STREPTOCOCCUS EQUI SUBSP. *ZOOEPIDEMICUS*

Swine Health Information Center

SUMMARY

FEBRUARY 2021

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.

LITERATURE REVIEW FEBRUARY 2021

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,[1, 2] and one in Manitoba, Canada.[3] A mortality event linked to *S. zooepidemicus* also occurred in pigs in Pennsylvania,[4] and epidemiological information suggests similar events may have occurred in other US locations.[5] *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.[6] Zoonotic infection with *S. zooepidemicus* occurs but is relatively uncommon.[6] Disease is mostly associated with consumption of unpasteurized dairy products or animal contact.[6] 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.[7] An overview of the clinical features seen among cases is shown below.

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

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

continued - Table 1. Clinical Features *S. zooepidemicus* Septicemia in Hong Kong, 1982-86				
Patient Information	Underlying Disease	Presenting Symptoms	Additional Isolation Site	Outcome
F, 58 yrs	—	Fever, mitral valve incompetence	—	Recovered
M, 55 yrs	Cellulitis, alcoholic neuropathy, plantar ulcers	Fever, weakness, cellulitis	Wound swab	Recovered
F, 71 yrs	Breast cancer, post-radiotherapy	Left arm lymphedema, cellulitis	—	Recovered
M, 5 yrs	Abdominal sepsis, descending colon adenocarcinoma, retro-peritoneal abscess	Persistent fever, cellulitis of left loin post-surgery	—	Recovered
M, 58 yrs	Gangrenous appendicitis	Persistent fever post-surgery	—	Recovered

* Adapted from Yuen, Seto, Choi, Ng, Ho, and Chau, 1990[49]

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 2. Characteristics of *S. zooepidemicus* Outbreaks in Swine			
Location	Clinical Signs	Postmortem Lesions	Diagnosis
Sichuan Prov., China, 1975[23]			
Indonesia, 1994[25]	Painful swelling of the joints, respiratory disturbances, diarrhea*	Polyarthritis, bronchopneumonia, pleuritis, epicarditis, endocarditis, meningitis	Culture, serogroup testing, biochemical testing, restriction enzyme digestion (*SmaI*), gel electrophoresis
Manitoba, 2019[26]	Rapid development of reluctance to stand, fever, lethargy, death Preceded by abortion in some cases	Rhinitis (mild, diffuse mucopurulent discharge), pulmonary edema, gall bladder edema, hemorrhagic lymphadenopathy (tan-colored to hemorrhagic) submandibular, cervical neck, bronchial	Gram stain, culture, and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry Genomic sequencing (Illumina MiSeq platform, 250x2)
Tennessee and Ohio, 2019[1, 2]	Sudden death, weakness, lethargy, fever	Splenomegaly (fibrinosuppurative perisplenitis with vasculitis), hemorrhagic lymph nodes, suppurative or fibrinosuppurative lymphadenitis, nephritis, myocarditis, hepatitis	Culture, histopathology, PCR Genomic sequencing (Illumina MiSeq platform, 250x2)
Pennsylvania, 2019[4, 30]	Sudden death	Splenomegaly (fibrinosuppurative), cloudy orange fluid in abdomen	Culture, MALDI-TOF mass spectrometry Genomic sequencing (Illumina MiniSeq platform, 150x2)

*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

Isolate	Clinical Signs		Postmortem Lesions		Mortality	
	Sows	Feeders	Sows	Feeders	Sows	Feeders
Swine	Fever, vomiting, inappetence, purulent nasal discharge, severe lethargy, severe depression	Fever, inappetence, lethargy, depression, reluctance to rise; neurologic signs in 3/6 pigs (twitching, seizures, paddling	Pulmonary, hepatic, splenic congestion with fibrin thrombi and intralesional bacterial colonies	Pulmonary congestion and/or hemorrhage; hepatic, renal, and splenic congestion, peritonitis (less severe)	5/5 died or euthanized by 56 hpi	6/6 died or euthanized by 72 hpi
Guinea pig	Fever, vomiting, inappetence, purulent nasal discharge, severe lethargy, severe depression	Fever, inappetence, lethargy, depression, reluctance to rise; neurologic signs in 2/6 pigs	Hepatic, splenic congestion (less severe)	Hepatic, splenic congestion (less severe)	4/5 died or euthanized by 96 hpi	2/6 euthanized by day 6
Equine	None	None	None	None	None	None

As described by Hau et al., 2021[83]

TREATMENT

Generally, β-hemolytic streptococci from **horses** are susceptible to β-lactams including penicillin, ampicillin, and ceftiofur, as well as trimethoprim-sulfonamide and oxytetracycline.[11] 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.[12] Similarly, a study of *S. zooepidemicus* isolates from Canada and the United States found that ceftiofur susceptibility did not change from 1989–2008.[13] Macrolide resistance, however, has been demonstrated in a *S. zooepidemicus* isolate from a **horse** in Germany (*mefA* and *msrD* genes).[14]

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).[1] 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).[3]

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.[15] 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.[16]

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 10^8 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[5]

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.[6]

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®, 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, guttural 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 guttural 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.[48] It has been described in other healthy animals, including pigs.[18] 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).[49] Additional studies have identified streptococci, but not *S. zooepidemicus*, in tonsils of healthy swine.[50, 51]

S. zooepidemicus causes infection in many species. In horses, respiratory disease (rhinitis, bronchitis, pneumonia) and endometritis are most commonly seen.[11, 48, 52] Infections also occur in cows,[53] sheep,[54] goats,[55] dogs,[56] cats,[57] monkeys,[9] llamas and alpacas,[58] guinea pigs,[59] chinchillas,[60] poultry,[61] pigs[2, 3, 8, 9], and humans. Although zoonotic infections are rare, they can be very serious (see *Public Health*).[48]

GEOGRAPHIC DISTRIBUTION

Historically, *S. zooepidemicus* has been the most common cause of swine streptococcal disease in China. A large outbreak occurred in 1975[8] and epidemics occurred regularly after that. *S. zooepidemicus* was also isolated from diseased pigs and monkeys in Indonesia in 1994.[9] In the early 2000s, *S. suis* became the predominant streptococcal species isolated in China[62] and it remains so today.[63]

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[3] and sudden death in the United States[1, 2] (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.[1]

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*.[64] A subsequent study of condemned free-range pigs with tuberculosis-like lesions showed similar results.[65]

The 1975 outbreak of *S. zooepidemicus* in China reported high mortality, with the death of 300,000 pigs.[8] 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.”[9] 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.[3] 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.”[66]

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.[2] In Pennsylvania, a livestock dealer’s holding facility (linked to a case of *S. zooepidemicus*) had a case fatality rate of 81%.[4]

