

# Rapid application of long-acting ceftiofur can prevent death losses associated with *Streptococcus equi* subspecies *zooepidemicus* in pigs

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## Summary

**Objective:** Introduction of *Streptococcus equi* subspecies *zooepidemicus* strains into naive populations results in field mortality rates of 30% to 50% over 5 to 10 days. Because of the rapid disease progression, our goal was to determine whether antibiotic intervention could control *S zooepidemicus* disease in a group of animals following development of clinical signs.

**Materials and methods:** Thirty-two pigs were challenged with *S equi* subsp *zooepidemicus*. Following the development of clinical signs, 16 were treated with long-acting, injectable ceftiofur. Seven unchallenged pigs served as controls.

Clinical signs were monitored following challenge and survival was compared between groups. Antibody titers were measured on day 0 and day 30 post challenge. On day 30 post challenge, 3 contact pigs were commingled with 2 treated animals to evaluate *S equi* subsp *zooepidemicus* transmission.

**Results:** Ceftiofur treatment eliminated clinical signs in 15 of 16 animals. However, multiple treatments were required to control disease in treated animals (2-3 doses providing 12-18 days of coverage). Antibody titers to *S equi* subsp *zooepidemicus* increased in challenged animals treated with ceftiofur, indicating sufficient exposure for immune stimulation.

No contact pigs developed clinical signs of *S equi* subsp *zooepidemicus* following exposure.

**Implication:** Rapid application of injectable antibiotics is a viable method to reduce losses due to the introduction of *S equi* subsp *zooepidemicus* into a naive group of pigs and may help prevent transmission to contact animals following recovery.

**Keywords:** swine, *Streptococcus equi* subspecies *zooepidemicus*, septicemia, antibiotic

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## Resumen - La aplicación rápida de ceftiofur de acción prolongada puede prevenir pérdidas por muertes asociadas con *Streptococcus equi* subespecie *zooepidemicus* en cerdos

**Objetivo:** La introducción de cepas de *Streptococcus equi* subespecie *zooepidemicus* en poblaciones libres da como resultado tasas de mortalidad en el campo del 30% al 50% durante 5 a 10 días. Debido a la rápida evolución de la enfermedad, nuestro objetivo fue determinar si la intervención con antibióticos podría controlar la enfermedad por *S zooepidemicus* en un grupo de animales después del desarrollo de signos clínicos.

**Materiales y métodos:** Treinta y dos cerdos fueron desafiados con *S equi* subsp *zooepidemicus*. Tras el desarrollo de

signos clínicos, 16 fueron tratados con ceftiofur inyectable de acción prolongada. Siete cerdos no desafiados sirvieron como controles. Los signos clínicos se monitorearon después de la exposición y se comparó la supervivencia entre los grupos. Los títulos de anticuerpos se midieron el día 0 y el día 30 después de la exposición. El día 30 después de la exposición, se mezclaron 3 cerdos contacto con 2 animales tratados para evaluar la transmisión de *S equi* subsp *zooepidemicus*.

**Resultados:** El tratamiento con ceftiofur eliminó los signos clínicos en 15 de 16 animales. Sin embargo, se requirieron múltiples tratamientos para controlar la enfermedad en los animales tratados (2-3 dosis que proporcionaron 12-18 días de cobertura). Los títulos de

anticuerpos contra *S equi* subsp *zooepidemicus* aumentaron en animales desafiados tratados con ceftiofur, lo que indica una exposición suficiente para la estimulación inmunológica. Los cerdos sin contacto desarrollaron signos clínicos de *S equi* subsp *zooepidemicus* después de la exposición.

**Implicación:** La aplicación rápida de antibióticos inyectables es un método viable para reducir las pérdidas debidas a la introducción de *S equi* subsp *zooepidemicus* en un grupo de cerdos sin tratamiento previo y puede ayudar a prevenir la transmisión a animales contacto después de la recuperación.

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## Résumé - L'administration rapide de ceftiofur à action prolongée peut prévenir les mortalités associées à *Streptococcus equi* ssp *zooepidemicus* chez les porcs

**Objectif:** L'introduction de souches de *Streptococcus equi* ssp *zooepidemicus* dans des populations naïves entraîne des taux de mortalité sur le terrain de 30% à 50% sur 5 à 10 jours. En raison de la progression rapide de la maladie, notre objectif était de déterminer si l'administration d'antibiotique pouvait limiter la maladie à *S equi* ssp *zooepidemicus* dans un groupe d'animaux après le développement de signes cliniques.

