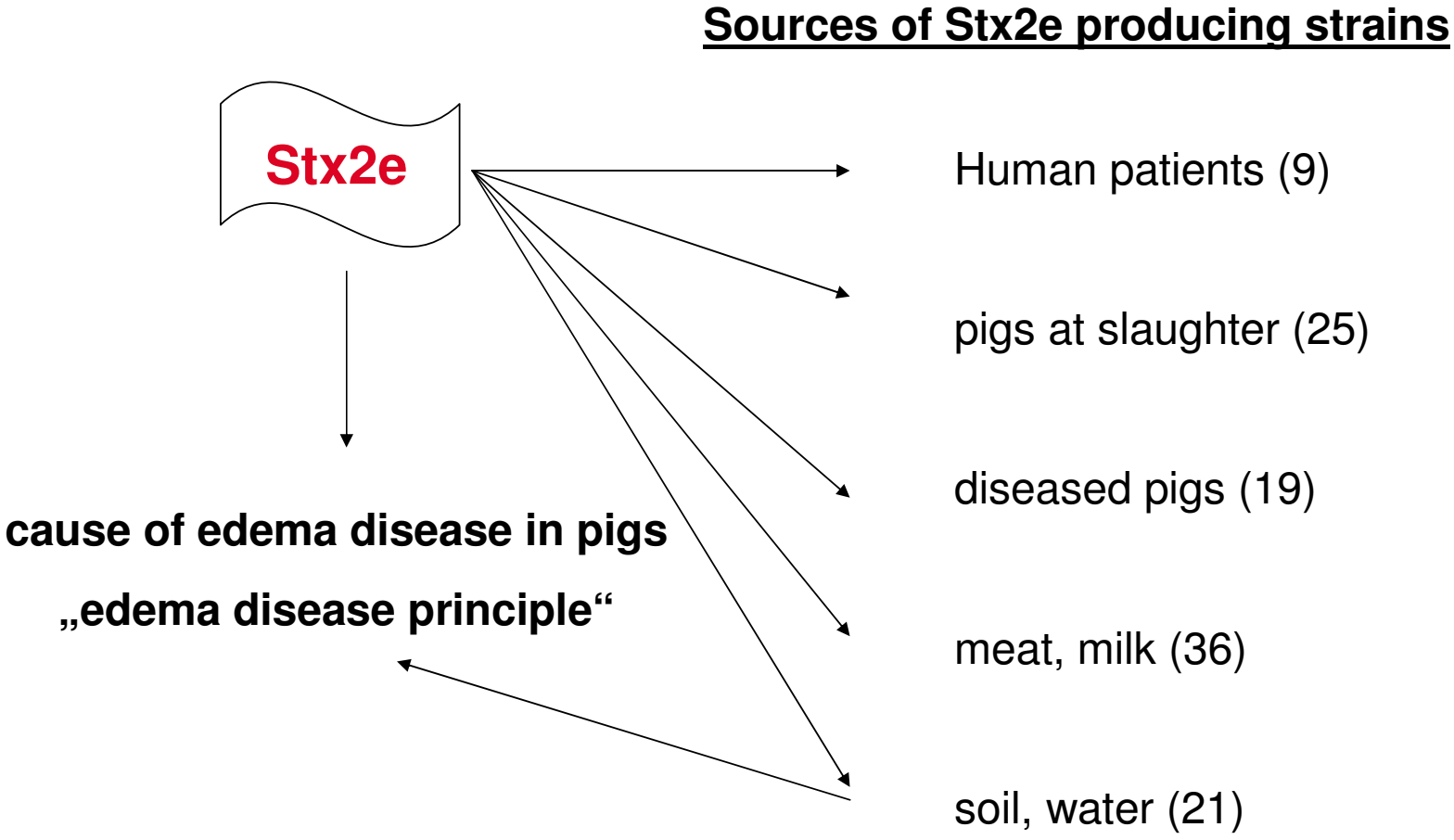
***Beutin L, et al. JCM 2004 42: 1099-1108*