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.[18] The genus *Streptococcus* is divided into more than 70 species that are classified by habitat, pathogenicity, physiological characteristics, macromolecule characteristics, and Lancefield group.[67] 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).[18] First described in 1933, Lancefield grouping is based on the identification of carbohydrate antigens associated with β-hemolytic streptococci.[68] There are 20 recognized serotypes, identified as A to H and K to V.[18] Some streptococci belong to more than one Lancefield group, based on more recent evidence of intergroup phage reactions and intergroup transduction between strains.[67]

CHARACTERISTICS OF *S. ZOOEPIDEMICUS*

Phenotype and Lancefield group have traditionally been used to identify streptococci.[68] *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.[11, 69, 70] However, primary cultures from lung or tracheal washes may be briefly mucoid,[69] making them grossly indistinguishable from *S. equi*.[11] *S. zooepidemicus* colonies recently isolated from US pigs were large, clear, and mucoid in appearance.[1]

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.[68] *S. zooepidemicus* belongs to Lancefield Group C, carrying *N*-acetylgalactosamine on the oligosaccharide side chains of its cell wall carbohydrate antigens.[67]

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.[67] There are four GCS and GGS species groups, containing 12 individual species based on 16S rRNA as shown in Table 4.[67] *S. zooepidemicus* and *S. equi* are very closely related (98% DNA homology).[48] *S. equi* is thought to be a descent of *S. zooepidemicus*.[71, 72]

Table 4. Characteristics of Group C and G Streptococci

Species/subspecies	Group	Hemolysis	Host(s)
S. canis	G	β	Animals, humans
S. dysgalactiae subsp. *dysgalactiae*	C (L)	α,β, γ	Animals
S. dysgalactiae subsp. *equisimilis*	C, G (A, L)	β	Humans
S. equi subsp. *equi*	C	β	Equids
S. equi subsp. *ruminatorum*	C	β	Animals, humans
S. equi subsp. *zooepidemicus*	C	β	Animals, humans
S. parauberis	C	α, γ	Fish, cattle
S. phocae subsp. *salmonis*	C (F, -)*	β	Salmon, pinnipeds
S. uberis	C (D, E, P, U, -)*	α, β, γ	Mostly cattle

* 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*.[73] *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.[73]

MAJOR VIRULENCE DETERMINANTS

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[74]

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

Fibronectin Binding Proteins[7, 16]

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[7, 16-20]

M protein is the major virulence determinant of GAS *(S. pyogenes)*.[67] 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[7, 16]

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[7, 16]

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[7, 16]

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.[79] 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 5. Major Virulence Determinants of *S. equi* and *S. zooepidemicus**

	Function	*S. equi*	*S. zoo*
Adherence Mechanisms			
Fibronectin-binding proteins	Attach and adhere to extracellular matrix components of host cells	FNE, FNE2, SFS	FNZ, FNZ2, SFS
Anti-Phagocytic Factors: Inhibition of Opsonization			
M-like proteins	Bind fibrinogen (FgBP) and IgG	SeM (FgBP + IgG)	SzM (FgBP only)
	Bind fibrinogen and Factor H	Se18.9	Se18.9 (rarely)
	Bind fibrinogen but not IgG	SzPSe	SzP
Anti-Phagocytic Factors: Immune System Evasion			
C5a peptidase	Cleaves, inactivates chemoattractant C52	ScpE	ScpZ
SpyCEP	Cleaves interleukin-8 and other chemokines to prevent neutrophil activation	SeCEP	SzoCEP
IgG Binding and Protein Inactivation			
G-like proteins	Type III IgG Fc receptor		ZAG
IgG-degrading enzymes	Cleaves hinge-region of IgG (IdeS)	IdeE/IdeE2	IdeZ/IdeZ2
	Removes core IgG glycans (EndoS)	EndoSe	EndoSz
Enzymes and Toxins			
Streptolysin S	Hemolytic toxin damages cell membrane and subcellular organelles	ND	
Streptococcal pyogenic toxins*	Cross-link MHC class II (on antigen-presenting cells) to T cell receptors, leading to T cell proliferation and release of inflammatory cytokines	SPEH, SPEI SPEK, SPEL	SPEK, SPEL SZEN, SZEP, SZEF

*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.[25]

Manitoba, Canada (April 2019)[3]

n 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).[8] 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.[66]

Tennessee and Ohio (September-October 2019)[1, 2, 5]

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 parvovirus 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.[8] 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*[82] were found (*szeF, szeL, szeM, szeN, szeP*).

Pennsylvania (December 2019)[4, 29, 30]

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.[11] 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.[11] 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.[5] 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[84]
- *szp*-knockout strain of *S. zooepidemicus* ATCC 35246; protective in BALB/c mice challenged ATCC 35246[85]
- Recombinant swinepox virus (rSPV-szp, M-like protein SzP inserted into swinepox virus genome); protective in ICR mice challenged with ATCC 35246[86]
- Purified recombinant SEZ C5a peptidase (expressed in *Escherichia coli*, rSCPZ); protective in BALB/c mice challenged with SEZ C55138[87]
- Purified recombinant GAPDH (glyceraldehyde-3-phosphate dehydrogenase, expressed in *E. coli*); protective in mice challenged with *S. zooepidemicus*[88]
- Purified recombinant protein CSP (cell surface protein, SeseC_00619, expressed in *E. coli*); protective in BALB/c mice challenged with SEZ C55138[89]
- Recombinant SzM (NC78) (SzM protein of strain NC78, expressed in *E.coli*); protective in ICR mice challenged with NC78 and W60[90]
- 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[91]
- *S. zooepidemicus* capsule-deficient mutant ΔhasB; protective in BALB/c mice challenged with SEZ C55138[92]
- 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[93]

- Porcine IL-18 capsid protein (Cap) of PCV2 and M-like protein (SzP) of *S. zooepidemicus* inserted into SPV genome to create a recombinant swinepox virus (rSPV-ICS), protective in Bama minipigs challenged with PCV2 and *S. zooepidemicus*[94]
- Recombinant GroEL chaperone protein (involved in biofilm formation, expressed in *E. coli*); protective in ICR mice challenged with ATCC 35246[95]
- Purified recombinant SeseC_01411 (a surface protein expressed in *E. coli*); protective in BALB/c challenged with *S. zooepidemicus*[96]
- Purified recombinant protein Sec_205 (an extracellular protein expressed in *E. coli*); protective in ICR mice challenged with SEZ C55138[97]
- Purified membrane anchored protein (MAP, expressed in *E. coli*) with an LPXTG-like cell wall motif; protective in BALB/c mice challenged with SEZ C55138[98]
- Recombinant fabF (3-oxoacyl-[acyl-carrier-protein] synthase, expressed in *E. coli*); protective in mice challenged with SEZ C55138[99]

CROSS-PROTECTION

The *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 *S. zooepidemicus* strains.[11] In horses, no cross-protection occurs between *S. zooepidemicus* and its closest relative, *S. equi*.[84]

GAPS IN PREPAREDNESS

The role of *S. zooepidemicus* in recent swine mortality events remains poorly understood. Stress seems to be a predisposing factor in some cases, but *S. zooepidemicus* has been confirmed as a potential primary pathogen. Notably, few cases of *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 *S. zooepidemicus*. In addition, misidentification of clincal swine specimens could be a concern. In a study of *S. suis* identification (biochemical testing vs. PCR), very high false-positive (71%) and false-negative (60%) rates were seen.[100] To develop preventive measures, the epidemiology of *S. zooepidemicus* in pigs must be further investigated.

