Survival of *Streptococcus equi* on surfaces in an outdoor environment

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**Abstract** — Management practices to prevent or control outbreaks of *Streptococcus equi* subsp. *equi* often include consideration of environment survival, but limited objective data are available. This study involved evaluation of *S. equi* persistence following inoculation of wood, metal, and rubber surfaces in an outdoor environment. Survival was short, ranging from < 1 to 3 d. There was no effect of rain (*P* = 0.33) or surface type (*P* = 0.95), but there was an effect of sunlight (*P* = 0.002). Outdoor survival of *S. equi* is poor, and prolonged quarantine of outdoor areas, particularly areas exposed to the sun, is probably unnecessary.

**Résumé** — Survie de *Streptococcus equi* sur les surfaces dans un environnement de plein air. Les pratiques de gestion pour prévenir ou contrôler les éclosions de *Streptococcus equi* ssp. *equi* incluent souvent la considération de la survie dans l’environnement, mais des données objectives limitées sont disponibles. Lors de cette étude, des surfaces en bois, en métal et en caoutchouc ont été inoculées dans un environnement de plein air et la persistance de *S. equi* a été évaluée. La survie a été courte, allant de < 1 à 3 jours. Il n’y avait aucun effet de pluie (*P* = 0.33) ou de type de surface (*P* = 0.95), mais il y avait un effet de lumière du soleil (*P* = 0.002). La survie de *S. equi* en plein air est mauvaise et une quarantaine prolongée des aires extérieures, particulièrement celles exposées au soleil, est probablement inutile.

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*Streptococcus equi* subsp. *equi* is an important equine pathogen and the cause of "strangles." This gram-positive facultative anaerobe is highly contagious and endemic in the horse population in most regions. Both sporadic cases and outbreaks can occur, and management of *S. equi* outbreaks can be difficult. While clinically infected and recovering animals are typically regarded as the main source of infection (1,2), a variety of areas are often addressed in the management of infected horses. One aspect that is frequently discussed is the environmental persistence of *S. equi*, although the role of the environment in transmission of strangles has not been described. While laboratory studies have indicated prolonged survival on surfaces and recommendations for prolonged quarantine of contaminated surfaces (3,4), some authors have hypothesized that environmental survival is poor (2). The authors are aware of only 2 reports evaluating survival of *S. equi*, neither of which was published in a peer-reviewed journal. The first study reported survival of *S. equi* for 63 d on wood at 2°C and 48 d on glass or wood at 20°C (3). The other study reported survival of up to 72 d on some surfaces (4). One concern with both of these studies is that the study conditions did not reflect normal environmental conditions on farms. Potentially relevant factors such as temperature changes, temperature extremes, sunlight, competing microflora, and surfaces commonly present on equine farms were not properly evaluated, and a need for field-based evaluation of survival of *S. equi* in the environment has been declared (1). The objective of this study was to evaluate survival of *S. equi* following experimental inoculation in conditions more representative of the equine farm environment.

**Materials and methods**

**Inoculum preparation**

The base inoculum was prepared by inoculation of *S. equi* subsp. *equi* into 100 mL of tryptone soy broth (TSB; BD Biosciences, Mississauga, Ontario). After 24 h of incubation at 35°C, 2 different types of surface inoculum were prepared. One was prepared by mixing equal volumes of 24-h culture and phosphate buffered saline (PBS, pH 7.4). The other was prepared by mixing equal volumes of 24-h culture and upper respiratory tract secretions collected from horses that had died or were euthanized from nonrespiratory tract disease and had not been treated with antimicrobials. The concentration of *S. equi*...
in the base inoculum was determined by serial dilution of the base inoculum in PBS, and inoculation of 100 μL of each dilution onto blood agar.

**Study site**
The study was performed at a site in an enclosed, gravel-based courtyard that was exposed to direct sunlight for most of the day. A steel frame structure was assembled and covered in bird netting to prevent contact with wildlife. Meteorological data (high temperature, low temperature, sunny/partly cloudy/cloudy, rain yes/no) were recorded daily. Barrier gown and gloves were worn whenever the study site was entered. The University of Guelph Biosafety Committee approved this study.

**Inoculation**
The following surfaces were evaluated: unpainted wood (2" × 4" spruce), painted wood (2" × 4" spruce with 2 coats of white latex fence paint), rubber feed bin, and metal feed bin. Wood surfaces were divided into 15-cm sections, while the circular bases of metal and rubber feed bins were divided into 8 sections. Feed bins were left upside down to prevent water accumulation. Wood pieces were raised off the ground on saw-horses.

For each replicate, 1 mL of S. equi suspension was inoculated onto 8 different sites on each study material. The inoculum was then spread over a 15-cm × 5-cm area using a sterile cotton-tipped swab. Both saline- and mucus-based S. equi suspensions were inoculated at the same time. Eight replicates were performed.

**Sampling**
A cotton-tipped swab was moistened with PBS and wiped thoroughly over the area to be sampled. Swabs were placed in liquid Stuart’s medium and transported directly to the laboratory. Sampling was performed immediately after inoculation (day 0), and on days 1, 3, 5, 7, 10, 14, 21, 28, 35, and 42, or until 2 negative samples were obtained from a specific surface type.