Many humans with Stx2e strains have **no symptoms** of gastrointestinal disease

**no significant association between Stx2e infection and diarrhea*****

****Friedrich AW, et al. JID 2002 185:74-84*

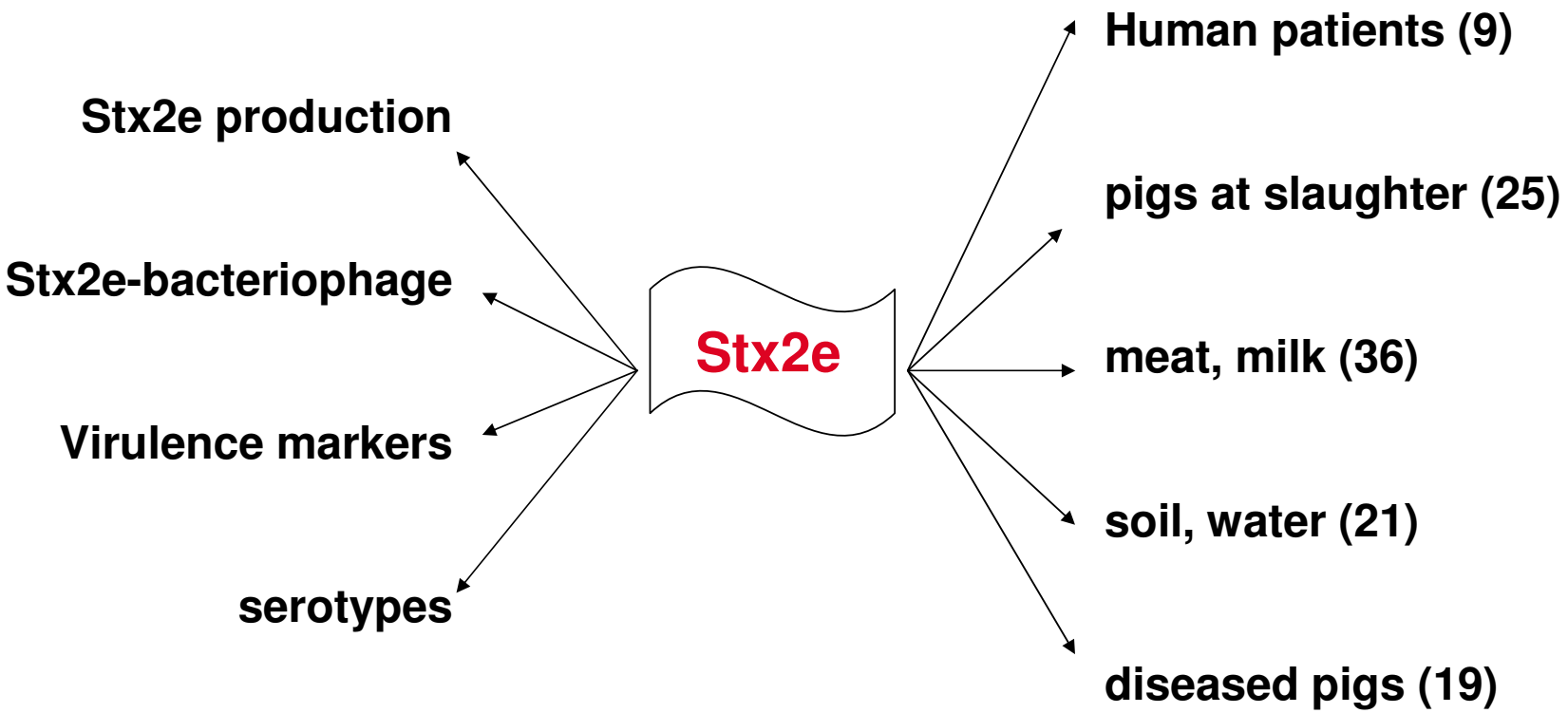
# Risk Assessment of 110 Stx2e strains from different sources for their role as human pathogens



