STREPTOCOCCUS EQUI SUBSP. ZOOEPIDEMICUS

SUMMARY

IMPORTANCE

- **Streptococcus equi** subsp. *zooepidemicus* (*S. zooepidemicus*) is a commensal found in the upper respiratory tract of horses, pigs, and other animals. As an opportunistic pathogen, it causes infections in many species.
- *S. zooepidemicus* infection in pigs was confined to Asia until recently, when several outbreaks were reported in North America. *S. zooepidemicus* should be considered an emerging pathogen in swine.

PUBLIC HEALTH

- Zoonotic disease due to *S. zooepidemicus* is relatively uncommon. Illness is mostly associated with consumption of unpasteurized dairy products or animal contact. Humans may develop pharyngitis, glomerulonephritis, skin/soft tissue infection, toxic shock syndrome, infectious arthritis, and invasive disease.
- In the 1980s, an outbreak of septicemia in Hong Kong was linked to pork consumption.

INFECTION IN SWINE

- Weakness, lethargy, fever, and rapidly escalating mortality are associated with *S. zooepidemicus* in pigs. Abortion has also been reported.
- Splenomegaly and lymphadenopathy may be observed at necropsy, and histologically, lesions consistent with septicemia are seen.
- In sows and feeder pigs experimentally infected with *S. zooepidemicus*, clinical signs begin within 24 hours post-infection (hpi), with severe depression and lethargy noted by 36 hpi. Pigs can also develop neurological disease.

TREATMENT

- *S. zooepidemicus* isolates from North American swine have been susceptible to many commonly used antibiotics including ceftiofur, enrofloxacin, penicillin, tiamulin, and tilmicosin.

CLEANING AND DISINFECTION

- *S. zooepidemicus* is likely susceptible to common disinfectants like 1% bleach, quaternary ammonium compounds, chlorhexidine, and Virkon® (potassium peroxymonosulfate).

PREVENTION AND CONTROL

- Prevention is aimed at eliminating stress and practicing good biosecurity. Pigs that are sick should receive treatment and supportive care to decrease the likelihood of secondary infection.

TRANSMISSION

- Modes(s) of transmission are not known for recent swine outbreaks.
- Transmission in other species can be due to direct contact with infected animals; indirect contact with contaminated equipment, housing, bedding, or clothing; and ingestion.
- Stressors such as overcrowding, transportation, and weaning may be related to development of disease.
PATHOGENESIS
- Pathogenesis of *S. zooepidemicus* infection is poorly understood.
- In horses, *S. zooepidemicus* is believed to enter through the nose or mouth and colonizes the mucosal surface and tonsillar tissue of the nasopharynx.

DIAGNOSIS
- Culture and biochemical testing remain common methods of identification. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has also been used to identify *S. zooepidemicus*. Next generation sequencing has been used for genetic characterization and phylogenetic analysis of recent outbreak isolates from pigs.
- A qPCR assay based on a conserved region of the *szM* gene has been recently developed, and it is highly sensitive and specific to virulent swine isolates. Samples to collect include lymph node, lung, liver, kidney, spleen, and tonsil.

EPIDEMIOLOGY
- The prevalence of *S. zooepidemicus* in US swine is not known.
- High mortality is clearly associated with *S. zooepidemicus* outbreaks in pigs, and all isolates obtained from North America have been closely related to ATCC 35246, a Chinese strain identified in 1975. However, the origin of current outbreaks remains unclear.

ETIOLOGY
- Streptococci are non-motile, Gram-positive bacteria that occur in pairs or chains.
- *S. zooepidemicus* is β-hemolytic, belonging to Lancefield group C. It is closely related to *S. equi* subsp. *equi* (*S. equi*), the cause of strangles in horses.
- Major virulence determinants of *S. zooepidemicus* include fibronectin binding proteins, M-like protein, complement fragment C5a peptidase, IgG-binding proteins, and pyogenic exotoxins.

HISTORY IN SWINE
- In 1975, an outbreak of *S. zooepidemicus* occurred in pigs in Sichuan Province, China. More than 300,000 pigs died. The next reported outbreak occurred in 1994, where pigs and monkeys in Indonesia were affected.
- In 2019, *S. zooepidemicus* caused a high mortality event in a large, vertically integrated swine system in Manitoba, Canada. There were in excess of 1000 sow deaths recorded, and the abortion rate was 11 times greater than normal.
- Also in 2019, outbreaks occurred in cull sows at a Tennessee slaughter plant (30% to 40% mortality) and in feeder pigs and cull sows at a buying station in Ohio (30% to 50% mortality).
- An additional 2019 case involved a cull sow that died at a livestock market in Pennsylvania. Traceback showed that other deaths had recently occurred at the consigner’s holding facility.

IMMUNITY
- Little is known about immunity to *S. zooepidemicus*.
- There are no commercially available *S. zooepidemicus* vaccines for animals.

GAPS IN PREPAREDNESS
- To develop preventive measures, the epidemiology of *S. zooepidemicus* in swine must be investigated. In the laboratory, swine isolates could be labeled unidentified or misidentified if they are not correctly typed to species level.
- Although *S. zooepidemicus* has been identified as a potential primary pathogen in pigs, where current outbreaks began and why they resolved remains unclear.
IMPORTANCE

*Streptococcus equi* subsp. *zooepidemicus* (*S. zooepidemicus*) is a commensal found in the upper respiratory tract of horses, pigs, and other animals. As an opportunistic pathogen, it causes infections in many species. Until recently, *S. zooepidemicus* infection in pigs was confined to Asia. However, outbreaks were reported in North America in 2019 – one each in Tennessee and Ohio, and one in Manitoba, Canada. A mortality event linked to *S. zooepidemicus* also occurred in pigs in Pennsylvania, and epidemiological information suggests similar events may have occurred in other US locations. *S. zooepidemicus* should be considered an emerging pathogen in swine.

PUBLIC HEALTH

*S. zooepidemicus* belongs to Lancefield Group C (see *Etiology*). In humans, Group C streptococci (GCS) cause a variety of clinical syndromes. Most infections are transmitted person-to-person. Zoonotic infection with *S. zooepidemicus* occurs but is relatively uncommon. Disease is mostly associated with consumption of unpasteurized dairy products or animal contact. Reported illness in humans includes pharyngitis, glomerulonephritis, skin/soft tissue infection, toxic shock syndrome, infectious arthritis, and invasive disease. Clinical syndromes and selected cases are reviewed in *Appendix A*.

Only one outbreak of *S. zooepidemicus* in humans has been linked to pork consumption. From 1982 to 1986, 14 cases of GCS septicemia were described in Hong Kong, 11 of which were attributed to *S. zooepidemicus*. Lyophilized specimens from five of these cases were compared to GCS isolates from septicemic pigs at an abattoir in the same district. All samples were analyzed using biotyping (API 20 Strep) and comparison of colonial morphology, capsular morphology, and chromosomal banding pattern following *Hind*III and *Eco*RI restriction endonuclease digestion. The samples from pigs and humans were determined to be identical. No animal contact was noted, nor was consumption of unpasteurized dairy, in any of the human cases. However, the authors note that consumption of raw/undercooked pork is popular in Hong Kong. An overview of the clinical features seen among cases is shown below.

