

The survival of *Streptococcus suis* on farm and veterinary equipment

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Summary—The ability of *Streptococcus suis* to survive and be transmitted on farm and veterinary equipment when exposed to a wide range of climactic and environmental stress factors was assessed. Pure cultures of *S. suis* were placed on surfaces or materials commonly found on farms and/or in veterinary practices and cultured at predetermined time intervals. Overall, isolates lived longer on rubber and plastic surfaces, especially when protected by hog manure. Isolates were viable at up to 55°C and could survive if kept frozen for up to 10 days. Repeated freezing and thawing was detrimental to the survival of the organism. Viable isolates were obtained for up to 4 days in aluminum hydroxide vaccines, but for less than 24 hours in oil-based products. *S. suis* survived for 10 days at 4°C when stored in porcine tissues or body fluids. No bacterial growth was detected in any disinfectant tested except 70% alcohol. The organism was shown to be able to be transmitted via fomites (such as manure-covered work boots, veterinary vehicles, and needles).

Minimizing disease problems requires knowledge of the spread of infectious disease. Pig flow schemes are changed (e.g., from continuous flow to all-in/all-out) to help reduce the microbial load in an existing facility. For such projects to succeed, we must know the ability of an infectious organism to withstand climactic and environmental stressors. Only then can we recommend the proper procedures for cleaning a building.

Streptococcus suis is a common organism found on hog operations. Because it can cause pneumonia, meningitis and arthritis, it is an important pathogen of swine.¹ Studies have demonstrated that the organism can survive in:

- feces for 104 days at 0°C
10 days at 9°C
- dust for 54 days at 0°C
25 days at 9°C.¹

It cannot survive for more than 24 hours in dust held at room temperature.¹ The incidence of problems caused by *S. suis* appears to be increasing on hog farms across the country, although it may also be that with improved producer awareness and knowledge of the conditions *S. suis* causes, it is being recognized more often. In either case, more information regarding environmental survival is needed. We conducted a number of experiments to assess the viability of *S. suis* after exposure to a wide range of climactic and environmental conditions similar to those found on hog operations as well as to examine the role of fomites typically encountered on farms or in veterinary clinics in transmitting the organism.

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Methods

A pure culture of gram-positive, catalase-negative, chained cocci isolated from a pig with meningitis was determined to be *S. suis*. Initially cultured on 5% sheep blood agar, it was further differentiated as a Group D *Streptococcus* by means of a positive-esculin reaction and the inability to grow in 6.5% sodium chloride broth. Cultures were confirmed to be *S. suis* with a positive reaction to *S. suis* type 2 antiserum using a plate agglutination test.²

To prepare test cultures, the culture was incubated overnight at 37°C in Casman's enrichment broth. The concentration of bacteria was determined to be 1.7×10^4 cfu/mL of broth. A 10 μ L dilution loop was placed into the broth culture and

Table 1.— Methods used to test surfaces with *S. suis* (all surfaces tested at both 20°C and 49°C)

Surface	With Manure	Without Manure
Unpainted plywood	✓	✓
Painted plywood		✓
Concrete	✓	✓
Plastic coated wire	✓	✓
Metal (hog snare)	✓	✓
Work boot	✓	

streaked onto 5% sheep blood agar for colony counting. A 0.2 mL aliquot of the broth was used as the standard inoculum for all survivability studies. All surfaces involved in this study were initially cultured on 5% sheep blood agar for the presence of *S. suis* and found to be negative using the biochemical tests previously mentioned.

Surface Studies

Surfaces encountered in typical hog operations were obtained from various farms (Table 1). A bleeding box from our practice, made of painted plywood and used to hold blood testing equipment, was also used as an experimental surface. All surfaces included in the study were tested at both room temperature (20°C) and at 49°C, which was achieved with a heat lamp suspended 12 inches above the surface being tested.

Most surfaces were tested both with and without a coating of manure (Table 1). Manure was obtained from a hog facility and found to be negative for *S. suis*. It had not been previously sterilized prior to shipment. Except for the bottom of the work boot, the standard inoculum was placed in two spots on each surface, one spot (5.1 cm in diameter and 0.25 cm wide) was covered with manure and the other was

left uncovered. The bottom of the work boot was inoculated with a mixture of 0.2 mL bacterial broth and manure and held at room temperature. The manure completely covered the inoculum. Cultures were taken every 4 hours on day of inoculation, every 6 hours on day 2 post-inoculation and every 24 hours on days 3-14 post-inoculation, or until a negative culture was obtained. Cotton swabs were rubbed three to four times gently over the surface where the inoculum had been placed. The manure cover was lifted using a sterile forceps in order to swab the inoculated area. The entire inoculated area was swabbed. Cultures were placed on 5% sheep blood agar and incubated overnight at 37°C.