**Matériels et méthodes:** Trente-deux porcs ont été infectés avec *S equi* ssp *zooepidemicus*. À la suite de l'apparition

de signes cliniques, 16 ont été traités avec du ceftiofur injectable à action prolongée. Sept porcs non infectés ont servi de témoins. Les signes cliniques ont été surveillés après provocation et la survie a été comparée entre les groupes. Les titres d'anticorps ont été mesurés au jour 0 et au jour 30 après la provocation. Au jour 30 après l'infection, trois porcs contact ont été mélangés avec deux animaux traités pour évaluer la transmission de *S equi* ssp *zooepidemicus*.

**Résultats:** Le traitement au ceftiofur a éliminé les signes cliniques chez 15 des 16 animaux. Cependant, plusieurs traitements ont été nécessaires pour maîtriser la maladie chez les animaux traités (2 à 3 doses fournissant 12 à 18 jours de

couverture). Les titres d'anticorps contre *S equi* ssp *zooepidemicus* ont augmenté chez les animaux provoqués traités avec du ceftiofur, indiquant une exposition suffisante pour une stimulation immunitaire. Aucun porc contact n'a développé de signes cliniques d'infection à *S equi* ssp *zooepidemicus* à la suite de l'exposition.

**Implication:** L'administration rapide d'antibiotiques injectables est une méthode viable pour réduire les pertes dues à l'introduction de *S equi* ssp *zooepidemicus* dans un groupe de porcs naïfs et peut aider à prévenir la transmission aux animaux contact après la guérison.

*Streptococcus equi* subspecies *zooepidemicus* is a zoonotic pathogen that causes infections in a variety of mammalian species, including pigs.<sup>1-4</sup> Historically, *S equi* subsp *zooepidemicus* infection has been sporadic in swine in the United States; however, in 2019, multiple introductions of a novel *S equi* subsp *zooepidemicus* strain occurred in the United States and resulted in mortality reaching 30% to 50% in affected groups of animals.<sup>3,4</sup> Diseased animals had clinical signs including fever, severe lethargy, and reluctance to rise.<sup>3,4</sup> Similar clinical signs were observed during experimental replication of disease, which progressed rapidly leading to 100% mortality 72 hours post infection.<sup>5</sup>

Currently, no *in vivo* studies have assessed the use of antimicrobials as an intervention strategy for *S equi* subsp *zooepidemicus* infection in pigs. Though *S equi* subsp *zooepidemicus* isolates, including the 2019 strains,<sup>3,4</sup> are largely susceptible to  $\beta$ -lactam antibiotics,<sup>6,7</sup> the rapid progression of disease could prevent antibiotic treatment from controlling losses associated with *S equi* subsp *zooepidemicus* introduction into a swine herd.

In this study, we investigated the use of long-acting ceftiofur crystalline free acid as an intervention strategy to control *S equi* subsp *zooepidemicus* infection in pigs. Our objective was to determine if rapid application of ceftiofur after the development of clinical signs could prevent losses associated with *S equi* subsp *zooepidemicus* infection.

## Animal care and use

This animal study was approved by the US Department of Agriculture (USDA) Agricultural Research Service National Animal Disease Center Institutional Animal Care and Use Committee.

## Materials and methods

### Animal study

Thirty-nine, 10-week-old pigs were divided into three groups. Group 1 animals were challenged with *S equi* subsp *zooepidemicus* (n = 16). Group 2 animals were challenged with *S equi* subsp *zooepidemicus* and treated with a weight-calculated dose of ceftiofur crystalline free acid (Excede; Zoetis) given intramuscularly (n = 16). Group 3 animals were not challenged (n = 7). Animals in groups 1 and 2 were inoculated intranasally and orally with 3 mL of  $2 \times 10^8$  colony forming units (CFU)/mL (1 mL per nostril and 1 mL orally). Animals in group 2 were treated with ceftiofur after the development of clinical signs on day 1 post challenge and again on day 5 post challenge when fevers started recurring. A third treatment was given only to animals developing a fever following retreatment. Body temperature was monitored twice daily following challenge by temperature chip (Destron Fearing). Following the challenge, animals were monitored every 4 hours excluding an 8-hour overnight period for the development of clinical signs including depression, reluctance to rise, and neurologic signs. Pigs were euthanized and necropsied if clinical signs became severe. At necropsy, the following samples were collected for culture: nasal swab, tonsil swab, serosal

swab, liver swab, splenic swab, joint fluid, cerebrospinal fluid (CSF), bronchoalveolar lavage fluid, and serum. Nasal and tonsil swabs were collected at 7- and 30-days post challenge in surviving animals to assess colonization.

