Streptococcus equi zooepidemicus
human infection outbreak

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S. equi subsp. zooepidemicus

- **Streptococcus equi** subspecies *zooepidemicus* (SZE) belongs to Lancefield group C beta-hemolytic

- considered a commensal and opportunistic pathogen of several warm blooded hosts, including humans, horses, canine and swine

- is an equine mucosal commensal responsible for upper respiratory tract also of pigs and other animals.

- human can host several subspecies (rare disease)
**S. equi** subsp. *zooepidemicus* in bovine and pigs

- Agent of bovine and ovine mastitis
- Confined in swine in Asia, several outbreaks in North America (2020) and in Canada (2019): to be considered emergent pathogen for swine?
- Sequence type (ST) 194 strains have been associated with outbreaks in China, USA and Canada causing high mortality in pigs.

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*Streptococcus equi* Subspecies *zooepidemicus* and Sudden Deaths in Swine, Canada

Matheus de O. Costa and Brad Lane
S. zooepidemicus human infection

- is a rare (underreported?) infection in humans associated with:
  - animal contact (horses)
  - consumption of unpasteurized milk and raw milk cheese
  - consumption of pork meat: septicaemia outbreak (Hong Kong, 1982-1986)
  - Thailand retrospective analysis (2021): 72.2% human patients had a history of eating raw pork products.

Zoonotic infection and clonal dissemination of Streptococcus equi subspecies zooepidemicus sequence type 194 isolated from humans in Thailand

Anusak Kedsin 1, Preechanika Choppjit 2, Rujrat Hatrongjit 3, Parichart Boueroy 3, Maysak Gottschalk 3
(2009) a large outbreak of nephritis in Brazil was attributed to consumption of unpasteurized cheese

(2013) glomerulonephritis outbreak in Monte Santo de Minas (Brazil) associated with the consumption of milk and its derived products.
SZE human infection

- Food borne transmission:
  - bovine/ovine mastitis
  - milk contamination
  - contamination of raw milk manufactured cheese
  - ingestion of contaminated raw milk and/or raw milk manufactured cheese
SZE human infection

- pharyngitis, glomerulonephritis, skin/soft tissue infection, toxic shock syndrome, infectious arthritis, endocarditis, meningitis,
- several studies have documented endophthalmitis as very rare complication of meningitis and bacteremia
- the eye target organ of SZE
- this finding confirmed in the here described human outbreak

Endogenous endophthalmitis complicating *Streptococcus equi* subspecies *zooepidemicus* meningitis: a case report
Dominik Madzar, Mareike Hage, Sebastian Möller, Martin Regensburger, De-Hyung Lee, Stefan Schwab, and Jonathan Jantsch
Diagnosis and treatment

- methods of detection/identification
  - culture and biochemical tests
  - mass spectrometry (MALDI-TOF MS)
  - NGS - next generation sequencing was used for genetic characterization and phylogenetic analysis

- treatment
  - Penicilline (treatment of choice)
  - Ceftriaxone
  - Gentamicin
  - Resistant to different antibiotics (e.g. amikacin, ampicillin.)
S. zooepidemicus
human infection
outbreak
description
Epidemic cluster of SZE human infection

- First human cases (3) reported by Infectious disease ward of Pescara GH in November 2021
- February 2022: routine lab checks of blood components from donors: platelets pool found positive for SZE
- 5 donors involved with specific medical history throat negative results
- April 2022 human cases increased to 27 with 4 deaths
- Majority of cases (18) were hospitalized patients and 4 were donors.
IZSAM: the PCR and species identification with MALD-TOF identified 19 strains from 17 patients confirmed the species *Streptococcus equi* subspecies *zooepidemicus* strain never reported to public database

- it was identified as the causative strain of the outbreak

- IZS validated the PCR test to detect *S. equi* and specifically the subspecies *zooepidemicus* on biological and food samples (bulk milk and unpasteurized cheeses).
Whole genome sequencing (WGS) data

- DNA extraction of 21 clinical strains from 19 patients
- WGS
- KmerFinder tool for species confirmation through WGS data
- Sequence Type ST61 was calculated using the MLST scheme (https://pubmlst.org/organisms/streptococcus-zooepidemicus)
- The comparative genomic analysis (CFSAN) confirmed the outbreak
SZE ST61 strain

- bibliographic search ST 61 on foodborne outbreaks

- ST61 was described in horse in Japan (Kinoshita et al., 2018)

- ST61 strain was compared to other public available SZE strains with no overlapping sequences
Epidemic cluster of SZE human infection

- the GH lab strengthened the donors' medical history to detect flu-like symptoms and consumption of dairy products.
- main symptoms: vomiting, nausea, abdominal pain, diarrhoea, osteo-muscular pain, septicaemia
- All cases came from the same geographical area with history of consumption of fresh cheeses purchased from street vendors, supermarket, cheese factors
- implicated fresh cheese manufactured by the establishment X located in the same geographic area.
Epidemiological investigation

- 9 cheese factories located in the area were inspected by the veterinary service with sampling of raw milk, dairy products and water

- SZE was found (culture) in bulk milk at cheese factory X
  
  - halted production of raw milk cheeses at the cheese factory X
  
  - raw milk dairy products placed under official detention
  
  - company withdrawal/recall all raw milk fresh cheese products
IZSAM Abruzzo Molise

- PCR confirmation of SZE culture positive results of bulk milk (sensitivity)

- PCR detection of SZE in raw milk manufactured cheese (giuncata) found negative with microbiological culture tests
Comparative Genomic analysis

- 21 sequences obtained from raw milk and raw milk cheese products, including a milk sample from a cow with mastitis detected in November 2021

- MLST → ST61

- a total of 42 sequences (21 clinical + 21 from food) were considered for the SNPs analysis

- the genomic distance was 0-3 SNPs
Lab investigation (human and animal)

- at cheese factory X owners and workers were screened with throat swabs for SZE and all were negative

- animal health inspection
  - absence of the ST61 strain in milk from cows and nasal and cloacal swabs on horses and chickens present in the farm
  - source of contamination: most likely a cow with mastitis detected during a check for mastitis at the end of November 2021, then excluded from milking and finally euthanized on 28 February 2022.
  - three sampling sessions for all lactating cows with negative results both culture and PCR.

Lab investigation (human and animal)
Lab investigation (milk and cheese)

- 8 strains isolated from unpasteurized cow's milk and 6 strains from seasoned unpasteurized cow's milk cheeses.

- SZE not found in 90dd seasoned unpasteurized cheese, pasteurized milk and derived cheeses.

- Results showed pasteurization is effective for the elimination of pathogen.

- Short and medium-term maturation of unpasteurized cheese not effective.
What’s next?

▪ Further genotypic characterization
  • to detect virulence genes compared to pathogenic strains described in the literature

▪ Joint veterinary and human clinical investigation (One Health)
  ▪ carry out round of checks on individual lactating and dry cattle
  ▪ carry out microbiological analysis of seasoned cheese, water and environment to rule out the persistence of the pathogen.
  ▪ perform clinical and microbiological checks of dairy plant’s employees
SZE an emerging risk?

- SZE may be a re-emerging zoonosis if unpasteurized milk is increasingly used for food production.

- Emerging risk in pigs: human infection associated with raw pork consumption

- Human infection associated with raw horse meat consumption

- Need of strict veterinary monitoring of raw milk and raw milk manufactured fresh cheeses

- Higher risk exposure of elderly people consuming raw milk manufactured fresh cheeses, with underlying co-morbidities

- New microbiological criterion in raw milk?