Media Studies

The standard inoculum was placed in each of:

- porcine semen (10 mL);
- urine (10 mL);
- whole blood (10 mL);
- a Precision Amies Culturette® (Precision Dynamics Corp. San Fernando, California);
- a 10 cc sample of infected aluminum hydroxide (from a 250 mL bottle); and
- a 10 cc sample of infected oil-based vaccine (from a 250 mL bottle).

Samples were held at room temperature and cultures were taken every 24 hours. The inoculum was also injected into the brain tissue (right hemisphere, mid-cerebrum) of a dead suckling piglet. The piglet had been dead for less than 12 hours prior to inoculation. These samples were stored at 4°C and cultured every 24 hours for 10 days or until a negative isolation was obtained. A cotton swab was placed once into each medium and then applied to a 5% sheep blood agar plate and incubated overnight at 37°C. The brain tissue was sampled by placing a swab into the same location (right hemisphere, mid-cerebrum) each time and cultured in a similar fashion.

Temperature Studies

A glass test tube containing 5 mL of the standard inoculum was placed in a beaker containing tap water and heated on a stove. A culture was taken during every 5°C increase in temperature up to 100°C. The standard inoculum was frozen at -20°C and thawed immediately, four consecutive times. A culture was taken after every thaw. No more than 10 minutes elapsed between thawing, culturing and refreezing. Another 0.2 mL aliquot was

Table 2.— *Streptococcus suis* survivability: Surface study

Surface	4h	8h	12h	16h	20h	24h	48h	72h
Painted plywood	-	-	-	-	-	-	NT	NT
Plywood	-	-	-	-	-	-	NT	NT
Plywood, heat	-	-	-	-	-	-	NT	NT
Plywood, manure	+	+	+	-	-	-	-	NT
Plastic flooring	+	+	+	+	+	-	-	-
Plastic, heat	-	-	-	-	-	-	NT	NT
Plastic, manure	+	+	+	+	+	+	-	-
Plastic, heat, manure	+	-	-	-	-	-	-	NT
Concrete	-	-	-	-	-	-	NT	NT
Concrete, heat	-	-	-	-	-	-	NT	NT
Concrete, manure	+	+	+	+	+	+	-	-
Concrete, heat, manure	+	-	-	-	-	-	-	NT
Metal	-	-	-	-	-	-	NT	NT
Metal, manure	+	+	-	-	-	-	-	NT
Rubber, manure	+	+	+	+	+	+	+	+

Key: + Growth detected; - No growth detected; NT not tested

kept frozen at -20°C for 10 consecutive days, thawed once and then cultured in a similar manner as above.

Disinfectant Studies

The inoculum was streaked over a Mueller Hinton agar similar to the technique involved in a Kirby Bauer antibiotic sensitivity assay.³ Seven disinfectants were diluted to recommended levels, sterile swabs were dipped in each disinfectant and placed on the agar surface.⁴ The ability of the disinfectant to inhibit the growth of the organism was determined by the presence or absence of bacteria around the swab. The disinfectants tested were:

- phenol (1-Stroke Environ®, Sanofi, Overland Park, Kansas);
- quaternary ammonium (Roccal-D®, Upjohn, Kalamazoo, Michigan);
- formaldehyde (DC & R®, Hess and Clark, Ashland, Ohio);
- chlorhexadine (Novalsan®, Fort Dodge, Iowa);
- 3% iodine;
- 70% alcohol; and
- 5% hypochlorite (Chlorox®, Chlorox Co., Oakland, California).

Mechanical Vector Survival

The standard inoculum (0.2 mL) was mixed with hog manure and smeared on a 4 sq in (26 cm²) area of the tread of the left front tire of a veterinary vehicle. The vehicle was driven on an asphalt at 35–40 miles per hour (56–64 km per hour) and the inoculated portion of the tread was cultured every half mile for a total of 3 miles (5 km). The swab was placed directly into the manure and then onto 5% sheep blood agar. The vehicle was then driven for 8 more miles (13 km) at 60–75 miles per hour (97–121 km per hour) and the tread cultured at the end of the trip. All cultures were held at 37°C overnight.