On day 30 post challenge, 2 animals (692 and 697) that had been treated only twice with ceftiofur were commingled with 3 negative control animals from group 3 in a clean animal room to evaluate transmission from recovered animals to naive contacts following treatment. To allow time for development of serologic response, animals were monitored for clinical signs as previously described until day 63 when they were euthanized. At necropsy, samples were collected as previously indicated to screen for *S equi* subsp *zooepidemicus*.

### Bacterial isolate and culture conditions

*Streptococcus equi* subsp *zooepidemicus* 19-031482-K1916623-LUNG1 (SRR10584760, <https://www.ncbi.nlm.nih.gov/sra/SRR10584760>) was isolated from a high mortality event in Tennessee in 2019.<sup>5,8</sup> *Streptococcus equi* subsp *zooepidemicus* inoculum was grown on trypticase soy agar with 5% sheep blood (Becton Dickinson) at 37°C with 5% CO<sub>2</sub>. Overnight *S equi* subsp *zooepidemicus* cultures were harvested in phosphate-buffered saline to an OD<sub>600</sub> = 0.42, which results in 10<sup>8</sup> to 10<sup>9</sup> CFU/mL. The final concentration of *S equi* subsp *zooepidemicus* in the inoculum was quantified by plating serial dilutions. Animal samples were plated on trypticase soy agar with 5% sheep blood and incubated overnight at 37°C with 5% CO<sub>2</sub> to assess

for *S equi* subsp *zooepidemicus*. Suspect colonies were confirmed to be *S equi* subsp *zooepidemicus* by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) at the National Veterinary Services Laboratories in Ames, Iowa.

### Serum antibody assessment

Serum was collected at the time of challenge and on day 30 post challenge from groups 1, 2, and 3. Serum was collected from contact pigs on day 0 (challenge), day 30 (commingling), and day 63 (approximately 4 weeks post commingling). All serum was stored at -80°C until enzyme-linked immunosorbent assays (ELISA) were performed.

For the ELISA, 96-well plates were coated with a 1:10 dilution of heat killed *S equi* subsp *zooepidemicus* ( $OD_{600} = 0.6$ ) in carbonate-bicarbonate buffer. Titer was determined by serially diluting swine serum. Antibody was detected using horseradish peroxidase conjugated

secondary antibody specific to the swine immunoglobulin heavy chain (1:20,000 dilution; SeraCare Life Sciences Inc) and tetramethylbenzidine substrate (Life Technologies). Optical density was measured at 450 nm with correction at 655 nm. Data were modeled with the nonlinear function of the  $\log_{10}$  dilution and the log (agonist)-versus-response variable slope four-parameter logistic model in GraphPad Prism (GraphPad Software) with endpoint titer interpolated using two times the average reading for gnotobiotic swine serum.

