

# Duration of efficacy of ceftiofur crystalline free acid sterile suspension against clinical disease in grower pigs challenged with *Actinobacillus pleuropneumoniae*

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## Summary

**Objective:** To evaluate the duration of efficacy of a single dose of ceftiofur crystalline free acid sterile suspension (CCFA-SS) against clinical disease in grower pigs inoculated intratracheally with *Actinobacillus pleuropneumoniae*.

**Materials and methods:** One hundred and ninety male piglets were blocked by weight and randomly assigned to 19 pens of 10 animals each, with three pens randomly assigned to each of six treatments. Pigs were challenged with *A pleuropneumoniae* at 7 to 8 weeks old (Day 0). Groups 1 through 5 were treated with a single intramuscular dose of CCFA-SS (5 mg ceftiofur

equivalents per kg body weight) on Days -13, -10, -7, -4, and -1, respectively. Group 6 pigs were challenged, untreated controls. A single pen of 10 unchallenged, untreated pigs acted as sentinels. The primary variable was removal rate (percent of pigs per pen that died or were euthanized because of severe illness by Day 9). Ancillary variables included demeanor and respiratory index scores, rectal temperature, and feed intake.

**Results:** Removal rate for controls was 89.3% by Day 1, and did not differ for groups treated with CCFA-SS on Days -13 and -10. However, 100% of pigs treated on Days -1 and -4 and approximately 77%

of the Day -7 group survived to study termination ( $P < .001$  versus controls). High initial losses within the control group precluded statistical analysis of ancillary variables.

**Implication:** Under the conditions of this study, the duration of efficacy of a single dose of CCFA-SS against *A pleuropneumoniae* is 7 days.

**Keywords:** swine, respiratory disease, pleuropneumonia, ceftiofur, mortality

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## Resumen – Duración de la eficacia de la suspensión estéril de ácido libre de ceftiofur cristalino contra la enfermedad clínica en cerdos de crecimiento probados con el *Actinobacillus pleuropneumoniae*

**Objetivo:** Evaluar la duración de la eficacia de una dosis única de la suspensión estéril de ácido libre de ceftiofur cristalino (CCFA-SS por sus siglas en inglés) contra la enfermedad clínica en cerdos de crecimiento inoculados intratraquealmente con *Actinobacillus pleuropneumoniae*.

**Materiales y métodos:** Ciento noventa lechones machos se organizaron en bloque por peso y al azar se les asignó a uno de 19 corrales de 10 animales cada uno, se asignaron al azar tres corrales para cada uno de los seis tratamientos. Los cerdos fueron retados con *A pleuropneumoniae* entre las

7 y 8 semanas de edad (Día 0). Los grupos del 1 al 5 fueron tratados con una dosis intramuscular única de CCFA-SS (5 mg de ceftiofur equivalentes por kg de peso corporal) los Días -13, -10, -7, -4, y -1, respectivamente. Los cerdos del grupo 6 fueron el control retado pero no tratado. Un solo corral de 10 cerdos no retados, ni tratados actuaron como centinelas. La variable principal fue el porcentaje de eliminación (porcentaje de cerdos por corral que murieron o recibieron eutanasia debido a enfermedad severa hasta el Día 9). Las variables auxiliares incluyeron calificaciones de comportamiento y problemas respiratorios, temperatura rectal, y consumo de alimento.

**Resultados:** El porcentaje de eliminación para los cerdos control fue de 89.3% al Día 1, y no difirió para los grupos tratados con CCFA-SS en los Días -13 y -10. Sin

embargo, 100% de los cerdos tratados en los Días -1 y -4 y aproximadamente el 77% del grupo del Día -7 sobrevivió hasta el final del estudio ( $P < .001$  versus controles). Las altas pérdidas iniciales dentro del grupo control impidieron el análisis estadístico de variables auxiliares.