**Culture**
Swabs were inoculated onto 2 Columbia blood agar (Oxoid, Nepean, Ontario) plates: 1 for aerobic culture at 35°C and the other for anaerobic culture in an anaerobic chamber at 37°C. The swab was then placed in 2 mL of Todd Hewitt broth (Oxoid) and incubated aerobically at 35°C for 24 h, followed by inoculation onto blood agar for anaerobic incubation. All culture plates were evaluated after 24 and 48 h of incubation. Colonies with the typical appearance of S. equi and beta-hemolysis were subcultured onto Columbia blood agar. S. equi was confirmed by Gram stain, catalase production, and an inability to ferment sorbitol. No attempt was made to enumerate or identify other bacteria.

**Analysis**
Samples were considered positive if S. equi was isolated using any method. Duration of survival was defined as the time from inoculation to the last sample from which S. equi was isolated.

**Results**
Eight replicates were performed between July 5 and Sept 21, 2007. The mean concentration of the S. equi inoculum was 2.1 × 10^8 colony forming units (CFU)/mL [standard deviation (s): 1.5 × 10^8, range: 1.3 × 10^7 to 4.8 × 10^8]. Survival was short, overall, ranging from < 1 to 3 d (Table 1).

There was no effect of rain (P = 0.33) or surface type (P = 0.95) on persistence. There was, however, an effect of sunlight, with survival of 24 h or less on 24/24 samples tested during periods where it was sunny throughout, versus 9/16 (56%) samples tested during partly cloudy and 20/24 (83%) samples tested during cloudy periods (P = 0.002). There was no effect of daily high or low temperature (P = 0.60 and 0.64, respectively).

There was no difference in persistence of S. equi in saline versus mucus inocula (P = 0.32). However, when the analysis was repeated categorizing outcome as < 1 d and ≥ 1 d, there was a significant difference (P = 0.024) with S. equi persisting longer in mucus.

**Discussion**
The short duration of survival of S. equi is in stark contrast with previous laboratory-based studies but is not surprising because it was reasonable to assume that outdoor environmental factors would have an adverse effect on S. equi survival. This is demonstrated by the significant effect of sunlight on S. equi persistence. This is not a surprising finding because of the susceptibility of bacteria to ultraviolet light and dessication; however, it supports the notion that laboratory-based studies are not adequate for evaluation of outdoor environmental persistence. The presence of environmental organisms may also have played a role in poor

<table>
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<th>2</th>
<th>3</th>
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<td>Saline</td>
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<td>1 (6.3%)</td>
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<tr>
<td>Metal</td>
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<tr>
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* Number of samples from which S. equi was grown.
persistence compared with laboratory studies since it has been suggested that *S. equi* survival decreases in the presence of soil bacteria, likely due to the presence of bacteriocins to which *S. equi* is susceptible (1).

There was no significant difference in survival times among different surface types, although it is possible that this is a result of inadequate statistical power rather than a true biological difference. Further study using different and greater numbers of surfaces would be useful. An aspect that was not evaluated is the ability to clean and disinfect surfaces. Unsealed wood surfaces are difficult or impossible to thoroughly clean and disinfect (5), and it is possible that painted surfaces, while having no effect on environmental survival, could be beneficial if cleaning and disinfection were evaluated concurrently. Similarly, the rougher surface of the rubber food bin and the increased likelihood of surface defects developing over time may make rubber bins more amenable to *S. equi* survival in the presence of disinfection compared with smooth metal surfaces. Regardless, the short survival in the outdoor environment may decrease the practical relevance of those concerns.

In this study, inoculation of *S. equi* in mucus resulted in significantly longer survival when < 24 h was used as the cutoff, but not when ≤ 24 h was the cutoff. The reason for this discrepancy is unclear, but it is reasonable to believe that mucus would provide a more hospitable environment for *S. equi* by providing both a nutritional source and physical protection from adverse environmental effects. While conflicting results were present, it is reasonable to recommend that mucus be used as an inoculum base for future persistence studies.

The use of nonsterile surfaces was more biologically appropriate, but did have some effects on study protocols. Because competing organisms were present, and in some situations were present as heavy growth, it is possible that *S. equi* was overlooked in some samples. However the use of aerobic and anaerobic culture environments, both of which are appropriate for *S. equi*, and enrichment culture, decreased the chance that viable *S. equi* was not detected.

One must take care in extrapolating the results of any experimental study to a clinical situation. While this study indicates that outdoor survival of *S. equi* is of much shorter duration than previously reported, it must be remembered that these results may only apply to the environmental conditions of this study. Survival may be different with different environmental conditions, such as lower temperatures and less sunlight. Additionally, these results should not be extrapolated to areas free of sunlight, such as in a barn, in shady outdoor areas, or within soil or grass. Further study of those environments is required. Further, survival on other surfaces such as in water or admixed with feed might be different. Regardless, it is clear that some previous assumptions concerning the persistence of *S. equi*, at least in some environments, are excessive.

Even with these results, it is difficult to make specific guidelines for management of pastures and paddocks potentially contaminated with *S. equi*. However, it is reasonable to assume that, at least in summer and in sunny conditions, prolonged (weeks) quarantine should not be required to significantly reduce or eliminate environmental contamination of solid contact surfaces.

**Acknowledgments**

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**References**