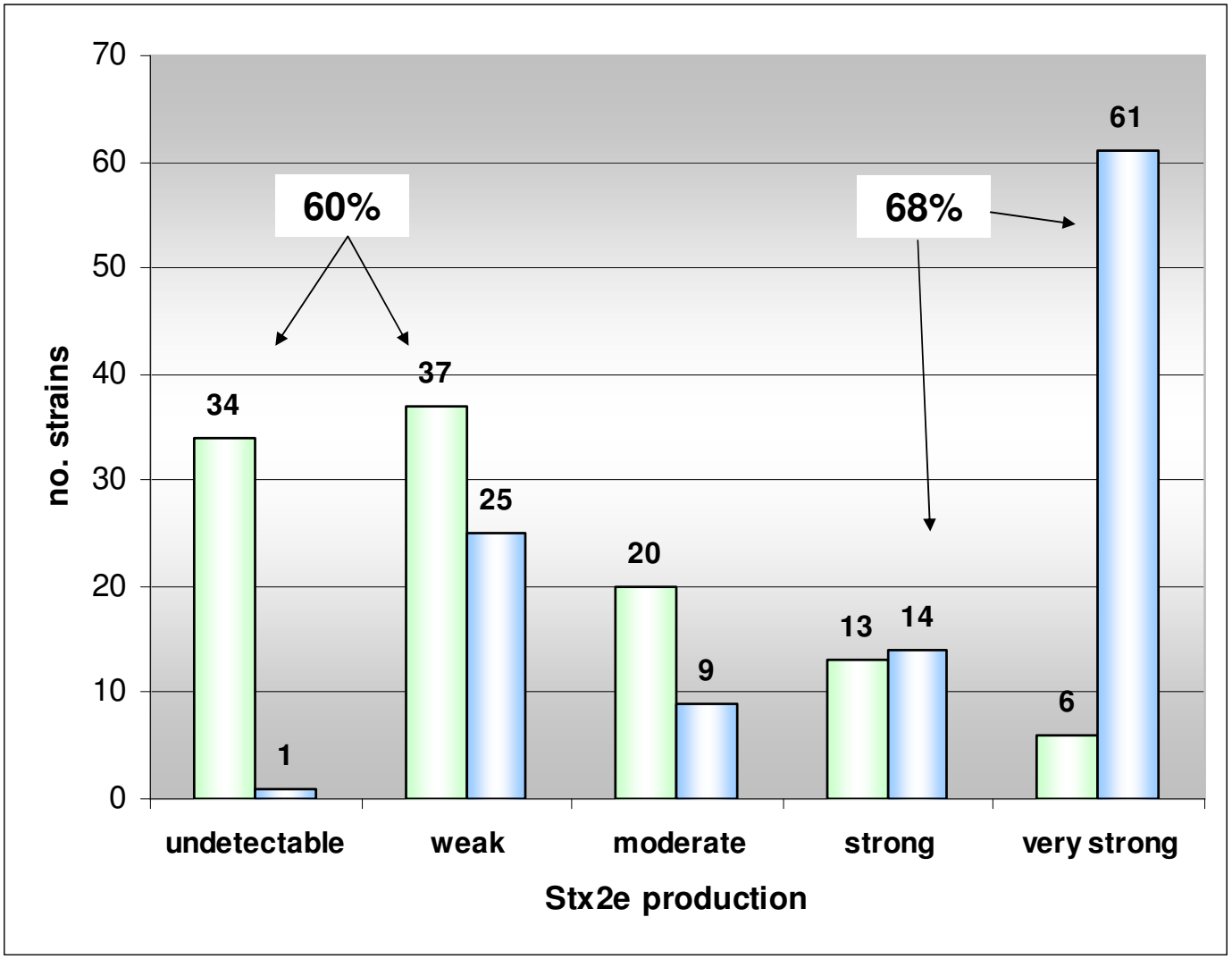
# Risk Assessment of 110 Stx2e strains from different sources for their role as human pathogens

**Properties of Stx2e producing strains**

**Sources of Stx2e producing strains**



# Stx2e production as measured by ELISA




Stx2e production is low in most strains without induction, but inducible by mitomycin C