Table 1. Clinical Features *S. zooepidemicus* Septicemia in Hong Kong, 1982-86

<table>
<thead>
<tr>
<th>Patient Information</th>
<th>Underlying Disease</th>
<th>Presenting Symptoms</th>
<th>Additional Isolation Site</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>F, 79 yrs</td>
<td>Respiratory tract infection, chronic obstructive airway disease, congestive heart failure</td>
<td>Sudden collapse Cardio-respiratory failure</td>
<td>Endotracheal aspirate</td>
<td>Died</td>
</tr>
<tr>
<td>M, 69 yrs</td>
<td>Chronic obstructive airway disease</td>
<td>Shortness of breath, wheezing</td>
<td>Sputum</td>
<td>Recovered</td>
</tr>
<tr>
<td>F, 55 yrs</td>
<td>—</td>
<td>Chills, rigor, pharyngitis, lymphad nopathy</td>
<td>Thorat swab</td>
<td>Recovered</td>
</tr>
<tr>
<td>F, 32 yrs</td>
<td>—</td>
<td>Tonsillitis, abdominal pain</td>
<td>Throat swab</td>
<td>Recovered</td>
</tr>
<tr>
<td>M, 65 yrs</td>
<td>Intravascular infection</td>
<td>Fever, abdominal distension, leaking abdominal aortic aneurysm</td>
<td>Abdominal aortic wall</td>
<td>Recovered</td>
</tr>
<tr>
<td>M, 62 yrs</td>
<td>Cirrhosis of the liver, portal hypertension</td>
<td>Fever, abdominal distension, weight loss, leaking abdominal aortic aneurysm</td>
<td>Abdominal aortic wall</td>
<td>Died</td>
</tr>
</tbody>
</table>
INFECTION IN SWINE

Clinical signs and postmortem lesions associated with known outbreaks of *S. zooepidemicus* in pigs are summarized below. Diagnostic tests used in each of the described outbreaks are also listed (see Diagnosis).

<table>
<thead>
<tr>
<th>Location</th>
<th>Clinical Signs</th>
<th>Postmortem Lesions</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sichuan Prov., China, 1975&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Painful swelling of the joints, respiratory disturbances, diarrhea&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Polyarthritis, bronchopneumonia, pleuritis, epicarditis, endocarditis, meningitis</td>
<td>Culture, serogroup testing, biochemical testing, restriction enzyme digestion (<em>SmaI</em>), gel electrophoresis</td>
</tr>
<tr>
<td>Indonesia, 1994&lt;sup&gt;25&lt;/sup&gt;</td>
<td>Rapid development of reluctance to stand, fever, lethargy, death Preceded by abortion in some cases</td>
<td>Rhinitis (mild, diffuse mucopurulent discharge), pulmonary edema, gall bladder edema, hemorrhagic lymphadenopathy (tan-colored to hemorrhagic) submandibular, cervical neck, bronchial</td>
<td>Gram stain, culture, and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry Genomic sequencing (Illumina MiSeq platform, 250x2)</td>
</tr>
<tr>
<td>Manitoba, 2019&lt;sup&gt;26&lt;/sup&gt;</td>
<td>Sudden death, weakness, lethargy, fever</td>
<td>Splenomegaly (fibrinosuppurative perisplenitis with vasculitis), hemorrhagic lymph nodes, suppurative or fibrinosuppurative lymphadenitis, nephritis, myocarditis, hepatitis</td>
<td>Culture, histopathology, PCR Genomic sequencing (Illumina MiSeq platform, 250x2)</td>
</tr>
<tr>
<td>Tennessee and Ohio, 2019&lt;sup&gt;1, 2&lt;/sup&gt;</td>
<td>Sudden death, weakness, lethargy, fever</td>
<td>Splenomegaly (fibrinosuppurative), cloudy orange fluid in abdomen</td>
<td>Culture, MALDI-TOF mass spectrometry Genomic sequencing (Illumina MiniSeq platform, 150x2)</td>
</tr>
<tr>
<td>Pennsylvania, 2019&lt;sup&gt;1, 30&lt;/sup&gt;</td>
<td>Sudden death</td>
<td>Splenomegaly (fibrinosuppurative), cloudy orange fluid in abdomen</td>
<td>Culture, MALDI-TOF mass spectrometry Genomic sequencing (Illumina MiniSeq platform, 150x2)</td>
</tr>
</tbody>
</table>

<sup>*</sup>No distinction made between signs seen in pigs vs. monkeys

In a recent study by Hu *et al.*, pigs infected with *S. zooepidemicus* were evaluated over a period of 14 days to understand disease progression. Three isolates were tested: one from the 2019 high mortality swine outbreak in Tennessee, one from a horse with pneumonia and a pleural abscess, and one from a guinea pig lymph node. For each isolate, five sows and six five-month-old pigs were oronasally inoculated and monitored for clinical signs. Clinical signs, postmortem lesions, and morality are described in Table 3.
Table 3. Characteristics of Experimental *S. Zooepidemicus* Infection in Pigs

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Clinical Signs</th>
<th>Postmortem Lesions</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sows Feeder</td>
<td>Sows Feeder</td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>Fever, vomiting, inappetence, purulent nasal discharge, severe lethargy, severe depression</td>
<td>Fever, inappetence, lethargy, depression, reluctance to rise; neurologic signs in 3/6 pigs (twitching, seizures, paddling)</td>
<td>Pulmonary, hepatic, splenic congestion with fibrin thrombi and intralesional bacterial colonies</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Fever, vomiting, inappetence, purulent nasal discharge, severe lethargy, severe depression</td>
<td>Fever, inappetence, lethargy, depression, reluctance to rise; neurologic signs in 2/6 pigs</td>
<td>Hepatic, splenic congestion (less severe)</td>
</tr>
<tr>
<td>Equine</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

As described by Hau et al., 2021

**TREATMENT**

Generally, β-hemolytic streptococci from horses are susceptible to β-lactams including penicillin, ampicillin, and ceftiofur, as well as trimethoprim-sulfonamide and oxytetracycline. In a 2009 study, no resistance to penicillin was noted in either *S. equi* or *S. zooepidemicus* from horses in Europe with lower respiratory disease. Similarly, a study of *S. zooepidemicus* isolates from Canada and the United States found that ceftiofur susceptibility did not change from 1989–2008. Macrolide resistance, however, has been demonstrated in a *S. zooepidemicus* isolate from a horse (*mef* and *msrD* genes).

Antimicrobial susceptibility testing was performed for *S. zooepidemicus* isolates from pigs in the 2019 Tennessee and Ohio outbreaks. Isolates were reportedly susceptible to ceftiofur, enrofloxacin, penicillin, tiamulin, and tilmicosin (via minimum inhibitory concentration, MIC testing). Isolates from the 2019 Manitoba outbreak in pigs were susceptible to ampicillin, ceftiofur, penicillin, and tilmicosin, but resistant to lincomycin, neomycin, and tetracycline (via Kirby-Bauer disk diffusion).