REFERENCES

1. Sitthicharoenchai P, Derscheid R, Schwartz K, et al. Cases of high mortality in cull sows and feeder pigs associated with *Streptococcus equi* subsp. *zooepidemicus* septicemia. *J Vet Diagn Invest*. Jul 2020;32(4):565-571. doi:10.1177/1040638720927669
2. Chen X, Resende-De-Macedo N, Sitthicharoenchai P, et al. Genetic characterization of *Streptococcus equi* subspecies *zooepidemicus* associated with high swine mortality in the United States. *Transboud Emerg Dis*. 2020-11-01 2020;67(6):2797-2808. doi:10.1111/tbed.13645
3. Costa MDO, Lage B. *Streptococcus equi* subspecies *zooepidemicus* and sudden deaths in swine, Canada. *Emerg Infect Dis*. 2020-10-01 2020;26(10):2522-2524. doi:10.3201/eid2610.191485
4. Pitcher P. *Streptococcus equi zooepidemicus*: An Emergent Swine Pathogen. US Department of Agriculture; 2020.
5. US Department of Agriculture. Emerging Risk Notice: *Streptococcus equi* subspecies *zooepidemicus*. Accessed February 21, 2021. https://www.aphis.usda.gov/animal_health/downloads/streptococcus-zooepidemicus-notice.pdf
6. Baracco GJ. Infections caused by group C and G streptococcus (*Streptococcus dysgalactiae* subsp. *equisimilis* and others): epidemiological and clinical aspects. *Microbiol Spectr*. Mar 2019;7(2)doi:10.1128/microbiolspec.GPP3-0016-2018
7. Yuen KY, Seto WH, Choi CH, Ng W, Ho SW, Chau PY. *Streptococcus zooepidemicus* (Lancefield group C) septicaemia in Hong Kong. *J Infect*. Nov 1990;21(3):241-50. doi:10.1016/0163-4453(90)93885-v
8. Feng Z, Hu J. Outbreak of swine streptococcosis in Sichuan province and identification of pathogen. *Anim*

Husbandry Vet Med Lett. 1977;2:7-12.
9. Soedarmanto I, Pasaribu FH, Wibawan IW, Lämmler C. Identification and molecular characterization of serological group C streptococci isolated from diseased pigs and monkeys in Indonesia. *J Clin Microbiol*. Sep 1996;34(9):2201-4. doi:10.1128/jcm.34.9.2201-2204.1996
10. Surendran Nair M, Byukusenge M, Li L, et al. Draft genome sequences of two virulent *Streptococcus equi* subsp. *zooepidemicus* swine isolates from Pennsylvania. *Microbiol Resour Announc*. 2020-10-15 2020;9(42)doi:10.1128/mra.00974-20
11. Waller A, Sellon D, Sweeney C, Timoney P, Newton J, Hines M. Streptococcal Infections. In: Sellon D, Long M, eds. *Equine Infectious Diseases*. 2nd ed. Saunders Elsevier; 2014:265-77:chap 28.
12. Bade D, Portis E, Keane C, et al. In vitro susceptibility of ceftiofur against *Streptococcus equi* subsp *zooepidemicus* and subsp *equi* isolated from horses with lower respiratory disease in Europe since 2002. *Vet Ther*. Winter 2009;10(4):E1-10.
13. Bade D, Sibert G, Hallberg J, Portis E, Boucher J, Bryson L. Ceftiofur susceptibility of *Streptococcus equi* subsp *zooepidemicus* isolated from horses in North America between 1989 and 2008. *Vet Ther*. Winter 2009;10(4):E1-7.
14. Haenni M, Lupo A, Madec JY. Antimicrobial resistance in *Streptococcus* spp. *Microbiol Spectr*. Mar 2018;6(2) doi:10.1128/microbiolspec.ARBA-0008-2017
15. Weese JS, Jarlot C, Morley PS. Survival of *Streptococcus equi* on surfaces in an outdoor environment. *Can Vet J*. Sep 2009;50(9):968-70.
16. Durham AE, Hall YS, Kulp L, Underwood C. A study of the environmental survival of *Streptococcus equi* subspecies *equi. Equine Vet J*. Nov 2018;50(6):861-864. doi:10.1111/evj.12840
17. Slater J. Bacterial Infections of the Equine Respiratory Tract. In: McGorum B, Dixon P, Robinson N, Schumacher J, eds. *Equine Respiratory Medicine and Surgery*. Saunders Elsevier; 2007:327-53:chap 23.
18. Spickler A. Streptococcosis. Center for Food Security and Public Health. Accessed February 14, 2021. https://www.cfsph.iastate.edu/Factsheets/pdfs/streptococcosis.pdf
19. Yi L, Wang Y, Ma Z, et al. Biofilm formation of *Streptococcu*s equi ssp. *zooepidemicus* and comparative proteomic analysis of biofilm and planktonic cells. *Curr Microbiol*. Sep 2014;69(3):227-33. doi:10.1007/s00284-014-0574-z
20. Hoopes JT, Stark CJ, Kim HA, Sussman DJ, Donovan DM, Nelson DC. Use of a bacteriophage lysin, PlyC, as an enzyme disinfectant against *Streptococcus equi*. *Appl Environ Microbiol*. Mar 2009;75(5):1388-94. doi:10.1128/aem.02195-08
21. Crawford S, Weese JS. Efficacy of endotracheal tube disinfection strategies for elimination of *Streptococcus zooepidemicus* and *Bordetella bronchiseptica*. *J Am Vet Med Assoc*. Nov 1 2015;247(9):1033-6. doi:10.2460/javma.247.9.1033
22. Sundberg P. SHIC Monitors as Pennsylvania Declares *S. zooepidemicus* as a Dangerous Transmissible Disease. American Association of Swine Veterinarians. Accessed February 21, 2021. https://www.aasv.org/news/story.php?id=12344
23. Nugroho W, Cargill CF, Putra IM, et al. Investigations of selected pathogens among village pigs in Central Papua, Indonesia. *Trop Anim Health Prod*. Jan 2016;48(1):29-36. doi:10.1007/s11250-015-0913-5
24. Carpio MM. Hemolytic streptococci in supramammary lymph nodes from healthy pigs from the Peruvian jungle. *Int J Zoonoses*. Jun 1978;5(1):62-4.
25. Salasia SI, Wibawan IW, Pasaribu FH, Abdulmawjood A, Lammler C. Persistent occurrence of a single *Streptococcus equi* subsp. *zooepidemicus* clone in the pig and monkey population in Indonesia. *J Vet Sci*. Sep 2004;5(3):263-5.
26. Anzai T, Walker JA, Blair MB, Chambers TM, Timoney JF. Comparison of the phenotypes of *Streptococcus zooepidemicus* isolated from tonsils of healthy horses and specimens obtained from foals and donkeys with pneumonia. *Am J Vet Res*. Feb 2000;61(2):162-6. doi:10.2460/ajvr.2000.61.162
27. Bannister MF, Benson CE, Sweeney CR. Rapid species identification of group C streptococci isolated from horses. *J Clin Microbiol*. Apr 1985;21(4):524-6. doi:10.1128/jcm.21.4.524-526.1985
28. Kudirkiene E, Welker M, Knudsen NR, Bojesen AM. Rapid and accurate identification of *Streptococcus equi*