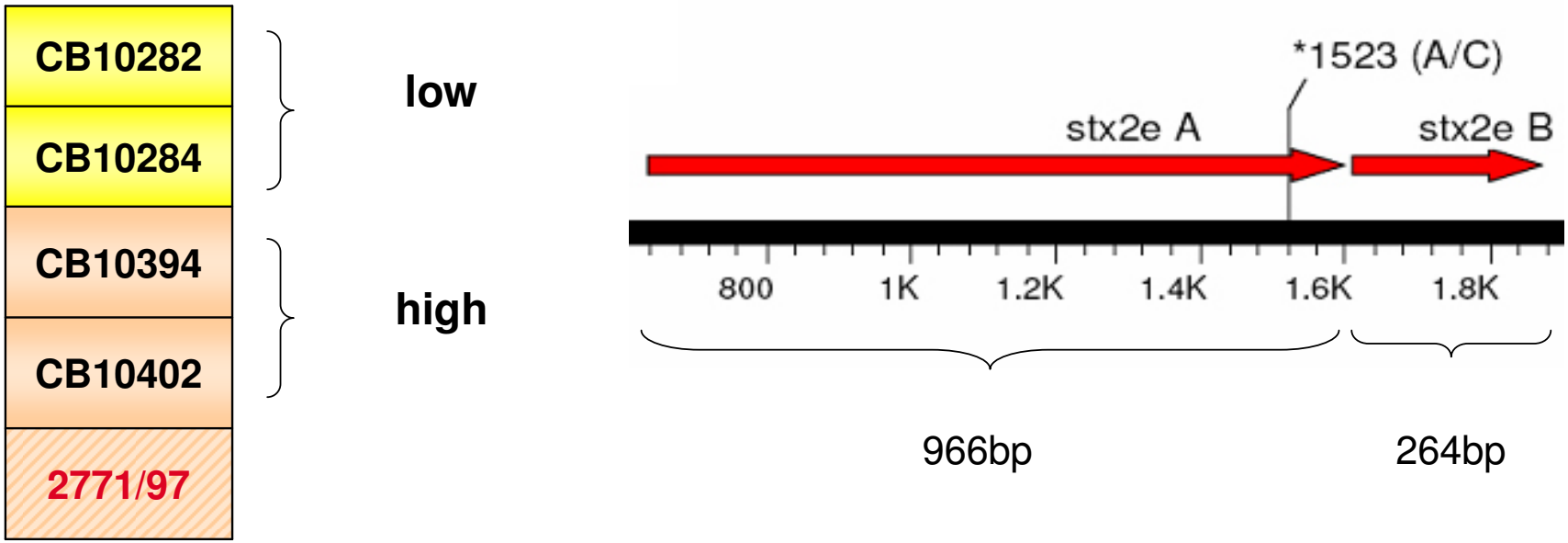
# Comparison of representative high and low Stx2e producers

| | Stx ELISA ($P_1$-g-EIA) | | | |
|---|---|---|---|---|
| | No induction | | + Mitomycin C | |
| Strain | ELISA | Highest dilution +ve | ELISA | Highest dilution +ve |
| CB10282 | undetectable | - | weak | 0 |
| CB10284 | undetectable | - | weak | 0 |
| CB10394 | weak | 1:4 | Very strong | 1:2048 |
| CB10402 | weak | 1:2 | Very strong | 1:1024 |
| 2771/97 | moderate | 1:32 | Very strong | 1:4096 |