### Statistical analysis

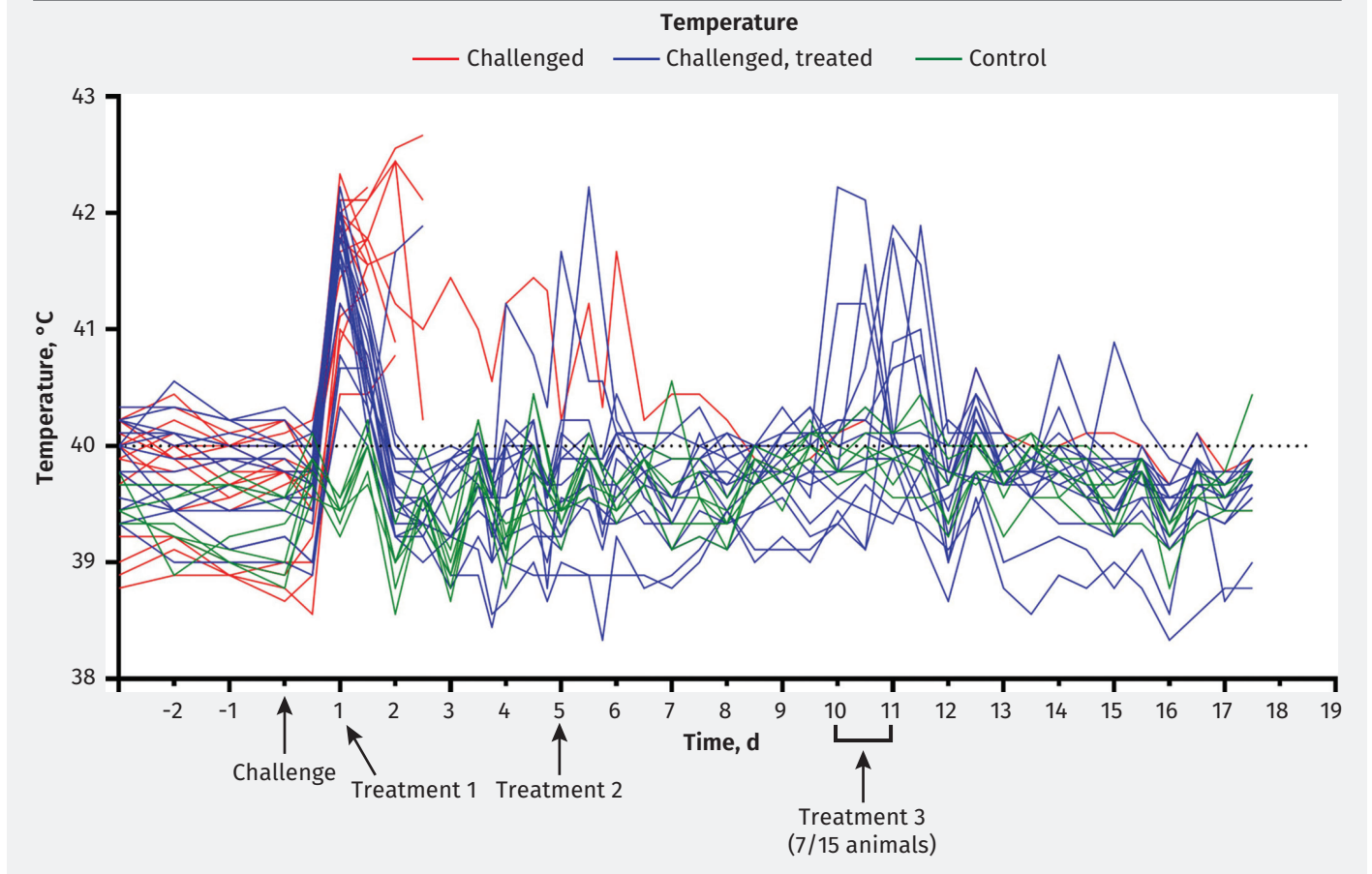
Statistical analysis was completed in GraphPad Prism 8. Survival analysis was performed by the Kaplan and Meier product limit method comparing survival curves using the log-rank test. Antibody titers were converted to  $\log_{10}$  values and compared using a two-way analysis of variance. A  $P$  value  $\leq .05$  was considered statistically significant.

## Results

### Clinical progression and survival

Challenge of pigs resulted in disease development by 24 hours post challenge (anorexia, fever, and depression) in group 1 and 2 animals. No clinical signs developed in nonchallenged animals (group 3). Following identification of clinical disease, group 2 animals were treated with a weight-calculated dose of ceftiofur. Most pigs (15 of 16) returned to normal behavior and normal body temperature by 24 hours post treatment (Figure 1). One animal (695) in group 2 developed neurologic signs (unable to rise, ataxic) following treatment and was euthanized 72 hours post challenge (Figure 2). *Streptococcus equi* subsp *zooepidemicus* was isolated from the CSF sample but absent from other collected samples. By day 5 post challenge (4 days post treatment), pigs were redeveloping clinical signs. They were

