Next Generation Sequencing – The Role of New Sequence Technologies in Shaping the Future of Veterinary Science

Hosted by the RCVS Charitable Trust
Streptococcus equi subsp. zooepidemicus:

More than just an opportunist?

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The streptococcal family tree

Streptococcus (Greek: streptos, chain; kokhos, berry) and S. zooepidemicus
More than one bad apple??

S. equi (strangles)

- Fatal canine pneumonia
- Non-strangles LN abscesses
- equine URT

S. equi and S. zooepidemicus

More than one bad apple??
Acute fatal haemorrhagic pneumonia

- Rare, but rapidly fatal canine disease
- Associated with *S. zooepidemicus* infection ($p=0.0003$)

*Photo: H Brooks*

*Acute fatal haemorrhagic pneumonia*

*Photo: L Woolford*

Gower and Payne 2012 Vet Rec.
‘Superantigens’

- Associated with increased morbidity and mortality of *S. pyogenes* infection of humans

**Activation**

(1/10⁵ or 10⁶ immune cells)

**Superantigen**

(5-20% immune cells)
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S. equi

Holden et al., 2009 PLoS Pathogens.

BHS5
Novel superantigens?
New ‘superantigens’

- 57/165 (34%) strains szeF +ve
- 31/165 (19%) strains szeN +ve
- 35/165 (21%) strains szeP +ve

But significantly not associated with acute fatal haemorrhagic pneumonia ($p=0.015$)

Paillot et al., 2010 Infect & Immun.  
Preistnall et al., 2010 Clin Vac. Immunol.
Sequencing *S. zooepidemicus*: the next generation
Icelandic epidemic

- Identified April 2010
- Approximately 80,000 horses affected
- Morbidity: 100%
  - Almost the entire horse population was sensitive to the infection indicating a new infectious agent
  - Lack of specific immunity
- Mortality: very low
- Incubation time: 1 - 4 weeks
  - depending on the infectious load
- Zoonoses
  - three cases including miscarriage
- Export of horses stopped
  - May 2010 to Jan 2011
Microbiological investigations

• Extensive virological investigations
  – Institute for Experimental Pathology at Keldur, Iceland
  – National Veterinary Institute in Uppsala, Sweden
  – Institute of Virology, Justus-Liebig-Universität in Giessen, Germany

• No viruses could be detected as a possible primary cause of the outbreak

• *Streptococcus equi* subsp. *equi*
  – Negative by culture and PCR tests

• Almost all clinical cases were positive for *Streptococcus equi* subsp. *zooepidemicus*
Illumina sequencing

- 3 multiplex libraries of 288 strains
- Sequence mapped to a reference genome, H70
- Core and accessory genome
  - Core SNPs used to generate a phylogeny with other sequenced S. zooepidemicus isolates
Main Icelandic clades

- Clade 4
- Clade 3
- Clade 2
- Clade 1
Clade 4

All Keldur 112

Sep, Oct

Sep, Oct and Nov

Oct

10 SNPs
Clade 3 – ST-248

10 SNPs

Efri 601
Borgarfjörour 311
Hrisdalur 311
Keldur 112
Reykjavik 110
Baer 110
Fakur 110
Dog: 110
Vatnesleysa 551
Clade 1 - ST-209

10 SNPs

Sweden 2008

Torfastaoir 801, Sorli 221
Brekka, Hella 850
Hleskogar 601, Dufur 566,
Baer 110

Hleskogar 601, Dufur 566,
Fakur 110, Hrisdalur 311
Gustur 200, Torfastaoir 801
Holar 551

Fakur 110, Hrisdalur 311,
Gustur 200, Torfastaoir 801
Holar 551

Hrisdalur 311, Efra 801,
Borgarfjörour 311,
Sandgeroi 245, Gufuol 380,
Hleskogar 601,
Keflavik 230, Gustur 200

Fossnes 801, Fakur 110,
Vatnsleysa 551, Husavik 641,
Brekka

Gustur 200, Dalvik 620,
Reykjanesbaer 641

110

Sandgeroi 245
Network analysis (Feb/Mar 2010)
ST-209 genome features

- Variant of SzeF
- Increases coughing and transmission??
S. zooepidemicus – an agile pathogen

• Causes disease in a huge variety of hosts
  – Adapted to horses

• Flexible and opportunistic
  – Usually sub-clinical
  – Non-equine disease can be severe

• Highly diverse
  – Constantly probing pathogenic boundaries

• Can we prevent it?
  • Vaccination
    • Needs to be broad coverage of strains
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