subspecies by MALDI-TOF MS. *Syst Appl Microbiol*. Jul 2015;38(5):315-22. doi:10.1016/j.syapm.2015.02.010
29. Mani RJ, Thachil AJ, Ramachandran A. Discrimination of *Streptococcus equi* subsp. *equi* and *Streptococcus equi* subsp. *zooepidemicus* using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J Vet Diagn Invest*. Sep 2017;29(5):622-627. doi:10.1177/1040638717702687
30. Kuchipudi SV, Surendran Nair M, Yon M, et al. A novel real-time PCR assay for the rapid detection of virulent *Streptococcus equi* subspecies *zooepidemicus*-an emerging pathogen of swine. *Front Vet Sci*. 2021;8:604675. doi:10.3389/fvets.2021.604675
31. Timoney JF, Artiushin SC. Detection of *Streptococcus equi* in equine nasal swabs and washes by DNA amplification. *Vet Rec*. Oct 25 1997;141(17):446-7. doi:10.1136/vr.141.17.446
32. Alber J, El-Sayed A, Lämmler C, Hassan AA, Weiss R, Zschöck M. Multiplex polymerase chain reaction for identification and differentiation of *Streptococcus equi* subsp. *zooepidemicus* and *Streptococcus equi* subsp. *equi. J Vet Med B Infect Dis Vet Public Health*. Dec 2004;51(10):455-8. doi:10.1111/j.1439-0450.2004.00799.x
33. Younan M, Estoepangestie AT, Cengiz M, Alber J, El-Sayed A, Lämmler C. Identification and molecular characterization of *Streptococcus equi* subsp. *zooepidemicus* isolated from camels (*Camelus dromedarius*) and camel milk in Kenya and Somalia. *J Vet Med B Infect Dis Vet Public Health*. Apr 2005;52(3):142-6. doi:10.1111/j.1439-0450.2005.00828.x
34. Båverud V, Johansson SK, Aspan A. Real-time PCR for detection and differentiation of *Streptococcus equi* subsp. *equi* and *Streptococcus equi* subsp. *zooepidemicus*. *Vet Microbiol*. Oct 6 2007;124(3-4):219-29. doi:10.1016/j.vetmic.2007.04.020
35. Priestnall SL, Erles K, Brooks HW, et al. Characterization of pneumonia due to *Streptococcus equi* subsp. *zooepidemicus* in dogs. *Clin Vaccine Immunol*. Nov 2010;17(11):1790-6. doi:10.1128/cvi.00188-10
36. North SE, Wakeley PR, Mayo N, Mayers J, Sawyer J. Development of a real-time PCR to detect *Streptococcus equi* subspecies *equi*. *Equine Vet J*. Jan 2014;46(1):56-9. doi:10.1111/evj.12088
37. Kinoshita Y, Niwa H, Katayama Y. Development of a loop-mediated isothermal amplification method for detecting *Streptococcus equi* subsp. *zooepidemicus* and analysis of its use with three simple methods of extracting DNA from equine respiratory tract specimens. *J Vet Med Sci*. Sep 2014;76(9):1271-5. doi:10.1292/jvms.14-0140
38. Cordoni G, Williams A, Durham A, Florio D, Zanoni RG, La Ragione RM. Rapid diagnosis of strangles (*Streptococcus equi* subspecies *equi*) using PCR. *Res Vet Sci*. Oct 2015;102:162-6. doi:10.1016/j.rvsc.2015.08.008
39. Moriconi M, Acke E, Petrelli D, Preziuso S. Multiplex PCR-based identification of *Streptococcus canis*, *Streptococcus zooepidemicus* and *Streptococcus dysgalactiae* subspecies from dogs. *Comp Immunol Microbiol Infect Dis*. Feb 2017;50:48-53. doi:10.1016/j.cimid.2016.11.011
40. Sun F, Ganguli A, Nguyen J, et al. Smartphone-based multiplex 30-minute nucleic acid test of live virus from nasal swab extract. *Lab Chip*. May 5 2020;20(9):1621-1627. doi:10.1039/d0lc00304b
41. Robinson C, Steward KF, Potts N, et al. Combining two serological assays optimises sensitivity and specificity for the identification of *Streptococcus equi* subsp. *equi* exposure. *Vet J*. Aug 2013;197(2):188-91. doi:10.1016/j.tvjl.2013.01.033
42. Knowles EJ, Mair TS, Butcher N, Waller AS, Wood JL. Use of a novel serological test for exposure to *Streptococcus equi* subspecies *equi* in hospitalised horses. *Vet Rec*. Mar 6 2010;166(10):294-7. doi:10.1136/vr.166.10.294
43. Rossdale Laboratories. Strangles (*Streptococcus equi*) ELISA. Accessed February 24, 2021. https://www.rossdales.com/laboratories/tests-and-diseases/strangles-streptococcus-equi-elisa
44. El-Hage CM, Bannai H, Wiethoelter AK, et al. Serological responses of Australian horses using a commercial duplex indirect ELISA following vaccination against strangles. *Aust Vet J*. Jul 2019;97(7):220-224. doi:10.1111/avj.12825
45. Arai S, Kim H, Watanabe T, et al. Assessment of pig saliva as a *Streptococcus suis* reservoir and potential source of infection on farms by use of a novel quantitative polymerase chain reaction assay. *Am J Vet Res*. Sep 2018;79(9):941-948. doi:10.2460/ajvr.79.9.941
46. Murase K, Watanabe T, Arai S, et al. Characterization of pig saliva as the major natural habitat of *Streptococcus suis* by analyzing oral, fecal, vaginal, and environmental microbiota. *PLoS One*. 2019;14(4):e0215983. doi:10.1371/journal.pone.0215983