**Figure 1:** Pig temperature following challenge with *Streptococcus equi* subsp *zooepidemicus*. Body temperature for each animal is plotted individually. Fever is indicated by the dashed line (40°C). All challenged animals developed a fever (> 40°C) following challenge. The challenged, treated pigs received treatment at 24 hours post challenge, which caused their temperatures to drop below 40°C. Temperatures rose again around 5 and 10 days post challenge and a second treatment of all pigs in the group was administered on day 5 post challenge, which brought temperatures back below 40°C. A third treatment was given only to pigs developing clinical signs or a fever (7 of 15 animals).



treated a second time and temperatures and behavior returned to normal. A third treatment was required in 7 of 15 surviving animals in the treatment group as they became febrile at 10- to 11-days post challenge (Figure 1). Following the third treatment, animals did not develop further signs of disease, though temperature fluctuations were common (Figure 1).

In contrast to group 2 animals, group 1 animals rapidly deteriorated without antibiotic intervention and 15 of 16 pigs were euthanized by 72 hours post challenge (Figure 2). *Streptococcus equi* subsp *zooepidemicus* was isolated from multiple systemic sites in all nontreated animals euthanized during the acute phase of infection.

Nasal and tonsil swabs were taken on day 7 and day 30 post challenge to evaluate colonization. On day 7, no *S equi* subsp *zooepidemicus* was isolated from any of the surviving animals in group 2 (n = 15). *Streptococcus equi* subsp *zooepidemicus* was isolated from nasal swabs

from the surviving nontreated animal (group 1, n = 1). By day 30 post challenge, *S equi* subsp *zooepidemicus* was not isolated from any of the surviving animals in group 1 (n = 1) or group 2 (n = 15).

### Commingling with naive contact animals

On day 30 post challenge, 3 naive pigs from the nonchallenged controls (group 3) were commingled with 2 treated, challenged animals (group 2) that had only received 2 doses of ceftiofur. None of the commingled pigs showed behavior changes following commingling consistent with *S equi* subsp *zooepidemicus* disease; however, they developed a transient fever (> 40°C; Figure 3), which lasted for 36 to 60 hours. By day 11 post commingling, all temperatures returned to normal and remained normal until the end of the study. At necropsy, nasal and tonsil swabs of contact animals were culture negative.

### Assessment of serum antibody titers

All groups had similar titers to *S equi* subsp *zooepidemicus* prior to challenge. At 30 days post challenge, a statistical increase in titer was seen in group 2 (15 of 16) and the surviving nontreated animal in group 1 ( $P < .001$ ; Figure 4). The titer of treated animals was comparable to that of the surviving nontreated animal on day 30 ( $P = .47$ ). No increase in titer was observed in the contact pigs following commingling ( $P = .07$ ).