**Implicación:** Bajo las condiciones de este estudio, la duración de la eficacia de una dosis única de CCFA-SS contra el *A pleuropneumoniae* es 7 días.

## Résumé – Durée de l'efficacité d'une suspension stérile de ceftiofur cristalline "free acid" lors d'un épisode clinique chez des porcs en croissance soumis à une infection défi avec *Actinobacillus pleuropneumoniae*

**Objetivo:** Évaluer la durée de l'efficacité d'une dose unique d'une suspension de ceftiofur cristalline (CCFA-SS) lors d'un épisode clinique de pleuropneumonie chez des porcs en croissance inoculés par voie intra-trachéale avec *Actinobacillus pleuropneumoniae*.

**Matériels et méthodes:** Un total de 190 porcelets mâles ont été regroupés par

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poids et répartis de manière aléatoire dans 19 parcs de 10 animaux chaque, et trois parcs assignés au hasard à chacun de six traitements. Les porcs ont été soumis à une infection défi avec *A pleuropneumoniae* à l'âge de 7 à 8 semaines (Jour 0). Les groupes 1 à 5 ont été traités par administration intramusculaire d'une dose unique de CCFA-SS (équivalent de 5 mg de ceftiofur par kg de poids corporel) aux Jours -13, -10, -7, -4, et -1, respectivement. Les animaux du groupe 6 ont servi de témoins infectés non traités. Dix animaux non infectés et non traités regroupés dans un parc unique ont servi de sentinelles. La variable principale était le taux de retrait (pourcentage de porcs par parc au Jour 9 mort ou euthanasié à cause de maladie sévère). Les variables accessoires incluaient l'apparence, les pointages d'index respiratoire, la température rectale, et la consommation de nourriture.

**Résultats:** Le taux de retrait pour les animaux témoins était de 89.3% au Jour 1, et était similaire à celui des groupes traités avec le CCFA-SS aux Jours -13 et -10. Toutefois, 100% des porcs traités aux Jours -1 et -4 et environ 77% des animaux du groupe traité au Jour -7 ont survécu jusqu'à la complétion de l'étude ( $P < .001$  en comparaison aux témoins). Les pertes initiales élevées dans le groupe témoin ont empêché d'effectuer des analyses statistiques sur les variables accessoires.

**Implication:** Dans les conditions expérimentales de la présente étude, la durée de l'efficacité d'une dose unique de CCFA-SS envers *A pleuropneumoniae* est de 7 jours.

**M**odern swine production is intensive. Very often, large groups of animals are housed in closed environments. This high stocking density favors transmission of airborne respiratory pathogens both within a herd and between herds. Respiratory disorders are regarded as the most serious disease problem in swine production.<sup>1</sup> The economic impact of acute bacterial swine respiratory disease at the herd level consists of mortality, decreased weight gain, decreased meat value (lung lesions), and disease-control expenditures, including medications and extra labor. Pathogens involved include *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, *Bordetella bronchiseptica*, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, and *Streptococcus suis*. Very often, more than one of these species may be isolated in an outbreak. Secondary

bacterial infections are also responsible for much of the illness associated with porcine reproductive and respiratory syndrome (PRRS).<sup>2,3</sup> The PRRS virus (PRRSV) is endemic throughout the world but does not tend to cause severe respiratory disease in the absence of bacterial coinfection.<sup>2,3</sup>

*Actinobacillus pleuropneumoniae* causes porcine pleuropneumonia, which is associated with substantial economic losses throughout the world. Research suggests that this disease causes an average 34% decrease in weight gain and a 26% decrease in feed efficiency in affected herds.<sup>1</sup> Pleuropneumonia may also cause significant mortality or condemnation at slaughter, with losses as high as 10% to 20% for each of these outcomes. Total losses due to pleuropneumonia have been estimated at 2% of the value at slaughter.