47. Costa G, Oliveira S, Torrison J. Detection of *Actinobacillus pleuropneumoniae* in oral-fluid samples obtained from experimentally infected pigs. *J Swine Health Prod*. 2012;20(2):78-81.
48. Timoney JF. The pathogenic equine streptococci. *Vet Res*. Jul-Aug 2004;35(4):397-409. doi:10.1051/vetres:2004025
49. O'Sullivan T, Friendship R, Blackwell T, et al. Microbiological identification and analysis of swine tonsils collected from carcasses at slaughter. *Can J Vet Res*. Apr 2011;75(2):106-11.
50. Lowe BA, Marsh TL, Isaacs-Cosgrove N, Kirkwood RN, Kiupel M, Mulks MH. Microbial communities in the tonsils of healthy pigs. *Vet Microbiol*. Jan 27 2011;147(3-4):346-57. doi:10.1016/j.vetmic.2010.06.025
51. Lowe BA, Marsh TL, Isaacs-Cosgrove N, Kirkwood RN, Kiupel M, Mulks MH. Defining the "core microbiome" of the microbial communities in the tonsils of healthy pigs. *BMC Microbiol*. Feb 7 2012;12:20. doi:10.1186/1471-2180-12-20
52. Mallicote M. Update on *Streptococcus equi* subsp *equi* infections. *Vet Clin North Am Equine Pract*. Apr 2015;31(1):27-41. doi:10.1016/j.cveq.2014.11.003
53. Sharp MW, Prince MJ, Gibbens J. *S zooepidemicus* infection and bovine mastitis. *Vet Rec*. Jul 29 1995;137(5):128. doi:10.1136/vr.137.5.128-b
54. Las Heras A, Vela AI, Fernández E, Legaz E, Domínguez L, Fernández-Garayzábal JF. Unusual outbreak of clinical mastitis in dairy sheep caused by *Streptococcus equi* subsp. *zooepidemicus*. *J Clin Microbiol*. Mar 2002;40(3):1106-8. doi:10.1128/jcm.40.3.1106-1108.2002
55. Pisoni G, Zadoks RN, Vimercati C, Locatelli C, Zanoni MG, Moroni P. Epidemiological investigation of *Streptococcus equi* subspecies *zooepidemicus* involved in clinical mastitis in dairy goats. *J Dairy Sci*. Mar 2009;92(3):943-51. doi:10.3168/jds.2008-1548
56. Priestnall S, Erles K. *Streptococcus zooepidemicus*: an emerging canine pathogen. *Vet J*. May 2011;188(2):142-8. doi:10.1016/j.tvjl.2010.04.028
57. Blum S, Elad D, Zukin N, et al. Outbreak of *Streptococcus equi* subsp. *zooepidemicus* infections in cats. *Vet Microbiol*. Jul 29 2010;144(1-2):236-9. doi:10.1016/j.vetmic.2009.12.040
58. Tibary A, Fite C, Anouassi A, Sghiri A. Infectious causes of reproductive loss in camelids. *Theriogenology*. Aug 2006;66(3):633-47. doi:10.1016/j.theriogenology.2006.04.008
59. Gruszynski K, Young A, Levine SJ, et al. *Streptococcus equi* subsp. *zooepidemicus* infections associated with guinea pigs. *Emerg Infect Dis*. Jan 2015;21(1):156-8. doi:10.3201/eid2101.140640
60. Mitchell CM, Johnson LK, Crim MJ, et al. Diagnosis, surveillance and management of *Streptococcus equi* subspecies *zooepidemicus* infections in chinchillas (*Chinchilla lanigera*). *Comp Med*. Aug 1 2020;70(4):370-375. doi:10.30802/aalas-cm-20-000012
61. Garmyn A, Van de Velde N, Braeckmans D, Ronsmans S, Boyen F, Verlinden M. An outbreak associated with *Streptococcus equi* subsp. *zooepidemicus* in layers: evidence of fecal rransmission. *Avian Dis*. Sep 1 2020;64(3):343-346. doi:10.1637/aviandiseases-D-19-00191
62. Wei Z, Li R, Zhang A, et al. Characterization of *Streptococcus suis* isolates from the diseased pigs in China between 2003 and 2007. *Vet Microbiol*. 2009-05-01 2009;137(1-2):196-201. doi:10.1016/j.vetmic.2008.12.015
63. Zhang B, Ku X, Yu X, et al. Prevalence and antimicrobial susceptibilities of bacterial pathogens in Chinese pig farms from 2013 to 2017. *Sci Rep*. 2019-12-01 2019;9(1)doi:10.1038/s41598-019-45482-8
64. Cardoso-Toset F, Gómez-Laguna J, Amarilla SP, et al. Multi-etiological nature of tuberculosis-like lesions in condemned pigs at the slaughterhouse. *PLoS One*. 2015;10(9):e0139130. doi:10.1371/journal.pone.0139130
65. Cardoso-Toset F, Gómez-Laguna J, Gómez-Gascón L, et al. Histopathological and microbiological study of porcine lymphadenitis: contributions to diagnosis and control of the disease. *Porcine Health Manag*. Dec 4 2020;6(1):36. doi:10.1186/s40813-020-00172-0
66. Canadian Swine Health Intelligence Network (CSHIN). Quarterly Producer Report: Q2 April-June 2019. Accessed February 21, 2021. https://www.cpc-ccp.com/uploads/userfiles/files/CSHIN%202019%20Q2%20Producer%20Report%20FINAL.pdf
67. Malke H. Genetics and pathogenicity factors of Group C and G streptococci. *Microbiol Spectr*. Mar 2019;7(2)