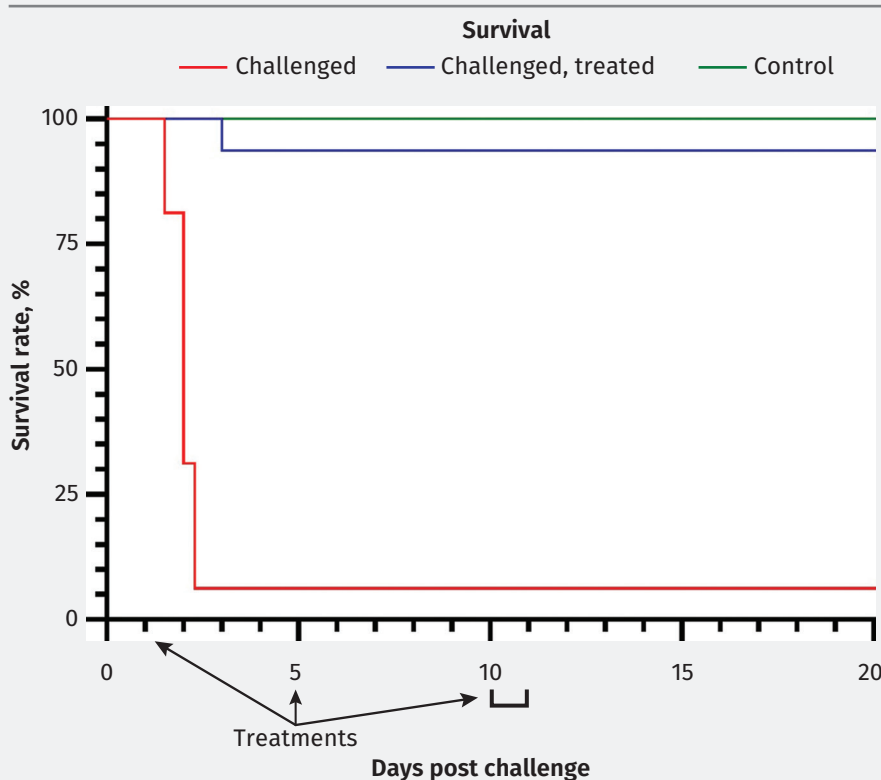
### Discussion

In 2019, introduction of *S equi* subsp *zooepidemicus* into groups of naive swine in North America resulted in severe systemic disease and mortality reaching 30% to 50%.<sup>3,4</sup> In this study, we evaluated antibiotic intervention to prevent severe death losses associated with *S equi* subsp *zooepidemicus* infection. We used injectable, long-acting ceftiofur, as the North American 2019 *S equi* subsp *zooepidemicus* isolates were found to be susceptible to  $\beta$ -lactam antibiotics.<sup>3,4</sup>

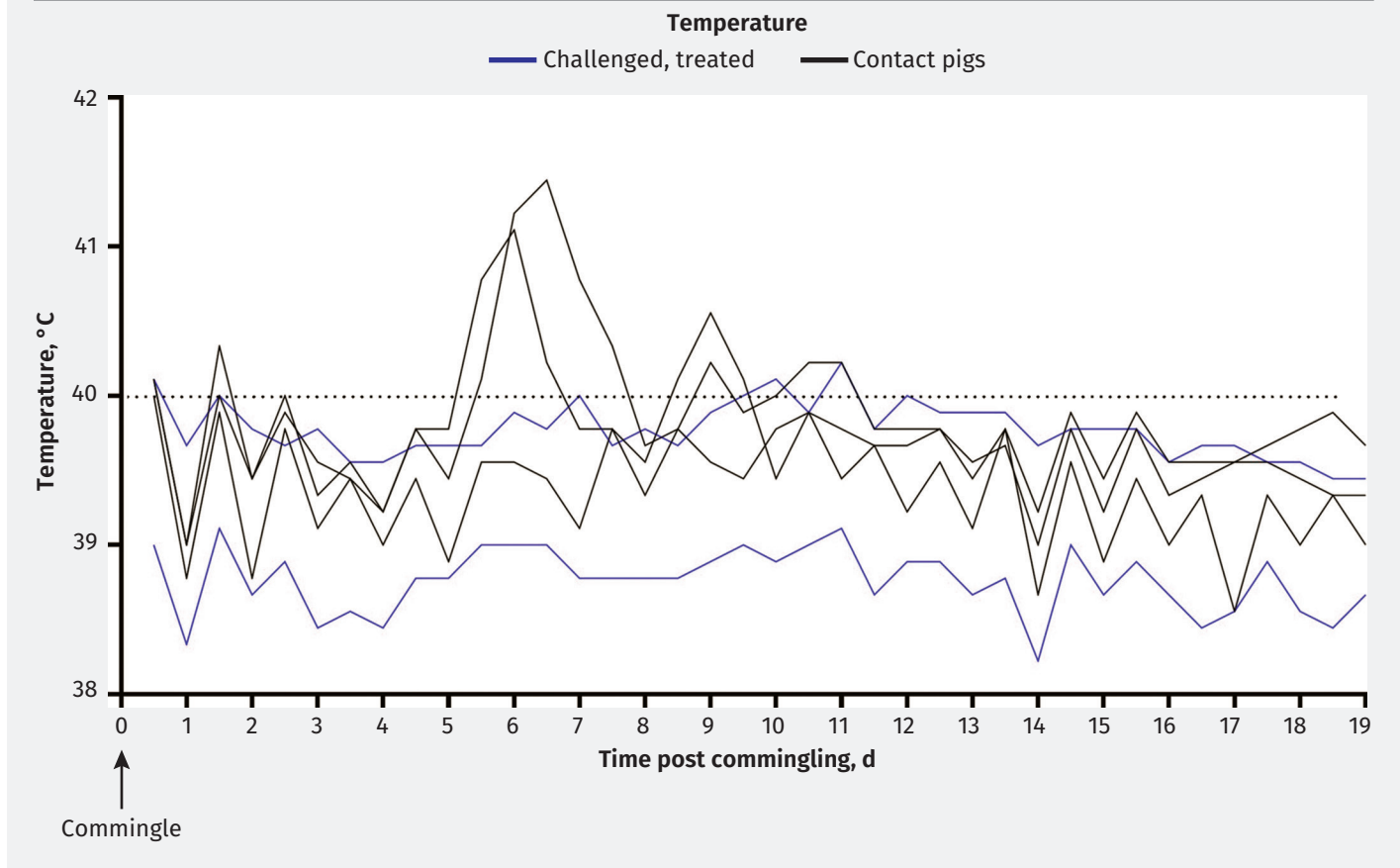
In nontreated animals, challenge with *S equi* subsp *zooepidemicus* produced clinical signs and mortality consistent with previous research.<sup>5</sup> All challenged animals rapidly developed clinical disease and 15 of 16 nontreated animals were euthanized within 72 hours post challenge. However, treatment with ceftiofur following identification of clinical disease improved survival (15 of 16 animals survived) and clinical signs completely resolved for 3 days. Though retreatment was necessary, animals did stabilize by 15 days post challenge and no animal required more than 3 ceftiofur treatments (12-18 days of therapeutic plasma levels based on product information). Additionally, when evaluating antibody titers, treated pigs developed titers similar to the surviving nontreated pig by 30 days post challenge. This indicates that immune stimulation was sufficient to develop an adaptive immune response even with antibiotic treatment, and the antibiotic treatment was able to prolong survival until the immune response could develop.