2771/97 = reference strain carrying stx2e phage P27

***Recktenwald J + Schmidt H. IAI 2002 70:1896-1908***

# *stx*2e gene sequence identity in high and low Stx2e producers



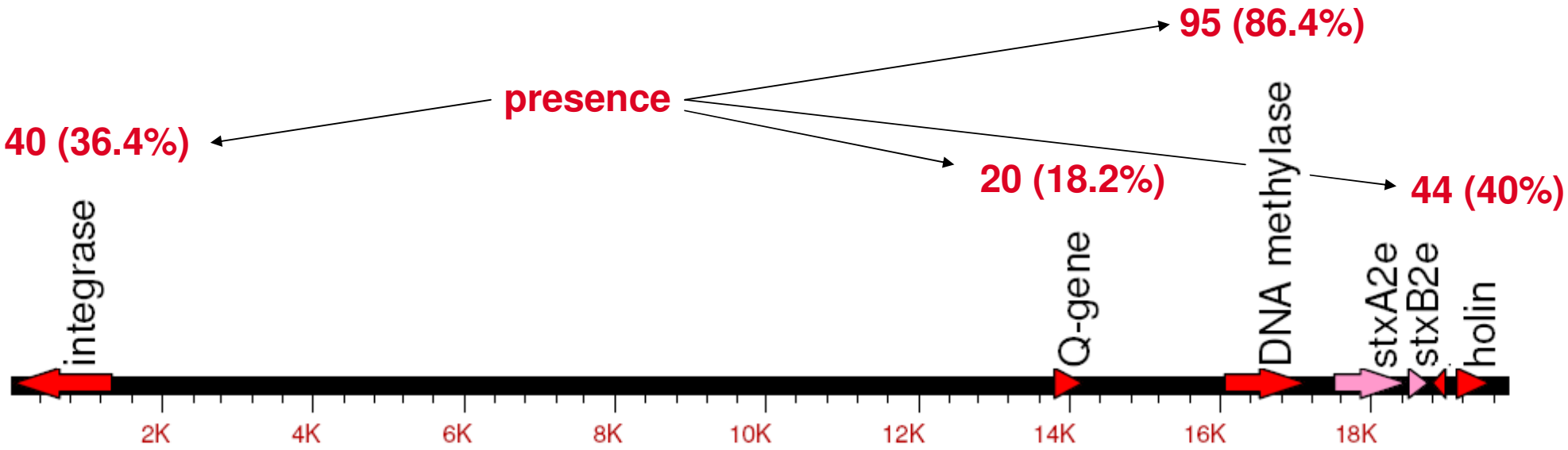

Highly similar to stx2e gene present on

inducible stx2e bacteriophage P27 strain 2771/97

# Differences in Stx2e production are related to mRNA transcription rates of *stx*2e genes in high and low producing strains.

| | RT-PCR relative quantification | | | |
|---|---|---|---|---|
| | No induction | | + Mitomycin C | |
| Strain | ELISA | Ratio *stx2*eA over *icdA* gene expression | ELISA | Ratio *stx2*eA over *icdA* gene expression |
| CB10282 | undetectable | 1.0 ± 0.0 | weak | 1.0 ± 0.2 |
| CB10284 | undetectable | 0.16 ± 0.1 | weak | 1.0 ± 0.2 |
| CB10394 | weak | 2.01 ± 0.4 | Very strong | 1179.0 ± 286 |
| CB10402 | weak | 1,19 ± 0.0 | Very strong | 9.84 ± 0.8 |
| 2771/97[c] | moderate | 5.71 ± 2.9 | Very strong | 151.86 ± 17.7 |