**CLEANING AND DISINFECTION**

**SURVIVAL**

No information was found about the survival of *S. zooepidemicus*. However, studies of *S. equi* show that it does not persist in the environment. In dry, sunny conditions, *S. equi* survived for less than one to three days on wood, metal, and rubber outdoors. Similarly, on experimentally inoculated objects (including wood, boots, clothing, and equipment), *S. equi* survived for up to two days in warm, dry weather, but in cold, wet weather survival time was increased to 34 days.
DISINFECTION

Little information was found on *S. zooepidemicus* disinfection. *S. equi* is inactivated by 1% bleach, quaternary ammonium compounds, chlorhexidine, and Virkon® (potassium peroxymonosulfate).17 Other disinfectants generally effective against streptococci include 70% ethanol, formaldehyde, glutaraldehyde, and iodine-based products.18 Efficacy of disinfectants could be affected by biofilm formation, which has been described in *S. zooepidemicus* ATCC 35246.19

A 2009 study investigated use of bacteriophage lysin PlyC against clinical isolates of *S. equi* and *S. zooepidemicus*. One μg of enzyme sterilized a 10⁸ CFU/ml culture of *S. equi* in 30 min, and PlyC retained full activity in the presence of hard water, organic matter, and non-ionic detergents.20 In 2015, disinfection of *S. zooepidemicus* in experimentally contaminated-endotracheal tubes was investigated. Bacterial load on the inner and outer surfaces was reduced by 0.5% chlorhexidine gluconate solution and accelerated hydrogen peroxide solution, but high-level disinfection (i.e., elimination of all vegetative bacterial growth) was not achieved.21

PREVENTION AND CONTROL

DISEASE REPORTING

*S. zooepidemicus* is not an OIE-listed disease. Individual states may have different disease reporting requirements. In early 2020, Pennsylvania designated *S. zooepidemicus* as a Dangerous Transmissible Disease, which provides legislative authority for reporting and penalties for noncompliance.22 Any suspicious clinical or necropsy findings should be reported to the USDA and your State Animal Health Official.

DISEASE PREVENTION

New animals should be quarantined for several weeks before introduction to the herd. However, utility of screening is unclear since the prevalence of *S. zooepidemicus* in healthy North American swine, in different types of production systems, is not known. While *S. zooepidemicus* has been reported in healthy pigs, in some studies it is much more common in clinically ill animals.23 Other studies report endemic persistence.24, 25

Pigs that are sick should receive treatment and supportive care to decrease the likelihood of secondary infection with *S. zooepidemicus*. Since stress may be a predisposing factor for *S. zooepidemicus* infection, pork producers should aim to prevent:

- Transport of injured or sick pigs (including malnourished pigs)
- Transport during extreme temperatures or inclement weather
- Overcrowding and loud noise exposure during transport
- Commingling of transported swine (which also allows direct contact)
- Extended hold times in lairage

DISEASE CONTROL

Outbreaks of *S. zooepidemicus* should be managed carefully to prevent disease spread. Affected premises should be isolated, and any contact premises identified via trace-forward or trace-back should be quarantined. Premises and possible fomites (equipment, vehicles, etc.) should be cleaned and disinfected regularly. Other standard biosecurity practices should be in place.

TRANSMISSION

Modes(s) of transmission are not known for recent swine outbreaks. Transmission in other species, such as horses, is due to direct contact with infected animals, and indirect contact with contaminated equipment, housing, bedding, or clothing. Bacteria can also be ingested via contaminated feed or water.11 In guinea pigs, oral abrasions are linked to *S.
zooepidemicus cervical lymphadenitis; illness in **dogs** has been linked to aerosols and environmental contamination; and infection in **camelids** has been associated with wound contaminated and ingestion (as cited by Sitthicharoenchai *et al.*).\(^1\) **Humans** are most often infected through consumption of unpasteurized dairy or animal contact.\(^8\)

Stressors such as overcrowding, poor nutrition, transportation, or weaning are thought to contribute to development of *S. zooepidemicus* infection in **horses**,\(^26\) and stress may have been a factor in recent North American swine outbreaks.

**PATHOGENESIS**

In **horses**, *S. zooepidemicus* enters through the nose or mouth and colonizes the mucosal surface and tonsillar tissue of the nasopharynx. Nasal shedding in horses is usually seen two to three days after onset of fever and persists for several weeks.\(^11\)

Pathogenesis is not completely understood but related to virulence determinants including M-like protein (prevents phagocytosis), fibronectin-binding protein (facilitates attachment and invasion), C5a peptidase and SpyCEP (involved in immune system evasion), G-like proteins and IgG degrading enzymes (bind IgG and inactivate proteins), and other enzymes and toxins that lead to an excessive inflammatory response (see **Major Virulence Determinants**).

In **pigs** experimentally infected with a swine *S. zooepidemicus* isolate, clinical signs began within 24 hours post-infection (hpi), with severe depression and lethargy noted by 36 hpi (see **Infection in Swine**).

**DIAGNOSIS**

**CULTURE AND IDENTIFICATION**

Culture and identification remain common methods to identify *S. zooepidemicus* infection. In **horses**, Columbia colistin-nalidixic acid (CNA) agar with 5% sheep or horse blood added is used.\(^11\) Biochemical test kits have been tested for identification\(^27\) of GCS in nasal, pharyngeal, or abscessed lymph node samples from **horses**. Several are commercially available (e.g., API\(^6\), bioMérieux).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been described to identify *S. equi* subspecies in various hosts/infection sites.\(^28\) A MALDI-TOF MS platform for subspecies-level identification of *S. equi* in clinical specimens from **horses** has also been developed.\(^29\)

In the 2019 Tennessee/Ohio outbreak of *S. zooepidemicus* in **pigs**, a comprehensive bacterial setup was used (nonselective agars, selective agars and broths, incubations at various atmospheric conditions) to isolate a wide range of potential pathogens. Heavy growth of clear, mucoid colonies surrounded by β-hemolysis was found in spleen and liver samples. Colonies were identified by classic biochemical testing and MALDI-TOF MS.\(^1\) Culture and MALDI-TOF MS were also used to detect *S. zooepidemicus* in **pigs** in the 2019 Manitoba outbreak.\(^3\) Next generation sequencing was used for genetic characterization and phylogenetic analysis of recent outbreak isolates from pigs.\(^1-3\)

**TESTS TO DETECT NUCLEIC ACIDS OR ANTIGEN**

Recently a quantitative polymerase chain reaction (qPCR) assay based on a conserved region of *szM* to detect highly virulent **swine** isolates was described. The assay was evaluated in porcine clinical samples including tissues, contact swabs, and isolated cultures. It was highly sensitive and specific, with no cross-reactivity to avirulent *S. zooepidemicus* isolates or *S. equi*.\(^30\)

Some tests described for *S. equi/S. zooepidemicus* diagnosis in other animals are shown below (in order of development, oldest to newest).