*Actinobacillus pleuropneumoniae* can cause peracute disease outbreaks and is an important cause of severe lung lesions associated with Apx toxin production.<sup>4</sup> Thus, *A pleuropneumoniae* is frequently used in bacterial respiratory disease models to investigate the efficacy of treatment regimens. Ceftiofur, a broad-spectrum, third-generation cephalosporin with excellent activity against the bacteria commonly involved in swine respiratory disease, is one of the most potent antibiotics against *A pleuropneumoniae* and is also highly active against *P multocida* and *S suis*.<sup>5-12</sup>

Ceftiofur sodium and ceftiofur hydrochloride require daily injections for several days, which add to the cost of treatment as well as increasing stress to the animal. Dosing compliance is also a challenge. Ceftiofur crystalline free acid sterile suspension (CCFA-SS), a new injectable formulation in a specially treated vegetable-oil vehicle, provides sustained release of ceftiofur and prolonged therapeutic activity from a single dose. The objective of this study was to measure the duration of effectiveness of a single dose of CCFA-SS (Excede for Swine; Pfizer Animal Health, Kalamazoo, Michigan) against clinical disease caused by *A pleuropneumoniae* respiratory infection in swine.

## Materials and methods

### Study animals

One hundred and ninety (190) castrated male Landrace × Yorkshire piglets, 3 to 4 weeks of age, were randomly assigned to 19 pens (10 piglets per pen) on arrival at a research facility in Michigan. Pigs were

obtained from a local specific-pathogen-free commercial source that was free of both *Mycoplasma hyopneumoniae* and PRRSV, and had no recent history of *A pleuropneumoniae* infection. Serum samples collected from three randomly selected pigs per pen on arrival were submitted to Biovet Inc (St Hyacinthe, Quebec, Canada) for testing by *A pleuropneumoniae* ELISA (APP serotype 5). A sample:positive (S:P) ratio  $\geq 0.50$  was considered positive and an S:P ratio  $\geq 0.40$  and  $< 0.50$  was considered suspect. Sera from all three pigs per pen were negative for antibodies to *A pleuropneumoniae* serotype 5.

Prior to arrival, all pigs had received chlorotetracycline in drinking water for 2 to 3 days postweaning, but no other routine treatments or vaccinations. No antibiotics were administered during the 7-day acclimation period before the study began.

### Housing and management

All pens were located in the same isolated room, which contained 20 segregated, raised pens divided into two equal rows by a central alley. Each pen measured approximately 1.5 m × 2.4 m, which provided 0.36 m<sup>2</sup> of space per pig. Pens were separated by solid partitions to prevent pig-to-pig contact between pens. Temperature, lighting, and negative-pressure ventilation were kept relatively uniform throughout the study by use of automatic environmental controls. Unpelleted feed and municipally sourced drinking water were provided ad libitum. Feed was manufactured on site and contained no antimicrobial additives.

### Study design

Pens were randomly assigned to six groups (Table 1), which included five CCFA-SS treatment groups and a negative (untreated) control group, with 30 animals (three pens) per treatment. An additional pen of 10 animals was retained as sentinels that received neither treatment nor bacterial challenge.

All pigs allocated to CCFA-SS treatment received the label dosage of 5 mg ceftiofur equivalents (CE) per kg body weight (BW) as a single intramuscular (IM) injection in the neck. To maintain blinding, personnel who administered treatments were not involved with animal removals, recording clinical observations, or pathological assessments.

All animals were observed daily prior to challenge. Pigs were excluded at randomization or during the pre-challenge observation period if they met any of the following

**Table 1:** Treatment design for a study including 190 nursery pigs challenged with *Actinobacillus pleuropneumoniae*\* after a single dose of ceftiofur†

Treatment group	Timing of treatment		No. of pens‡	Pigs/group
	Interval to challenge (days)	Study day		
1	13	-13	3	30
2	10	-10	3	30
3	7	-7	3	30
4	4	-4	3	30
5	1	-1	3	30
6§	NA	0	3	30
Sentinel¶	NA	NA	1	10

\* APP; intratracheally administered on Day 0 at 7 to 8 weeks of age.