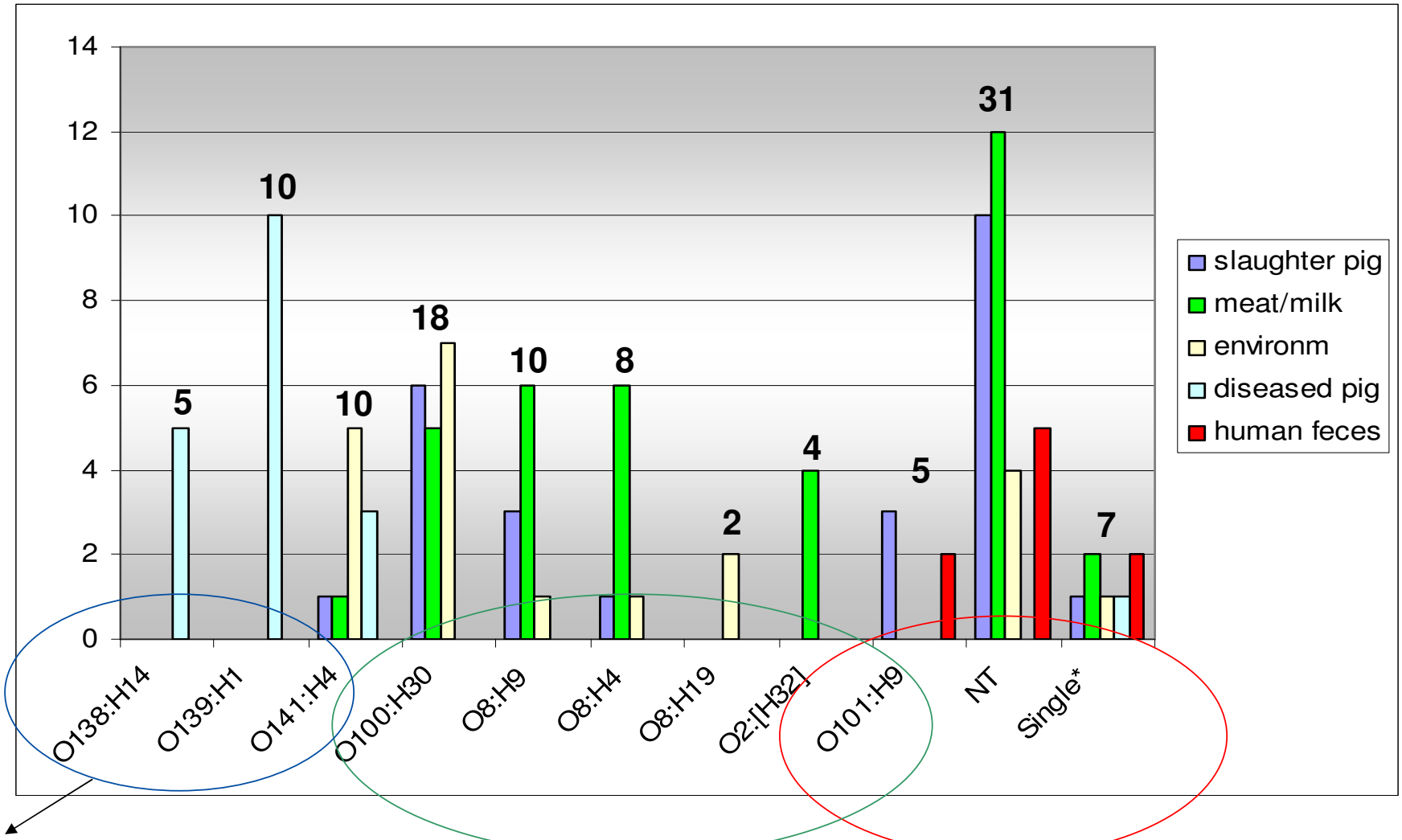
# Phage P27 is the origin of *stx*2e genes in strains of different serotype, origin and pathotype