- PCR to detect *S. equi* in **horses** (nasal swabs) based on *seM*, which encodes for the M-like protein SeM\(^31\)
- Multiplex PCR to detect *S. equi* in **horses** based on *sodA* gene (encodes for manganese-dependent superoxide
dismutase, which provides defense against oxidative stress) and seeH and seeI genes (encode for the pyrogenic mitogens SePE-H and SePE-I, which are present in S. equi and S. pyogenes but absent in S. zooepidemicus)\(^{32}\)

- Multiplex PCR to identify different strains of S. zooepidemicus in camels and camel milk, based on polymorphisms in the 16S-23S rDNA intergenic space region, and the virulence gene szp\(^{33}\)
- Multiplex RT-PCR to detect S. equi in horses based on sodA, seeH, and seeI genes (nasal swabs, tracheal wash fluid)\(^{34}\)
- PCR to detect superantigen (SAg) genes (szeF, szeN, szeP) in S. zooepidemicus from necropsy lung washes of dogs\(^{35}\)
- RT-PCR assay to detect S. equi in horses (nasal swabs, nasopharyngeal swabs, abscess swabs, gullet pouch washes and tracheal washes) based on the eqbE gene, which forms part of the integrative conjunctive element Se2 (ICESe2) and enhances iron acquisition; found in S. equi but not S. zooepidemicus\(^{36}\)
- Loop-mediated isothermal amplification (LAMP) method for detection of S. zooepidemicus in horses (respiratory tract samples) based on detection of sorD, the gene that encodes sorbitol-6-phosphate 2-dehydrogenase and enables fermentation of sorbitol (present in S. zooepidemicus, not in S. equi)\(^{37}\)
- Multiplex PCR and qPCR assays to detect S. equi in horses based on presence of ICESe2 in nasopharyngeal swabs and gullet pouch washes\(^{38}\)
- Multiplex PCR assay to differentiate streptococci in dogs with respiratory disease based on presence of CnISR and CnSodA (found in S. canis), SodA (found in S. zooepidemicus), and Dys (found in S. dysgalactiae)\(^{39}\)
- Portable, smartphone-based system to perform end-point fluorescence detection (LAMP) assays on a microfluidic chip, for pen-side detection of respiratory pathogens in nasal swabs from horses (S. equi, S. zooepidemicus, equine herpesviruses 1 and 4 (EHV1), and equine influenza virus H3N8)\(^{40}\)

**TESTS TO DETECT ANTIBODY**

There are no antibody tests described for detection of virulent S. zooepidemicus in pigs. Paired titers may be useful for comparing exposure vs. infection status. For S. equi, serum titers peak about five weeks post-exposure, and can remain elevated for more than six months.\(^{11}\)

Enzyme-linked immunosorbent assays (ELISAs) are typically used to screen asymptomatic horses for strangles before they are introduced to a disease-free population, to determine if vaccination is needed, or to assess exposure during an outbreak. Most target SeM, a major virulence factor, and cannot differentiate infected from vaccinated animals. There are several commercially available assays (IDEXX/EBI, Innovative Diagnostics). SeM-based ELISAs remain problematic, however, since SzM (found in S. zooepidemicus) is nearly identical to SeM and cross-reactivity is not well understood. As cited by Robinson *et al.*,\(^{41}\) pre-incubating sera with heat-killed S. zooepidemicus can remove cross-reactive antibodies to SzM; however, background reduction has not been implemented in current SeM-based ELISAs leading to the potential for false positive results.

An indirect ELISA (iELISA) that targets two S. equi protein fragments (SEQ2190 and SeM) has been described.\(^{42}\) It was found to have superior sensitivity and specificity compared to an iELISA that targets only SeM.\(^{41}\) This assay is available in at least one laboratory.\(^{43}\) The ability of an S. equi vaccine to interfere with detection of antibodies (via the SEQ2190 and SeM duplex iELISA) was found to be clinically insignificant.\(^{44}\)

**SAMPLES**

Collection of fixed and fresh tissues is recommended including lymph node, lung, liver, kidney, spleen, and tonsil.\(^{5}\) Oral fluids have not been evaluated for detection of S. zooepidemicus. However, S. suis has been found in swine saliva,\(^{45, 46}\) and detected in oral fluid samples,\(^{47}\) which is unsurprising since many streptococci are upper respiratory tract commensals.
EPIDEMIOLOGY

SPECIES AFFECTED

*S. zooepidemicus* is best known as a commensal found in the upper respiratory tract of horses. It has been described in other healthy animals, including pigs. Yet, in a culture study of 395 swine tonsils collected at slaughter, *S. zooepidemicus* was found much less frequently than other streptococci including *S. equisimilis*, *S. porcinus*, and *S. suis* (1.3% vs. 29.4%, 19.5%, and 53.7% respectively). Additional studies have identified streptococci, but not *S. zooepidemicus*, in tonsils of healthy swine. *S. zooepidemicus* causes infection in many species. In horses, respiratory disease (rhinitis, bronchitis, pneumonia) and endometritis are most commonly seen. Infections also occur in cows, sheep, goats, dogs, cats, monkeys, llamas and alpacas, guinea pigs, chinchillas, poultry, pigs, and humans. Although zoonotic infections are rare, they can be very serious (see Public Health).

GEOGRAPHIC DISTRIBUTION

Historically, *S. zooepidemicus* has been the most common cause of swine streptococcal disease in China. A large outbreak occurred in 1975 and epidemics occurred regularly after that. *S. zooepidemicus* was also isolated from diseased pigs and monkeys in Indonesia in 1994. In the early 2000s, *S. suis* became the predominant streptococcal species isolated in China and it remains so today.

Until recently, *S. zooepidemicus* was not reported in pigs outside of Asia. In 2019, *S. zooepidemicus* was linked to sudden death and abortion in Canada and sudden death in the United States (see History in Swine).

MORBIDITY AND MORTALITY

The prevalence of *S. zooepidemicus* in US swine is unknown. The Iowa State University Veterinary Diagnostic Lab (ISU VDL) has reportedly identified only six clinical cases of *S. zooepidemicus* in pigs since 2010.

In a study of pigs condemned due to tuberculosis-like lesions at a slaughterhouse in Spain, streptococci were cultured from nearly one third of tissue samples, including submandibular lymph nodes, lungs, liver, and spleen. Of 65 streptococci isolates, *S. suis* was isolated most frequently, followed by *S. porcinus*, *S. dysgalactiae* subsp. *equisimilis*, *S. zooepidemicus*, *S. agalactiae*, and *S. alactolyticus*. A subsequent study of condemned free-range pigs with tuberculosis-like lesions showed similar results.

The 1975 outbreak of *S. zooepidemicus* in China reported high mortality, with the death of 300,000 pigs. Authors of the 1994 report on *S. zooepidemicus* in pigs and monkeys in Indonesia stated that “most of the [diseased] animals died within a few days.” The number of cases is not stated.

In the second quarter of 2019, *S. zooepidemicus* was linked to several swine mortality events in Canada. The *S. zooepidemicus* outbreak at four sow farms in Manitoba, Canada was characterized by sudden death, with an excess of 1000 sow deaths occurring over a 12-week period, and abortion, at a rate 11 times greater than normal. A packing plant in Manitoba reported an increase in sows “dead on arrival” at the end of July. A US processing plant also flagged an increase in sow deaths linked to *S. zooepidemicus*. Around the same time, an assembly yard in western Canada reported shipments with an increase in pigs “dead on arrival.”