doi:10.1128/microbiolspec.GPP3-0002-2017
68. Facklam R. What happened to the streptococci: overview of taxonomic and nomenclature changes. *Clin Microbiol Rev*. Oct 2002;15(4):613-30. doi:10.1128/cmr.15.4.613-630.2002
69. Velineni S, Desoutter D, Perchec AM, Timoney JF. Characterization of a mucoid clone of *Streptococcus zooepidemicus* from an epizootic of equine respiratory disease in New Caledonia. *Vet J*. Apr 2014;200(1):82-7. doi:10.1016/j.tvjl.2014.01.014
70. Lindsay AM, Zhang M, Mitchell Z, et al. The *Streptococcus equi* prophage-encoded protein SEQ2045 is a hyaluronan-specific hyaluronate lyase that is produced during equine infection. *Microbiology (Reading)*. Feb 2009;155(Pt 2):443-449. doi:10.1099/mic.0.020826-0
71. Holden MT, Heather Z, Paillot R, et al. Genomic evidence for the evolution of *Streptococcus equi*: host restriction, increased virulence, and genetic exchange with human pathogens. *PLoS Pathog*. Mar 2009;5(3):e1000346. doi:10.1371/journal.ppat.1000346
72. Waller AS, Robinson C. *Streptococcus zooepidemicus* and *Streptococcus equi* evolution: the role of CRISPRs. *Biochem Soc Trans*. Dec 2013;41(6):1437-43. doi:10.1042/bst20130165
73. Webb K, Jolley KA, Mitchell Z, et al. Development of an unambiguous and discriminatory multilocus sequence typing scheme for the *Streptococcus zooepidemicus* group. *Microbiology (Reading)*. Oct 2008;154(Pt 10):3016-3024. doi:10.1099/mic.0.2008/018911-0
74. Turner CE, Bubba L, Efstratiou A. Pathogenicity factors in group C and G streptococci. *Microbiol Spect*. 2019-05-24 2019;7(3)doi:10.1128/microbiolspec.gpp3-0020-2018
75. Timoney JF, Artiushin SC, Boschwitz JS. Comparison of the sequences and functions of *Streptococcus equi* M-like proteins SeM and SzPSe. *Infect Immun*. Sep 1997;65(9):3600-5. doi:10.1128/iai.65.9.3600-3605.1997
76. Bergmann R, Jentsch MC, Uhlig A, et al. Prominent binding of human and equine fibrinogen to *Streptococcus equi* subsp. *zooepidemicus* is mediated by specific SzM types and is a distinct phenotype of zoonotic isolates. *Infect Immun*. Dec 17 2019;88(1)doi:10.1128/iai.00559-19
77. D'Gama JD, Ma Z, Zhang H, et al. A conserved streptococcal virulence regulator controls the expression of a distinct class of M-like proteins. *mBio*. Oct 22 2019;10(5)doi:10.1128/mBio.02500-19
78. Walker RL, Runyan CA. Identification of variations in SzP proteins of *Streptococcus equi* subspecies *zooepidemicus* and the relationship between protein variants and clinical signs of infection in horses. *Am J Vet Res*. Aug 2003;64(8):976-81. doi:10.2460/ajvr.2003.64.976
79. Paillot R, Darby AC, Robinson C, et al. Identification of three novel superantigen-encoding genes in *Streptococcus equi* subsp. *zooepidemicus*, szeF, szeN, and szeP. *Infect Immun*. Nov 2010;78(11):4817-27. doi:10.1128/iai.00751-10
80. Ma Z, Peng J, Yu D, et al. A streptococcal Fic domain-containing protein disrupts blood-brain barrier integrity by activating moesin in endothelial cells. *PLoS Pathog*. May 2019;15(5):e1007737. doi:10.1371/journal.ppat.1007737
81. Ma Z, Geng J, Zhang H, et al. Complete genome sequence of *Streptococcus equi* subsp. *zooepidemicus* strain ATCC 35246. *J Bacteriol*. 2011-10-01 2011;193(19):5583-5584. doi:10.1128/jb.05700-11
82. Xu B, Zhang P, Zhou H, Sun Y, Tang J, Fan H. Identification of novel genes associated with anti-phagocytic functions in *Streptococcus equi* subsp. *zooepidemicus*. *Vet Microbiol*. Jun 2019;233:28-38. doi:10.1016/j.vetmic.2019.04.023
83. Hau S, Lantz K, Stuart K, et al. Replication of clinical *Streptococcus equi* subspecies *zooepidemicus* disease in sows and feeder pigs. presented at: 52nd Annual Meeting of the American Association of Swine Veterinarians; 2021; Virtual.
84. Timoney JF, Walker J, Zhou M, Ding J. Cloning and sequence analysis of a protective M-like protein gene from *Streptococcus equi* subsp. *zooepidemicus*. *Infect Immun*. Apr 1995;63(4):1440-5. doi:10.1128/iai.63.4.1440-1445.1995
85. Hong-Jie F, Fu-yu T, Ying M, Cheng-ping L. Virulence and antigenicity of the szp-gene deleted *Streptococcus equi* ssp. *zooepidemicus* mutant in mice. *Vaccine*. Jan 1 2009;27(1):56-61. doi:10.1016/j.vaccine.2008.10.037
86. Lin HX, Huang DY, Wang Y, Lu CP, Fan HJ. A novel vaccine against *Streptococcus equi* ssp. *zooepidemicus*

infections: the recombinant swinepox virus expressing M-like protein. *Vaccine*. Sep 16 2011;29(40):7027-34. doi:10.1016/j.vaccine.2011.07.074