One animal in the treated group developed neurologic signs and was euthanized following the first antibiotic treatment. *Streptococcus equi* subsp *zooepidemicus* was isolated from the animal's CSF, but not from any of the other collected samples. This may be because *S equi* subsp *zooepidemicus* had already

**Figure 2:** Survival following a challenge with *Streptococcus equi* subspecies *zooepidemicus*. Most animals in the challenged group were euthanized by 72 hours post challenge (15 of 16). In contrast, most animals treated with ceftiofur recovered and only 1 animal was euthanized due to worsening clinical signs.



**Figure 3:** Body temperature following commingling of 2 challenged, treated pigs with 3 contact pigs. Body temperature for each animal is plotted individually. Fever is indicated by the dashed line (40°C). Transient fevers lasting 36-60 hours were noted in all 3 contact animals following commingling. All temperatures returned to normal by day 11 post commingling and remained normal for the rest of the study period.



crossed the blood-brain barrier by the time of treatment, and the treatment was able to clear *S equi* subsp *zooepidemicus* from the systemic sites but not within the central nervous system. This indicates the importance of early detection of disease and rapid initiation of treatment to prevent *S equi* subsp *zooepidemicus* losses.

To assess whether treated pigs could serve as a reservoir for infection in naive pigs introduced following stabilization of *S equi* subsp *zooepidemicus*, we commingled 3 of the nonchallenged controls with 2 pigs that recovered following 2 treatments with ceftiofur. No contact animals developed anorexia or depression; however, all 3 developed a transient fever between day 1 and 11 post commingling that lasted 36 to 60 hours. Peak temperatures were not as high in contact pigs as were seen in primary challenged pigs (average 41.04°C versus 41.75°C, respectively), which could be due to a smaller exposure dose. Additionally, titers were evaluated to detect an exposure event. The absence

of a rise in titers indicates any potential *S equi* subsp *zooepidemicus* exposure in the contact pigs did not stimulate an adaptive immune response. In other work, we have observed commingling of untreated pigs that survived an *S equi* subsp *zooepidemicus* challenge with naive contact pigs led to transmission in 2 of 3 contact animals, with one developing severe disease.<sup>9</sup> Overall, the data from this study does not support a large exposure risk from previously infected, treated animals. In our experiment, the barn maintained high hygiene and animals were moved into a clean room for the commingling assessment, which minimized the potential for environmental exposure.