† Ceftiofur crystalline free acid sterile suspension administered once by intramuscular injection in the neck 1 to 13 days before APP challenge, at a dose of 5 mg ceftiofur equivalents per kg body weight.

‡ All groups housed 10 per pen.

§ Controls: challenged, no treatment given.

¶ No treatment given, not challenged.

NA = not applicable.

three criteria: manifested visible abnormalities that could affect study conduct or outcome (eg, hernia or lameness); had a rectal temperature > 40.1°C on at least one occasion within 2 days before challenge and showed clinical signs of disease; or had received systemically active antibiotic treatment (other than the test drug) within 18 days before challenge.

Immediately before *A pleuropneumoniae* challenge on Day 0, three pigs per pen were randomly selected for collection of blood to determine the concentration of ceftiofur and desfuroylceftiofur-related metabolites in plasma. Each pig (excluding the 10 sentinels) was then inoculated with *A pleuropneumoniae* serotype 5 (Pfizer strain collection #200–18062). Pigs were observed for clinical signs of abnormal demeanor and respiration at 4- to 5-hour intervals for the 24-hour period immediately post challenge and at least twice daily for 9 days thereafter. All observations were scored according to the system shown in Table 2 by trained study personnel who were blinded to treatment.

Rectal body temperatures of all pigs were taken once daily from Day -2 to Day 9 at approximately the same time of day. Feed consumption by pen was measured daily from Day -4 to Day 9.

Pigs were removed and humanely euthanized if they exhibited either a demeanor

score of 3 or a respiratory index score of 3 on two consecutive observations, or a score of 3 for both signs concurrently on any single observation. On euthanasia (either at removal or at the end of the study), all animals were necropsied by a pathologist blinded to treatment. In addition to gross pathology, microbiological confirmation by culture of affected tissue was used to support the diagnosis of *A pleuropneumoniae* pneumonia.

Serum samples for serological testing were obtained from all pigs that survived to

study termination. Samples were submitted to Biovet Inc for testing by *A pleuropneumoniae* ELISA (APP serotype 5).

This study was conducted under close veterinary supervision and was pre-approved by an Institutional Animal Care and Use Committee.

### Preparation of challenge inoculum

The *A pleuropneumoniae* challenge strain was revived from lyophilization by streaking onto brain-heart infusion (BHI) plus 2% Supplement C (Difco; Becton Dickinson Company, Franklin Lakes, New Jersey) agar plates. Supplement C, a desiccated yeast concentrate containing hematin yeast extract, L-glutamine, coenzyme, growth factor, and cocarboxylase, is used to supplement media for cultivating fastidious organisms with exacting growth requirements, and provides for a more robust growth of *A pleuropneumoniae* than BHI with nicotinamide adenine dinucleotide alone. After incubation for 18 to 24 hours at 37°C in an atmosphere of 5% CO<sub>2</sub>, colonies from the area of individual growth were selected with a sterile loop and inoculated into 9 mL BHI broth containing 2% Supplement C. The culture was vortexed, then added to 41 mL of BHI broth (containing 2% Supplement C) and incubated at 37°C (without CO<sub>2</sub>) in a rotary shaker at 200 rpm until it equated to approximately 2 × 10<sup>9</sup> colony forming units (CFU) per mL (by predetermined optical density). The culture was then diluted 1:250 (to approximately 8 × 10<sup>6</sup> CFU per mL) in BHI broth without Supplement C. Final inoculum concentration was verified by viable plate counts both before and after inoculation of pigs. This

**Table 2:** Scoring system for pigs challenged intratracheally with *Actinobacillus pleuropneumoniae* at 7 to 8 weeks of age, or not challenged