87. Wei Z, Fu Q, Chen Y, et al. *Streptococcus equi* ssp. *zooepidemicus* C5a peptidase, a putative invasin, induces protective immune response in mice. *Res Vet Sci*. Oct 2013;95(2):444-50. doi:10.1016/j.rvsc.2013.03.026
88. Fu Q, Wei Z, Liu X, Xiao P, Lu Z, Chen Y. Glyceraldehyde-3-phosphate dehydrogenase, an immunogenic *Streptococcus equi* ssp. *zooepidemicus* adhesion protein and protective antigen. *J Microbiol Biotechnol*. Apr 2013;23(4):579-85. doi:10.4014/jmb.1209.09037
89. Fu Q, Wei Z, Chen Y, Xiao P, Lu Z, Liu X. Identification of a surface protective antigen, CSP of *Streptococcus equi* ssp. *zooepidemicus*. *Vaccine*. Feb 27 2013;31(10):1400-5. doi:10.1016/j.vaccine.2012.12.079
90. Velineni S, Timoney JF. Characterization and protective immunogenicity of the SzM protein of *Streptococcus zooepidemicus* NC78 from a clonal outbreak of equine respiratory disease. *Clin Vaccine Immunol*. Aug 2013;20(8):1181-8. doi:10.1128/cvi.00069-13
91. Velineni S, Timoney JF. Identification of novel immunoreactive proteins of *Streptococcus zooepidemicus* with potential as vaccine components. *Vaccine*. Aug 28 2013;31(38):4129-35. doi:10.1016/j.vaccine.2013.06.100
92. Wei Z, Fu Q, Chen Y, et al. The capsule of *Streptococcus equi* ssp. *zooepidemicus* is a target for attenuation in vaccine development. *Vaccine*. Jun 29 2012;30(31):4670-5. doi:10.1016/j.vaccine.2012.04.092
93. Velineni S, Timoney JF, Russell K, et al. Clones of *Streptococcus zooepidemicus* from outbreaks of hemorrhagic canine pneumonia and associated immune responses. *Clin Vaccine Immunol*. Sep 2014;21(9):1246-52. doi:10.1128/cvi.00222-14
94. Lin HX, Ma Z, Yang XQ, Fan HJ, Lu CP. A novel vaccine against porcine circovirus type 2 (PCV2) and *Streptococcus equi* ssp. *zooepidemicus* (SEZ) co-infection. *Vet Microbiol*. Jun 25 2014;171(1-2):198-205. doi:10.1016/j.vetmic.2014.03.018
95. Yi L, Wang Y, Ma Z, et al. Identification and characterization of a *Streptococcus equi* ssp. *zooepidemicus* immunogenic GroEL protein involved in biofilm formation. *Vet Res*. Apr 18 2016;47:50. doi:10.1186/s13567-016-0334-0
96. Xie H, Wei Z, Ma C, Li S, Liu X, Fu Q. Characterization of SeseC_01411 as a surface protective antigen of *Streptococcus equi* ssp. *zooepidemicus*. *Res Vet Sci*. Jun 2018;118:517-521. doi:10.1016/j.rvsc.2018.05.007
97. Liang H, Tang B, Zhao P, et al. Identification and characterization of a novel protective antigen, Sec_205 of *Streptococcus equi* ssp. *zooepidemicus*. *Vaccine*. Feb 1 2018;36(6):788-793. doi:10.1016/j.vaccine.2017.12.072
98. Tang B, Liang H, Gao X, et al. Identification of a surface protective antigen, MAP of *Streptococcus equi* subspecies *zooepidemicus*. *Res Vet Sci*. Jun 2019;124:387-392. doi:10.1016/j.rvsc.2019.04.021
99. Yi L, Yang W, Sun L, Li J, Li X, Wang Y. Identification of a novel protective antigen, 3-oxoacyl-[acyl-carrier-protein] synthase II of *Streptococcus equi* ssp. *zooepidemicus* which confers protective effects. *Comp Immunol Microbiol Infect Dis*. May 19 2020;71:101493. doi:10.1016/j.cimid.2020.101493
100. Meekhanon N, Kaewmongkol S, Jirawattanapong P, et al. High rate misidentification of biochemically determined *Streptococcus* isolates from swine clinical specimens. *J Vet Med Sci*. Apr 16 2019;81(4):567-572. doi:10.1292/jvms.18-0678
101. Duca E, Teodorovici G, Radu C, et al. A new nephritogenic streptococcus. *J Hyg (Lond)*. 1969-12-01 1969;67(4):691-698. doi:10.1017/s0022172400042145
102. Barnham M, Thornton TJ, Lange K. Nephritis caused by *Streptococcus zooepidemicus* (Lancefield group C). *Lancet*. Apr 30 1983;1(8331):945-8. doi:10.1016/s0140-6736(83)92078-0
103. Balter S, Benin A, Pinto SW, et al. Epidemic nephritis in Nova Serrana, Brazil. *Lancet*. May 20 2000;355(9217):1776-80. doi:10.1016/s0140-6736(00)02265-0
104. Pinto SW, Sesso R, Vasconcelos E, Watanabe YJ, Pansute AM. Follow-up of patients with epidemic poststreptococcal glomerulonephritis. *Am J Kidney Dis*. Aug 2001;38(2):249-55. doi:10.1053/ajkd.2001.26083
105. Sesso R, Pinto SW. Five-year follow-up of patients with epidemic glomerulonephritis due to *Streptococcus*

zooepidemicus. *Nephrol Dial Transplant*. Sep 2005;20(9):1808-12. doi:10.1093/ndt/gfh904
106. Pinto SWL, Mastroianni-Kirsztajn G, Sesso R. Ten-year follow-up of patients with epidemic post infectious glomerulonephritis. *PLOS ONE*. 2015-05-11 2015;10(5):e0125313. doi:10.1371/journal.pone.0125313
107. Nicholson ML, Ferdinand L, Sampson JS, et al. Analysis of immunoreactivity to a *Streptococcus equi* subsp. *zooepidemicus* M-like protein to confirm an outbreak of poststreptococcal glomerulonephritis, and sequences of M-like proteins from isolates obtained from different host species. *J Clin Microbiol*. Nov 2000;38(11):4126-30. doi:10.1128/jcm.38.11.4126-4130.2000
108. Beres SB, Sesso R, Pinto SW, et al. Genome sequence of a Lancefield group C *Streptococcus zooepidemicus* strain causing epidemic nephritis: new information about an old disease. *PLoS One*. Aug 21 2008;3(8):e3026. doi:10.1371/journal.pone.0003026
109. Kittang BR, Pettersen VK, Oppegaard O, et al. Zoonotic necrotizing myositis caused by *Streptococcus equi* subsp. *zooepidemicus* in a farmer. *BMC Infect Dis*. 2017-12-01 2017;17(1)doi:10.1186/s12879-017-2262-7
110. Breiman RF, Silverblatt FJ. Systemic *Streptococcus equi* infection in a horse handler--a case of human strangles. *West J Med*. Sep 1986;145(3):385-6.
111. McKeage MJ, Humble MW, Morrison RB. *Streptococcus zooepidemicus* cellulitis and bacteraemia in a renal transplant recipient. *Aust N Z J Med*. Apr 1990;20(2):177-8. doi:10.1111/j.1445-5994.1990.tb01299.x
112. Hashikawa S, Iinuma Y, Furushita M, et al. Characterization of Group C and G streptococcal strains that cause streptococcal toxic shock syndrome. *J Clin Microbiol*. 2004-01-01 2004;42(1):186-192. doi:10.1128/jcm.42.1.186-192.2004
113. Korman TM, Boers A, Gooding TM, Curtis N, Visvanathan K. Fatal case of toxic shock-like syndrome due to Group C streptococcus associated with superantigen exotoxin. *J Clin Microbiol*. 2004-06-01 2004;42(6):2866-2869. doi:10.1128/jcm.42.6.2866-2869.2004
114. Saleh M, Vialette V. Toxic shock syndrome related to *Streptococcus equi* subsp *zooepidemicus*. *BMJ Case Rep*. Sep 6 2013;2013doi:10.1136/bcr-2013-200566
115. González Terán B, Roiz MP, Ruiz Jimeno T, Rosas J, Calvo-Alén J. Acute bacterial arthritis caused by group C streptococci. *Semin Arthritis Rheum*. Aug 2001;31(1):43-51. doi:10.1053/sarh.2001.21405
116. Miles B, Tuomela K, Sanchez J. Severe Group C Streptococcus infection in a veterinarian. *IDCases*. © 2020 Published by Elsevier Ltd.; 2021:e01036.
117. Edwards AT, Roulson M, Ironside MJ. A milk-borne outbreak of serious infection due to *Streptococcus zooepidemicus* (Lancefield Group C). *Epidemiol Infect*. Aug 1988;101(1):43-51. doi:10.1017/s0950268800029204
118. Bradley SF, Gordon JJ, Baumgartner DD, Marasco WA, Kauffman CA. Group C streptococcal bacteremia: analysis of 88 cases. *Rev Infect Dis*. Mar-Apr 1991;13(2):270-80. doi:10.1093/clinids/13.2.270
119. Kuusi M, Lahti E, Virolainen A, et al. An outbreak of *Streptococcus equi* subspecies *zooepidemicus* associated with consumption of fresh goat cheese. *BMC Infect Dis*. Feb 27 2006;6:36. doi:10.1186/1471-2334-6-36
120. Poulin MF, Boivin G. A case of disseminated infection caused by *Streptococcus equi* subspecies *zooepidemicus*. *Can J Infect Dis Med Microbiol*. 2009:59-61. vol. 2.
121. Eyre DW, Kenkre JS, Bowler IC, McBride SJ. *Streptococcus equi* subspecies *zooepidemicus* meningitis--a case report and review of the literature. *Eur J Clin Microbiol Infect Dis*. Dec 2010;29(12):1459-63. doi:10.1007/s10096-010-1037-5
122. Rajasekhar A, Clancy CJ. Meningitis due to group C Streptococcus: a case report and review of the literature. *Scand J Infect Dis*. Aug 2010;42(8):571-8. doi:10.3109/00365541003754428
123. Madžar D, Hagge M, Möller S, et al. Endogenous endophthalmitis complicating *Streptococcus equi* subspecies *zooepidemicus* meningitis: a case report. *BMC Res Notes*. May 5 2015;8:184. doi:10.1186/s13104-015-1133-9
124. Kawakami V, Rietberg K, Lipton B, et al. Notes from the field: fatal infection associated with equine exposure — King County, Washington, 2016. *MMWR*. 2016-08-05 2016;65(30):788. doi:10.15585/mmwr.mm6530a5
125. Björnsdóttir S, Harris SR, Svansson V, et al. Genomic dissection of an Icelandic epidemic of respiratory disease in horses and associated zoonotic cases. *mBio*. Aug 1 2017;8(4)doi:10.1128/mBio.00826-17
126. Zahlanie Y, Almatrafi M, Filkins L, Hsiang MS. Possible canine source of *Streptococcus equi* subspecies