High mortality was seen in the US outbreaks that occurred in 2019. In Ohio, feeder pigs and cull sows at a buying station experienced mortality of 30% to 50% over a period of eight to 10 days, and a Tennessee abattoir reported the deaths of 30% to 40% of sows in lairage. In Pennsylvania, a livestock dealer’s holding facility (linked to a case of *S. zooepidemicus*) had a case fatality rate of 81%.
ETIOLOGY

CHARACTERISTICS OF STREPTOCOCCI

Streptococci are Gram positive bacteria in the family Streptococcaceae. They are non-motile, non-sporeforming cocci that often occur in pairs or chains. The genus *Streptococcus* is divided into more than 70 species that are classified by habitat, pathogenicity, physiological characteristics, macromolecule characteristics, and Lancefield group. Three types of hemolysis are seen: α, β, and γ. β-hemolytic strains cause most acute streptococcal disease and are divided into two groups based on colony size (large-forming vs. small-forming). First described in 1933, Lancefield grouping is based on the identification of carbohydrate antigens associated with β-hemolytic streptococci. There are 20 recognized serotypes, identified as A to H and K to V. Some streptococci belong to more than one Lancefield group, based on more recent evidence of intergroup phage reactions and intergroup transduction between strains.

CHARACTERISTICS OF *S. ZOOEPIDEMICUS*

Phenotype and Lancefield group have traditionally been used to identify streptococci. *S. zooepidemicus* is β-hemolytic. Most strains produce hyaluronic acid, giving a mucoid appearance. In horses, colonies from the tonsil are non-mucoid due to the production of hyaluronidase. However, primary cultures from lung or tracheal washes may be briefly mucoid, making them grossly indistinguishable from *S. equi*. *S. zooepidemicus* colonies recently isolated from US pigs were large, clear, and mucoid in appearance.

Streptococci are catalase negative. Most strains of *S. zooepidemicus* hydrolyze esculin and starch, and ferment ribose, sorbitol, and lactose. Some, but not all, ferment trehalose. *S. zooepidemicus* belongs to Lancefield Group C, carrying N-acetylgalactosamine on the oligosaccharide side chains of its cell wall carbohydrate antigens.

CLASSIFICATION OF GROUP C STREPTOCOCCI

Group G streptococci (GGS) are closely related to GCS; both belong to the pyrogenic streptococci group. Group A streptococci (GAS), an important cause of illness humans, also belong to the pyrogenic group. Both GCS and GGS are related to GAS (80% homology), and contain genes acquired through horizontal transfer. There are four GCS and GGS species groups, containing 12 individual species based on 16S rRNA as shown in Table 4. *S. zooepidemicus* and *S. equi* are very closely related (98% DNA homology). *S. equi* is thought to be a descent of *S. zooepidemicus*.

<table>
<thead>
<tr>
<th>Table 4. Characteristics of Group C and G Streptococci</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species/subspecies</strong></td>
</tr>
<tr>
<td><em>S. canis</em></td>
</tr>
<tr>
<td><em>S. dysgalactiae</em> subsp. <em>dysgalactiae</em></td>
</tr>
<tr>
<td><em>S. dysgalactiae</em> subsp. <em>equisimilis</em></td>
</tr>
<tr>
<td><em>S. equi</em> subsp. <em>equi</em></td>
</tr>
<tr>
<td><em>S. equi</em> subsp. <em>ruminatorum</em></td>
</tr>
<tr>
<td><em>S. phoca</em> subsp. <em>salmonis</em></td>
</tr>
<tr>
<td><em>S. parauberis</em></td>
</tr>
<tr>
<td><em>S. uberis</em></td>
</tr>
</tbody>
</table>

* Designation - indicates no Lancefield group

A multilocus sequence typing (MLST) scheme, based on seven highly conserved housekeeping genes (*arc, nrdE, proS, spi, tdk, tpi and yqiL*), has also been developed for classifying *S. zooepidemicus*. *S. zooepidemicus* is quite diverse, with more than 500 sequence types (STs) listed in PubMLST (pubmlst.org, accessed 2/22/21). In one study, when grouped by ST, some *S. zooepidemicus* strains were more commonly found in the respiratory tract while others were found in the reproductive tract.
Major virulence determinants of streptococci involve:

- Attachment and adherence to the extracellular matrix components of the host
- Resistance to phagocytosis
- Immunoglobulin binding and inactivation of other proteins (e.g., α2-macroglobulin)
- Dissemination facilitated by enzymes and toxins

Major virulence determinants of *S. zooepidemicus* are described below and summarized in Table 5.

**Fibronectin Binding Proteins**

Most GCS and GGS bind fibronectin, which mediates adhesion to host cells and cell internalization. *S. zooepidemicus* produces three fibronectin binding proteins—FNZ, FNZ2, and SFS. In some isolates the *fnz* gene has undergone a one-nucleotide deletion, giving rise to *fne* gene which encodes FNE in *S. equi*. FNE is functional but has reduced fibronectin-binding capability compared to FNZ. SFS is produced by both *S. equi* and *S. zooepidemicus*, and a similar counterpart is found in GAS.

**M-Like Proteins**

M protein is the major virulence determinant of GAS (*S. pyogenes*). M-proteins are multifunctional, binding fibrinogen, IgG, and perhaps other targets. Human GCS and GGS are known to express M or M-like proteins, encoded by the *emm* gene. GGS of animal origin do not express M or M-like proteins. However, GCS are known to produce the functionally similar proteins SeM and SzPSe (produced by *S. equi*), and SzM and SzP (produced by *S. zooepidemicus*). SeM is quite conserved, while SzM is heterogeneous. SzM shares near identity with SeM across the C-terminal two-thirds of this protein, but it lacks the N-terminal variable domain. Distinct SzM variants have recently been associated with human disease, but an earlier study showed no link between SzM types and clinical disease in horses. An *szM*-adjacent transcription factor, *sezV*, is thought to be required for *szM* expression.

**Complement Fragment C5a Peptidase**

Complement fragment C5a is a chemotaxin that helps recruit phagocytes to infection sites. The C5a peptidase gene scpA has been detected in human GGS, but not in animal GGS. In GCS, *S. zooepidemicus* produces ScpZ (isolated from a pig with septicemia), and *S. equi* produces ScpE, both homologues of ScpA. The *scp* genes of *S. equi* and *S. zooepidemicus* are associated with a transposon, rather than being within the mga regulon encoding the *emm* gene as seen in *S. pyogenes*.

**IgG-Binding and Protein Inactivation**

Protein G contributes to adherence, inhibition of phagocytosis, and host mimicry in human GCS and GGS. The role of G-like proteins in pathogenesis is not fully known, but likely related to inhibition of phagocytosis via proteinase-complexed α2M. There are other IgG-binding proteins that are functionally different from protein G, such as MAG and MIG, described in *S. dysgalactiae*, and ZAG, described in *S. zooepidemicus*.

**Pyrogenic Exotoxins**

Streptococcal pyrogenic exotoxins (SPEs) are SAgs that activate T cells and play a role in acute infection. *S. pyogenes* produces 11 SAgs: SPEA, SPEC, SPEG, SPEH, SPEI, SPEJ, SPEK, SPEL, SPEM, SSA, SMEZ. In one study, about half of *S. zooepidemicus* isolates associated with human disease produced at least one SAg. SPEs associated with *S. equi* and *S. zooepidemicus* include:
- SPEK and SPEL, found in both *S. equi* and *S. zooepidemicus*
- SPEH and SPEI, found in *S. equi* only
- SZEN, SZEP, and SZEF, found in *S. zooepidemicus* only

**Other Factors**22

The Fic domain-containing protein BifA was recently shown to be required for development of meningitis in mice (i.e., allowing *S. zooepidemicus* to cross the blood-brain-barrier), and for penetration of a human brain endothelial monolayer in a culture model.