We investigated the transmission of *S. suis* via a contaminated needle and syringe. A 5 mL tube of whole blood drawn from a sow during a herd visit was initially cultured and found to be negative for *S. suis*. A 0.1 mL aliquot of inoculum was placed in a tube of whole blood. Previously, five other tubes were filled with 5 mL of blood from the same sow and designated as experimental tubes 1-5. An 18-gauge 1.5 inch needle on a 12 mL syringe was placed into the tube containing the inoculum. One mL of blood was drawn, expelled into a waste receptacle and the syringe was flushed three times with sterile saline. The needle was then placed into the first experimental tube and the procedure was repeated until blood had been drawn from all tubes using the same needle and syringe. At no time did the needle enter a

Table 3.— *Streptococcus suis* survivability: Media study

Media (Temp. °C)	1d	2d	3d	4d	5d	6d	7d	8d	9d	10d
Oil (4°)	+	-	-	NT	NT	NT	NT	NT	NT	NT
Aluminum hydroxide (4°)	+	+	+	+	-	-	-	NT	NT	NT
Amies culturette (20°)	+	+	+	+	+	+	+	-	NT	NT
Urine (20°)	+	+	+	+	+	+	+	+	+	+
Semen (20°)	+	+	+	+	+	+	+	+	+	+
Whole blood (20°)	+	+	+	+	+	+	+	+	+	+
Brain (20°)	+	+	+	+	+	+	+	+	+	+

Key: + Growth detected; - No growth detected; NT not tested

new experimental tube before it was flushed three times with saline. At the end of the procedure, the five experimental tubes were cultured for the presence or absence of *S. suis*.

Results

Surface Studies

S. suis was detected longest when inoculated onto rubber or plastic surfaces, particularly in the presence of manure and when stored at room temperature (Table 2). Survival times of up to 48 hours were recorded on the bottom of manure-coated rubber boots, 24 hours on manure-coated plastic flooring, and up to 20 hours on clean plastic flooring. The organism survived less than 4 hours on clean concrete and plywood and less than 2 hours on painted plywood.

The presence of an exogenous heat source (heat lamp) decreased the organism's survivability, no matter what surface was used or whether or not manure was present.

Media Studies

S. suis was viable for up to 10 days in porcine whole blood, brain tissue, urine or semen and up to 7 days in an Amies culturette when held at room temperature (20°C). The organism survived in aluminum hydroxide vaccine up to 4 days at 4°C, but only 24 hours when exposed to an oil-based vaccine at the same temperature (Table 3).

Disinfectant Studies

No bacterial growth was detected in disinfectants except for 70% alcohol.

Temperature Studies

When exposed to water temperatures of greater than 55°C, the organism was killed. If frozen and thawed more than twice, the organism died; however, it was still viable after being kept frozen for 10 days.

Mechanical Vector Studies

S. suis was detected on the tread of the truck tire after 3 miles (5 km) when driven at a speed of 35–40 miles per hour (56–64 km per hour), but not after 8 more miles (13 km) at 60–75 miles per hour (97–120 km per hour).

S. suis was isolated from the first four of five experimental tubes of whole blood when the same needle and syringe was used to draw a sample out of the previous tube, even though the needle was flushed three times between samples with sterile saline.

Discussion

Manure contact prolonged the survivability of *S. suis* on all surfaces examined, probably because the manure protects the organism from drying and heat. *S. suis* survived longer on surfaces if manure was present, so it is important to thoroughly remove manure from all areas and disinfect when cleaning facilities. Although the technique used to test disinfectants was not standardized, it is a good indicator of a disinfectant's effectiveness. It could then serve as a quick, preliminary assessment of the effectiveness of a disinfectant, which could be further tested using an official protocol. Iodine, hypochlorite and quaternary ammonia compounds are not as effective in the presence of manure or urine.

S. suis is inactivated by water or exogenous heat sources at 55°C and higher. Encourage producers to purchase hot water power washers for their farms, not only to enhance the killing of microorganisms, but because power washers reduce the amount of time needed to clean facilities. Because the organism lives for up to 10 days in chilled brain tissue, dead pigs should be promptly removed and properly destroyed. The organism also survived quite well in porcine body fluids, i.e., urine, whole blood and semen. Animals can develop into long-term carriers of the bacterium, shedding the organism over a long period of time.

S. suis appears to be easily transmitted via fomites. Because the organism was capable of surviving on a hog cable snare

for up to 4 hours even when not covered with manure, encourage producers to periodically dip hog snares in an effective disinfectant when blood testing a large number of sows to avoid potential contamination between animals. Veterinarians should also be aware of the possibility that *S. suis* can be transmitted via a needle, particularly when bleeding large numbers of sows with the same syringe. It is recommended that practitioners use an individual syringe and needle for each animal tested. Contaminated needles placed repeatedly in a bottle of aluminum hydroxide-based vaccine could also result in bacterial proliferation in the product. Therefore, it is important to keep a single needle in the stopper of the bottle of vaccine during periods of use to prevent old needles from entering the bottle of sterile liquid. Finally, suspected cases of *S. suis* submitted to diagnostic labs should include cultures taken with Amies culturettes due to the prolonged viability of the organism in this medium (7 days).

Although this is a small study, it raises some interesting points on the survivability of a common swine pathogen. Further studies are necessary to assess how well *S. suis* survives under conditions common to swine units, where high concentrations of dust, temperature fluctuations and large populations of animals are present. The studies should be repeated using different concentrations of the bacterial inoculum, as well.

References

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