zooepidemicus causing meningitis in an infant. *IDCases*. 2019:e00568.
127. Latorre M, Alvarez M, Fernández JM, Berdonces P, Llanos A, Cisterna R. A case of meningitis due to "*Streptococcus zooepidemicus*". *Clin Infect Dis*. Nov 1993;17(5):932-3. doi:10.1093/clinids/17.5.932
128. Ferrandière M, Cattier B, Dequin PF, Hazouard E, Legras A, Perrotin D. Septicemia and meningitis due to *Streptococcus zooepidemicus*. *Eur J Clin Microbiol Infect Dis*. Apr 1998;17(4):290-1. doi:10.1007/bf01699990
129. Downar J, Willey BM, Sutherland JW, Mathew K, Low DE. Streptococcal meningitis resulting from contact with an infected horse. *J Clin Microbiol*. Jun 2001;39(6):2358-9. doi:10.1128/jcm.39.6.2358-2359.2001
130. Shah SS, Matthews RP, Cohen C. Group C streptococcal meningitis: case report and review of the literature. *Pediatr Infect Dis J*. Apr 2001;20(4):445-8. doi:10.1097/00006454-200104000-00016
131. Ural O, Tuncer I, Dikici N, Aridogan B. *Streptococcus zooepidemicus* meningitis and bacteraemia. *Scand J Infect Dis*. 2003;35(3):206-7. doi:10.1080/00365540310000076
132. Bordes-Benítez A, Sánchez-Oñoro M, Suárez-Bordón P, et al. Outbreak of *Streptococcus equi* subsp. *zooepidemicus* infections on the island of Gran Canaria associated with the consumption of inadequately pasteurized cheese. *Eur J Clin Microbiol Infect Dis*. Apr 2006;25(4):242-6. doi:10.1007/s10096-006-0119-x
133. Pati S, Al-Araji A, Orendi J. Atypical presentation of *Streptococcus zooepidemicus* bacteraemia and secondary meningitis. *Clin Neurol Neurosurg*. 2007:475-6. vol. 5.
134. Jovanović M, Stevanović G, Tošić T, Stošović B, Zervos MJ. *Streptococcus equi* subsp. *zooepidemicus* meningitis. *J Med Microbiol*. Mar 2008;57(Pt 3):373-375. doi:10.1099/jmm.0.47487-0
135. Minces LR, Brown PJ, Veldkamp PJ. Human meningitis from *Streptococcus equi* subsp. *zooepidemicus* acquired as zoonoses. *Epidemiol Infect*. Mar 2011;139(3):406-10. doi:10.1017/s0950268810001184
136. Mori N, Guevara JM, Tilley DH, Briceno JA, Zunt JR, Montano SM. *Streptococcus equi* subsp. *zooepidemicus* meningitis in Peru. *J Med Microbiol*. Feb 2013;62(Pt 2):335-337. doi:10.1099/jmm.0.050245-0
137. Pelkonen S, Lindahl SB, Suomala P, et al. Transmission of *Streptococcus equi* subspecies *zooepidemicus* infection from horses to humans. *Emerg Infect Dis*. Jul 2013;19(7):1041-8. doi:10.3201/eid1907.121365
138. Watson JR, Leber A, Velineni S, Timoney JF, Ardura MI. Recurrent *Streptococcus equi* subsp. *zooepidemicus* bacteremia in an infant. *J Clin Microbiol*. Sep 2015;53(9):3096-9. doi:10.1128/jcm.01306-15
139. Aida Z, Lamia A, Souheil Z, et al. Meningitis due to *Streptococcus equi* in a 73 year old woman with an osteodural defect. *IDCases*. © 2020 The Author(s). 2020:e00779.

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.[6] **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.[101]
- 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.[102]
- 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.[103] 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.[104] At the 5-year follow up, 56 cases were re-examined; approximately 30% were hypertensive and 49% had reduced kidney function.[105] 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.[106] Antibodies to the M-like protein Szp5058 were found in convalescent sera from 33/44 cases tested.[107] 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.[108] In addition, proteins such as streptokinase (which are implicated in post-streptococcal glomerulonephritis due to GAS) were highly divergent in strain MGCS10565.[108]

Skin and soft tissue infections due to *S. zooepidemicus* are less common than other GCS, and usually involve animal exposure.[6] 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.[109]
- 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.”[110]
- A 1990 case report from Australia described *S. zooepidemicus* cellulitis in a renal transplant patient who had contact with horses at a show. [111]

GCS and GGS have been occasionally identified in cases of **streptococcal toxic shock syndrome** (STSS).[6] 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.[112]
- 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.[113]
- 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.[114]

GCS are a known cause of **infectious arthritis** in humans, but *S. zooepidemicus* has been isolated infrequently.[6] 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.[115]
- 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.[116]

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.[6] 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.[117]
- 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]