<table>
<thead>
<tr>
<th>Function</th>
<th><em>S. equi</em></th>
<th><em>S. zooepidemicus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adherence Mechanisms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibronectin-binding proteins</td>
<td>Attach and adhere to extracellular matrix components of host cells</td>
<td>FNE, FNE2, SFS</td>
</tr>
<tr>
<td><strong>Anti-Phagocytic Factors: Inhibition of Opsonization</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-like proteins</td>
<td>Bind fibrinogen (FgBP) and IgG</td>
<td>SeM (FgBP + IgG)</td>
</tr>
<tr>
<td></td>
<td>Bind fibrinogen and Factor H</td>
<td>Se18.9</td>
</tr>
<tr>
<td></td>
<td>Bind fibrinogen but not IgG</td>
<td>SzPSe</td>
</tr>
<tr>
<td><strong>Anti-Phagocytic Factors: Immune System Evasion</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5a peptidase</td>
<td>Cleaves, inactivates chemoattractant C52</td>
<td>ScpE</td>
</tr>
<tr>
<td>SpyCEP</td>
<td>Cleaves interleukin-8 and other chemokines to prevent neutrophil activation</td>
<td>SeCEP</td>
</tr>
<tr>
<td><strong>IgG Binding and Protein Inactivation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-like proteins</td>
<td>Type III IgG Fc receptor</td>
<td>ZAG</td>
</tr>
<tr>
<td>IgG-degrading enzymes</td>
<td>Cleaves hinge-region of IgG (IdeS)</td>
<td>IdeE/IdeE2</td>
</tr>
<tr>
<td></td>
<td>Removes core IgG glycans (EndoS)</td>
<td>EndoSe</td>
</tr>
<tr>
<td><strong>Enzymes and Toxins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptolysin S</td>
<td>Hemolytic toxin damages cell membrane and subcellular organelles</td>
<td>ND</td>
</tr>
<tr>
<td>Streptococcal pyogenic toxins*</td>
<td>Cross-link MHC class II (on antigen-presenting cells) to T cell receptors, leading to T cell proliferation and release of inflammatory cytokines</td>
<td>SPEH, SPEI</td>
</tr>
</tbody>
</table>

*As described by Turner, Bubba, and Efstratiou, 2019.16

**HISTORY IN SWINE**

**Sichuan Province, China (1975)**23, 24

In 1975, a *S. zooepidemicus* outbreak in Sichuan Province led to the death of 300,000 pigs. Respiratory disease and sudden death were observed. The genome of strain ATC 35246, which caused the outbreak was described in 2011. Virulence-associated genes identified included *szm*, *fbpZ*, *skc*, *has* operon (related to hyaluronic acid production), and *scl*.

**Bali, Indonesia (1994)**25

In early 1994, an outbreak of arthritis, diarrhea, and respiratory disease occurred in pigs and monkeys in Bali, Indonesia. Most animals died within a few days of becoming ill. Cases were first reported in pigs in a small village on Bali. Over the next weeks to months, the outbreak spread to surrounding swine and a monkey population. Within 3 months, cases were seen in pigs on two additional islands, Sumatra (to the northwest) and Sulawesi (to the northeast). Postmortem lesions included polyarthritis, bronchopneumonia, pleuritis, epicarditis, endocarditis, and meningitis.
S. *zooepidemicus* was isolated from 30 pigs and four monkeys from April–July. Despite some phenotypic variation, all isolates were biochemically identical. Digestion with *Sma*I revealed identical DNA patterns for 32 isolates. In the remaining isolates, from two different pigs on Bali, there were differences seen in two fragments. Control strains had DNA profiles unrelated to outbreak strains. In 2004, endemicity of *S. zooepidemicus* was confirmed in Indonesian monkeys and swine.

**Manitoba, Canada (April 2019)**

In April 2019, a *S. zooepidemicus* outbreak occurred in four loose-housed, commercial swine farms in Manitoba, Canada. Farms were part of a large, vertically integrated swine system with more than 9000 sows. The outbreak was characterized by sudden death, with 1000 excess sow deaths occurring over a 12-week period, and abortion, at a rate 11 times greater than normal. Clinical signs developed rapidly; sows were reportedly healthy during morning checks, but became unwilling to stand, febrile, and lethargic, with death occurring in hours. Others aborted first, then went on to develop similar signs. Outbreaks seemed to be exacerbated by comingling/stress. No signs of illness were seen in pigs from affected sows. Postmortem lesions included rhinitis, pulmonary edema, gall bladder edema, and hemorrhagic lymphadenopathy of the submandibular, cervical, and bronchial lymph nodes.

Gram-positive cocci were found in heart and lymph node imprints, and *S. zooepidemicus* was cultured from liver, kidney, heart, brain, lung, spleen, and submandibular lymph nodes. Sequencing showed that all isolates were similar to ATCC 35246 (previously associated with a high mortality outbreak of septicemia in China). All isolates were identified as ST194. Virulence factors identified included *szm*, *szp*, *lmb*, *fbpZ*, *skc*, *has* operon, and *mga* regulon.

Around the same time, additional *S. zooepidemicus*-linked swine deaths were described. An assembly yard in western Canada experienced an increase in pigs “dead on arrival” in the second quarter of 2019, and a Manitoba packing plant noted an increase in sows “dead on arrival” in July 2019.

**Tennessee and Ohio (September-October 2019)**

In 2019, the first swine outbreaks of *S. zooepidemicus* septicemia occurred in the United States. In late September, mortality of 30% to 40% was seen in cull sows in lairage over a five- to seven-day period at a Tennessee slaughter plant. Deaths were also detected in an epidemiologically linked buying station in Ohio, where mortality of 30% to 50% was seen in feeder pigs and cull sows over eight to 10 days. Clinical signs in pigs included weakness, lethargy, and high fever. Splenomegaly and hemorrhagic lymph nodes were noted at necropsy.

Three cases were submitted to the ISU VDL. Microscopic lesions were consistent with acute bacterial septicemia (vasculitis, fibrin thrombi, fibrinosuppurative polyserositis, intralesional bacteria). *S. zooepidemicus* was isolated from spleen, lung, and kidney. Next-generation sequencing identified *S. zooepidemicus* and porcine partetravirus (a parovirus previously known as hokovirus).

Whole genome sequencing was performed on 24 *S. zooepidemicus* isolates, including eight from the outbreak, an outbreak-unrelated swine isolate from Arizona, and 15 *S. zooepidemicus* isolates from other animal species (six equine, three feline, three guinea pig, one canine, one caprine, one chinchilla). An additional 24 isolates from GenBank, from different countries and years, were included in the phylogenetic analysis.

Results showed that the eight isolates from Tennessee and Ohio were indistinguishable, suggesting a common source. These isolates were identified as ST194 and clustered together with the Chinese strain ATCC 35246, which has previously caused high mortality outbreaks in China. There were also three guinea pig-associated human cases from Virginia (unassigned ST) that were closely related to the outbreak isolates. The swine isolate from Arizona belonged to ST340 and was distant to the outbreak.