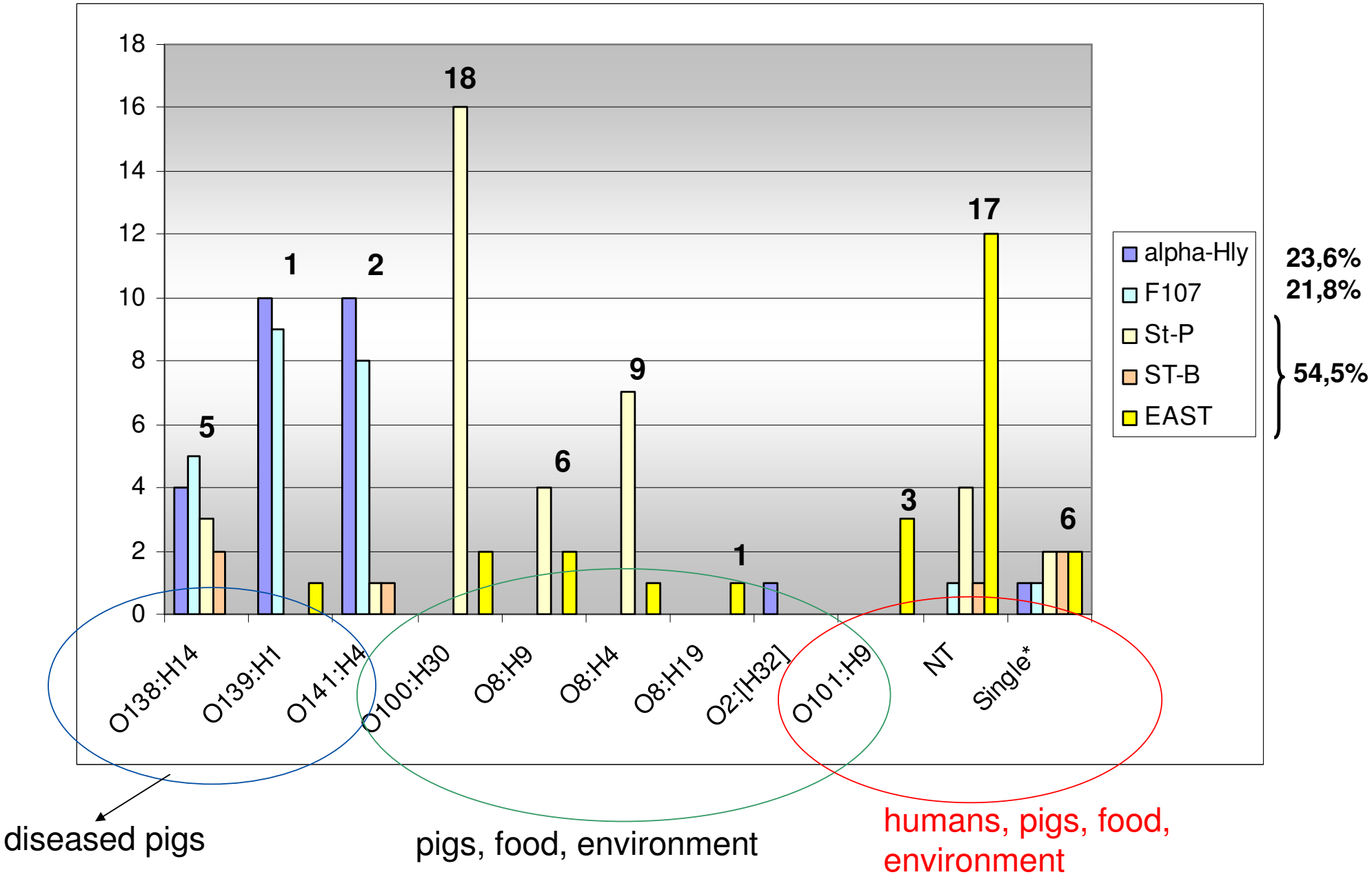

109 (99.1%) strains carried P27 specific gene sequences, only 7 (6.4%) were positive for all P27 sequences searched here. Cryptic phages or recombinants are frequent

Quantity of Stx2e production was not dependent on the integrity of the P27 genome or presence of genes like Q or presence of tRNA region upstream of stx2eA.

# Stx2e strains from humans do not belong to serotypes most frequently found in food, pigs and in the environment



# >50% of Stx2e strains are positive for heat stable enterotoxins (STI, STII and EAST1) that could play a role in diarrheal disease



# Are Stx2e strains pathogenic for humans?

All *stx*2e strains are negative for other types of Shiga toxins. Strains producing only Stx2e are not associated with diarrhea or HUS in humans.

Diarrhea in humans infected with Stx2e strains could be caused by heat-stable enterotoxins that are present in more than 50% of the natural Stx2e isolates.

The major serotypes of Stx2e producing strains present in pigs, in food and in the environment are frequently occurring and it is likely that humans get in contact with these.

In contrast, these strains are very rarely isolated from humans indicating that most of these cannot colonize/or cause disease in the human host.

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