Overall, this study indicated that early treatment of *S equi* subsp *zooepidemicus*-infected animals with injectable ceftiofur can reduce clinical signs, reduce mortality, and provide time for the immune system to respond with an adaptive immune response. While we used ceftiofur, there is no reason to expect that treatment with other  $\beta$ -lactam

antibiotics for the same duration would provide different efficacy. It is important to provide treatment rapidly after the detection of clinical signs. It is essential to provide treatment parenterally when clinical signs are present because acutely ill animals are off feed and do not drink, so antibiotics provided in feed or water would be ineffective.

## Implications

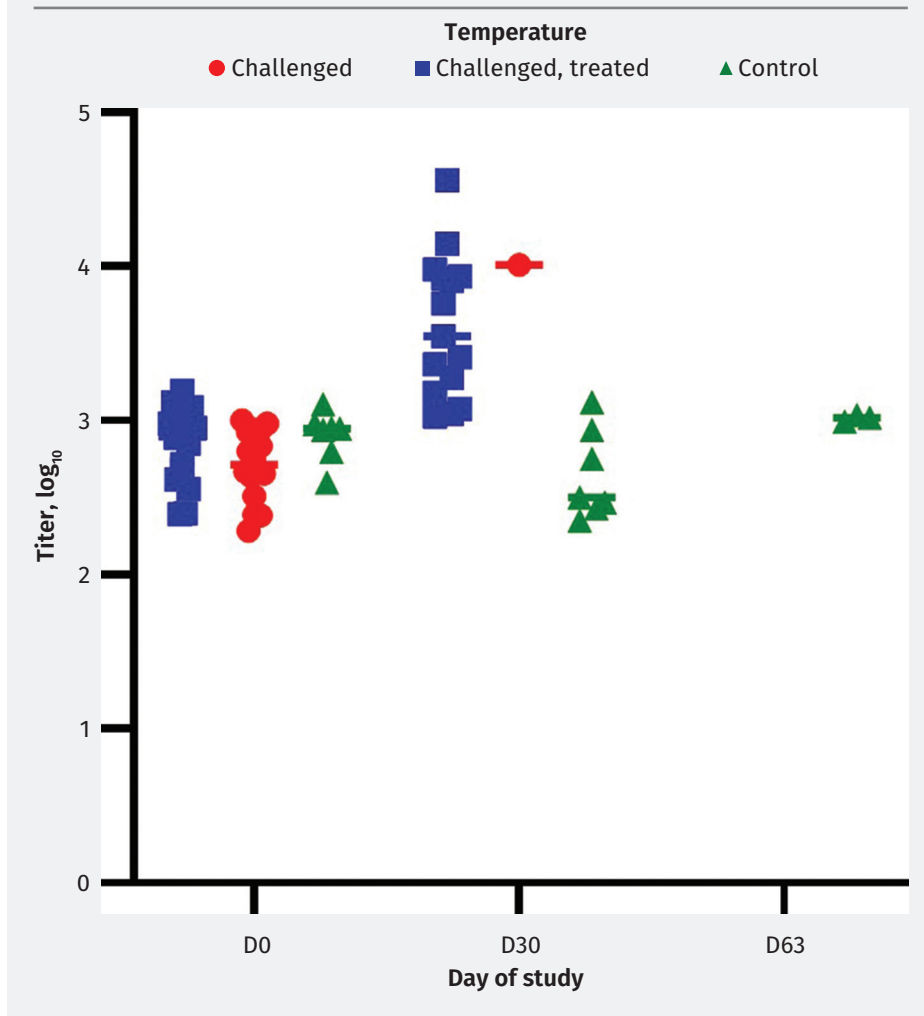
Under the conditions of this study with an *S equi* subsp *zooepidemicus* challenge:

- Long-acting ceftiofur reduced mortality.
- Ceftiofur treatment protected animals without preventing an antibody response.
- Exposure of naive pigs to recovered pigs did not result in disease.

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**Figure 4:** Antibody titers of pigs challenged with *Streptococcus equi* subsp *zooepidemicus*. Antibody titers were assessed on the day of challenge (D0) and the day of commingling (D30). Titters in the control pigs used as contacts were also measured on day 63 (33 days after commingling). An increased titer was noted in surviving challenged, treated animals (n = 15) and the surviving challenged animal ( $P < .001$ ). The titer of commingled challenged, treated pigs on day 63 was comparable to control pigs on day 0 and day 30 ( $P = .74$  and  $P = .07$ , respectively).



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### Conflict of interest

None reported.

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