Comparative genomic analysis showed that the outbreak isolates and two Chinese isolates (ATCC 35246 and CY) had similar genomic islands and virulence genes, including the M-like protein genes *szp* and *szm*, the Fic domain-containing...
protein BifA, the fimbrial subunit protein encoding gene fszF, and the protective antigen-like protein coding gene spaZ. None of the SAg genes that have been previously identified in S. zooepidemicus were found (szeF, szeL, szeM, szeN, szeP).

Pennsylvania (December 2019) S. zooepidemicus was detected in a cull sow at a livestock market in Pennsylvania in December 2019. Routine African swine fever/classical swine fever (ASF/CSF) surveillance on-site identified splenomegaly, with fibrin and pus present, as well as a large amount of cloudy, orange fluid in the abdomen. Culture showed heavy growth of S. zooepidemicus. The sow was malnourished and consigned with several others by a small livestock dealer. Traceback showed that additional pigs at the dealer’s holding facility had died. Additionally, surviving sows were sent to auction, and three were known to be sent to slaughter. The overall case fatality rate was 81% (17/21). Sequencing showed that isolates from this outbreak were genetically similar to S. zooepidemicus ATCC 35246. Virulence determinants identified included the scl1 gene and M-protein.

IMMUNITY

POST-EXPOSURE

Immunity to S. equi persists for five years or more in most horses following recovery. Surface-exposed proteins elicit IgG response, including SeM, Se44.2, Se46.8, Se45.5, and Se42.0. No information was found on post-exposure immunity to S. zooepidemicus in animals.

VACCINES

There are both killed and modified live vaccines available for S. equi, which are administered to horses based on risk. Vaccination of horses against S. equi does not confer immunity to S. zooepidemicus. According to the USDA, there are currently no commercial vaccines available for S. zooepidemicus in any species. The diversity of S. zooepidemicus has complicated vaccine development efforts; however, autogenous vaccines may be an option for pigs in the future.

Experimental vaccines that have been described for S. zooepidemicus include the following (shown below in order of development, oldest to newest).

- Recombinant protein SzPW60 (M-like protein gene from strain W60); protective in ICR mice challenged with W60 but not S. equi CF32
- szp-knockout strain of S. zooepidemicus ATCC 35246; protective in BALB/c mice challenged ATCC 35246
- Recombinant swinepox virus (rSPV-szp, M-like protein SzP inserted into swinepox virus genome); protective in ICR mice challenged with ATCC 35246
- Purified recombinant SEZ C5a peptidase (expressed in Escherichia coli, rSCPZ); protective in BALB/c mice challenged with SEZ C55138
- Purified recombinant GAPDH (glyceraldehyde-3-phosphate dehydrogenase, expressed in E. coli); protective in mice challenged with S. zooepidemicus
- Purified recombinant protein CSP (cell surface protein, SeseC_00619, expressed in E. coli); protective in BALB/c mice challenged with SEZ C55138
- Recombinant SzM (NC78) (SzM protein of strain NC78, expressed in E. coli); protective in BALB/c mice challenged with NC78 and W60
- Subset of S. zooepidemicus proteins isolated from a novel mucoid clone (SzNC78, ST307) from an outbreak of equine respiratory disease; various combinations tested, protective in ICR mice challenged with SzW60
- S. zooepidemicus capsule-deficient mutant ΔhasB; protective in BALB/c mice challenged with SEZ C55138
- Combinations of conserved hyaluronidase (HylC), serine protease (ScpC), and variable SzM and SzP from S. zooepidemicus strain NC78 (recombinant SzM, SzM plus SzP, or HylC plus ScpC); protective in ICR mice
challenged with canine strain\textsuperscript{93}

- Porcine IL-18 capsid protein (Cap) of PCV2 and M-like protein (SzP) of \textit{S. zooepidemicus} inserted into SPV genome to create a recombinant swinepox virus (rSPV-ICS), protective in Bama minipigs challenged with PCV2 and \textit{S. zooepidemicus}\textsuperscript{84}
- Recombinant GroEL chaperone protein (involved in biofilm formation, expressed in \textit{E. coli}); protective in ICR mice challenged with ATCC 35246\textsuperscript{95}
- Purified recombinant SeseC\textsubscript{01411} (a surface protein expressed in \textit{E. coli}); protective in BALB/c challenged with \textit{S. zooepidemicus}\textsuperscript{86}
- Purified recombinant protein Sec\textsubscript{205} (an extracellular protein expressed in \textit{E. coli}); protective in ICR mice challenged with SEZ C55138\textsuperscript{97}
- Purified membrane anchored protein (MAP, expressed in \textit{E. coli}) with an LPXTG-like cell wall motif; protective in BALB/c mice challenged with SEZ C55138\textsuperscript{86}
- Recombinant fabF (3-oxoacyl-[acyl-carrier-protein] synthase, expressed in \textit{E. coli}); protective in mice challenged with SEZ C55138\textsuperscript{99}

CROSS-PROTECTION

The \textit{S. zooepidemicus} population is highly diverse. Prolonged, repeated upper airway infections are common in young foals, suggesting that immunity is not acquired until individuals have been infected with many different \textit{S. zooepidemicus} strains.\textsuperscript{11} In horses, no cross-protection occurs between \textit{S. zooepidemicus} and its closest relative, \textit{S. equi}.\textsuperscript{84}

GAPS IN PREPAREDNESS

The role of \textit{S. zooepidemicus} in recent swine mortality events remains poorly understood. Stress seems to be a predisposing factor in some cases, but \textit{S. zooepidemicus} has been confirmed as a potential primary pathogen. Notably, few cases of \textit{S. zooepidemicus} have been reported in US swine. Whether testing reflects the actual prevalence is unclear. In the clinical laboratory, GCS isolates may not be speciated, leading to an underestimate of the disease burden of \textit{S. zooepidemicus}. In addition, misidentification of clinical swine specimens could be a concern. In a study of \textit{S. suis} identification (biochemical testing vs. PCR), very high false-positive (71\%) and false-negative (60\%) rates were seen.\textsuperscript{100} To develop preventive measures, the epidemiology of \textit{S. zooepidemicus} in pigs must be further investigated.

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APPENDIX A:
SELECTED CASE REPORTS OF S. ZOOEPIDEMICUS IN HUMANS

S. zooepidemicus most often caused pharyngitis (fever, sore throat, cervical lymphadenopathy) in humans, and it is typically linked to consumption of unpasteurized dairy. Glomerulonephritis can sometimes occur several weeks after the initial illness. Reported outbreaks include the following.

- In 1968, an outbreak of post-streptococcal glomerulonephritis was documented in P. Neamtz, Romania. Of 85 patients with pharyngitis, about 1/3 developed renal complications 2–3 weeks after the illness began. The outbreak was linked to milk from cows with mastitis at a dairy farm that supplied the town.

- In 1982, 5 people on a small farm in North Yorkshire developed fever and upper respiratory tract infection. Three subsequently developed nephritis. The family reported regularly consuming unpasteurized milk from their cows. However, 4 additional people living on the farm did not become ill. There was active mastitis in some cows in the dairy herd at the time of the outbreak.

- A large outbreak of post-streptococcal glomerulonephritis occurred in 1997–98 in Nova Serrana, Brazil, linked to unpasteurized local cheese. Of 253 cases, 7 required dialysis and there were 4 deaths. A 2-year follow up evaluated 134 of the original cases, and found that 3 required continuing dialysis, and 30–40% had hypertension and/or persistent renal dysfunction. At the 5-year follow up, 56 cases were re-examined; approximately 30% were hypertensive and 49% had reduced kidney function. A 10-year follow up, of 60 cases (out of 134 from 1998), showed that hypertension remained in about half of cases, but renal function had stabilized and was not significantly different.
from community controls. Antibodies to the M-like protein Szp5058 were found in convalescent sera from 33/44 cases tested. The genome of the causative strain, *S. zooepidemicus* MGCS10565, was sequenced in 2008. While the genome was similar to GAS in many ways, it lacked prophages that encode the majority of strain-specific gene content, and a gene related to streptococcal pyrogenic exotoxin B (SpeB), a suspected causative antigen for post-streptococcal glomerulonephritis. In addition, proteins such as streptokinase (which are implicated in post-streptococcal glomerulonephritis due to GAS) were highly divergent in strain MGCS10565.

**Skin and soft tissue infections** due to *S. zooepidemicus* are less common than other GCS, and usually involve animal exposure. Reported cases include the following.

- In 2017, sepsis, multi-organ failure, and necrotizing myositis were described in a 73-year-old Norwegian farmer. He reported having sores and abrasions on his fingers and having direct contact with two Shetland ponies at his stable. The ponies did not show signs of illness, and no nasopharyngeal swabs were collected.
- A 1986 case report from California described a 56-year-old man with pain and swelling on the right side of his face. He had developed fever, night sweats, and a dry cough prior. The man was a horse caretaker with 20 years of experience, and he lived close to a stable. His case was described as “human strangles.”
- A 1990 case report from Australia described *S. zooepidemicus* cellulitis in a renal transplant patient who had contact with horses at a show.

GCS and GGS have been occasionally identified in cases of **streptococcal toxic shock syndrome** (STSS). Only in a few instances has *S. zooepidemicus* been the cause.

- Necrotizing fasciitis, and underlying cirrhosis, lead to the death of a 64-year-old man in Japan in 2004. *S. zooepidemicus* was identified as the cause of STSS. Animal exposures were unknown.
- A report from 2004 describes a 63-year-old Australian man who developed left thigh pain and swelling while on an airplane flight. Shortly after he experienced fever, rigor, a rapidly progressing skin rash on his trunk and limbs, and death. He had received an IM injection of prochlorperazine in the left thigh for presumed acute labyrinthitis two days prior. He also had frequent contact with horses.
- In 2013, a case of STSS was identified in a 57-year-old man with multiple underlying conditions that worked at a horse farm. He initially developed influenza-like illness but was later admitted to the hospital with respiratory failure. He developed septic shock but responded to treatment and recovered in 6 weeks.

GCS are a known cause of **infectious arthritis** in humans, but *S. zooepidemicus* has been isolated infrequently. Reported cases include the following.

- In 2001, 2 cases of acute arthritis due to *Streptococcus dysgalactiae* subsp. *equisimilis* were reported in Spain. A review of the literature identified 22 additional cases of GCS arthropathy. In 2 of these, *S. zooepidemicus* was identified as the cause. In one case the patient reported collecting cervical cultures from infertile mares 4 days before the onset of symptoms, one of which was positive for GCS.
- In 2021, a case report was published for a recently retired veterinarian with severe GCS infection. The patient, a 65-year-old male who was previously healthy, developed septic polyarthritis, native mitral valve endocarditis, and lumbar discitis/osteomyelitis. An animal source was presumed but not identified.

**Invasive disease** caused by GCS and GGS appears to be increasing, especially in people with underlying disease. Primary bacteremia due to *S. zooepidemicus* is most often associated with animals or animal products. Reported outbreaks include the following.

- An outbreak of 11 cases of bacteremia was described in West Yorkshire, England, in 1988. Endocarditis and meningitis were also identified in many cases. Seven patients died; all were greater than 70-years-old. All cases had consumed unpasteurized milk from a dairy herd with mild, intermittent mastitis.
- A 1999 literature review identified 88 cases of bacteremia caused by GCS. Of these, 21 reported exposure to ani-
mals or animal products (unpasteurized milk, agricultural work, work as a butcher, other animal contact) and were diagnosed with *S. zooepidemicus*. Five cases of *S. zooepidemicus*-endocarditis, with animal exposure, were also described.\(^{118}\)

- An outbreak of *S. zooepidemicus* septicemia, linked to unpasteurized goat cheese, occurred in Finland in 2003. Seven cases were identified, one of which had purulent arthritis. All were previously healthy, and no deaths occurred.\(^{119}\)

- A report from 2009 examined a case of bacteremia, with complications involving meningitis, mitral endocarditis, and blindness (due to bilateral endophthalmitis) in a 59-year-old Canadian woman. She had multiple underlying conditions. Sporadic contact with horses was noted, and she recovered with some permanent sequelae.\(^{120}\)

- A 2010 case report described wound infection and subsequent meningitis, caused by *S. zooepidemicus*, in a 79-year-old man who was trampled by his horses. A further literature review identified 20 cases of *S. zooepidemicus* meningitis. Animal contact was noted in all but one, with suspected transmission due to inhalation, ingestion of unpasteurized dairy, and wound inoculation.\(^{121}\)

- Another case report from 2010 detailed a case of meningitis in an 83-year-old horse trainer from Florida. He had a history of hypertension, pacemaker placement, and penicillin allergy. He survived following a 4-week-course of ceftriaxone. No horses with GCS were identified on the farm following an epidemiological investigation by the state. A literature review identified 36 cases of GCS meningitis, about 1/3 reported horse exposure and about 1/5 reported ingestion of dairy products.\(^{122}\)

- A 2015 case report documented endogenous endophthalmitis in a 73-year-old man that had contact with a sick horse in Germany.\(^{123}\)

- In 2016, 2 cases of *S. zooepidemicus* were described in King County, Washington. A 37-year-old woman (patient A), who operated a horse riding and boarding facility, developed mild pharyngitis and a cough. Around the same time, a horse at the facility (horse A) developed upper respiratory disease. A 71-year-old woman (patient B), who was patient A’s mother, also developed an upper respiratory infection. She had lived in the same household as patient A, and had contact with horse A. Several weeks later, she developed vomiting and diarrhea, was found unconscious, and was transported to the hospital where she died. Both women were previously healthy.\(^{124}\)

- A nationwide outbreak of *S. zooepidemicus*-associated respiratory disease occurred in Iceland in 2010, spreading through 77,000 horses. All equine activities were stopped, and a self-imposed ban on equine exports was instated. 257 isolates were analyzed, and ST209 was thought to be responsible for the epidemic. Concurrent with this epidemic ST209 was isolated from a human case of septicemia.\(^{125}\)

- A 2019 case report documented meningitis possibly caused by *S. zooepidemicus* in a 6-month-old girl. She presented with fever, vomiting, fussiness, decreased energy, and imbalance during crawling. She also had a respiratory infection two weeks prior. Her only animal contact was with two pet dogs that recently had respiratory infections.\(^{126}\)

- Additional cases of invasive disease linked to *S. zooepidemicus* were found in the literature.\(^{127-139}\)