Score	Demeanor	Respiratory index
0	Normal	None (normal)
1	Mild depression: listless; minimally alert and reactive; slight reluctance to rise or move; eating	Slight: increased respiratory rate, abdominal breathing
2	Moderate depression: appears inappetent; rough coat; difficulty in rising or moving	Moderate: elevated respiratory rate, labored abdominal breathing
3	Severe depression: recumbent, unable to rise	Severe: rapid abdominal breathing, open-mouth breathing, or both; nasal discharge

strain of *A pleuropneumoniae* is sensitive to ceftiofur, with a minimum inhibitory concentration value of  $< 0.03 \mu\text{g CE per mL}$ .

### Administration of inoculum

Approximately  $4 \times 10^7$  CFU of *A pleuropneumoniae* in 5 mL of BHI broth was administered by intratracheal inoculation. Delivery was performed using a 10-mL syringe and a rubber catheter (approximately 40 cm long with a single-eye opening at the distal end). Upon manual restraint of each pig and aided by a speculum, the catheter was inserted into the mouth and advanced caudally, following the line of the ventral side of the soft palate, directly into the trachea. The inoculum was injected with sufficient pressure to ensure delivery to the trachea. The catheter was then closed by crimping the rubber tubing and the syringe was removed from the tube. Using the same syringe, air was expressed into the catheter to ensure full delivery of the inoculum deep within the lung. The catheter was then removed and the pig released.

### Assay for ceftiofur and desfuoyl-ceftiofur-related metabolites in plasma

Approximately 10 mL of blood from each selected pig was collected in heparin-treated tubes, then immediately placed on ice until centrifuged. The plasma was divided into two vials of approximately equal quantities and stored frozen at or below  $-20^\circ\text{C}$  pending liquid chromatographic assay.<sup>13</sup>

### Data analysis

This study was a one-way randomized controlled clinical trial, with pigs nested within pens. Therefore, the experimental unit was pen, with three replicates per treatment. The primary outcome of interest was mortality. Ancillary variables included clinical demeanor, respiratory index, rectal temperature, and feed intake. Mortality was analyzed as the proportion of pigs within each treatment-pen combination that died or required euthanasia due to *A pleuropneumoniae* disease. This proportion was transformed using a Freeman-Tukey arcsine transformation, then analyzed by weighted ANOVA. The weights were  $n + 0.5$ , in which  $n$  equals the number of pigs in each treatment-pen combination. ANOVA was performed using the MIXED procedure in SAS Version 6, release 6.12 (SAS Institute, Cary, North Carolina), with a fixed treatment effect and a random residual error. Statistical significance was set at  $\alpha = 0.05$ .

## Results

Due to several pigs meeting the study exclusion criteria, Groups 1, 2, 3, 5, and 6 started the *A pleuropneumoniae* challenge and subsequent observation period with 29 pigs instead of 30. One pig was later removed from Group 4 during the 9-day postchallenge period because of an injury. Therefore, all mortality analyses were based on 29 pigs per group.

The *A pleuropneumoniae* challenge caused a peracute, severe pleuropneumonia (including substantial respiratory distress and clinical depression) within approximately 4 hours of challenge. As a result, 89.3% of the untreated control pigs (Group 6) died or were euthanized within 24 hours of challenge (Table 3). Mortality rates were lower for pigs that had been treated with CCFA-SS on Days -1, -4, and -7 (Table 3;  $P < .001$ ), with no pigs lost among the 58 pigs treated on Days -1 and -4. Mortality rates for pigs treated on Days -10 and -13 were similar to that of controls. One of the 10 sentinel pigs also died. Gross pathology and microbiological culture of the lungs confirmed that all deaths were caused by *A pleuropneumoniae* infection.

The substantial mortality within the first 24 hours of challenge among the control and Day -10 and Day -13 treatment groups limited the available data on ancillary variables, precluding statistical analysis. However, by 24 hours after challenge, prevalence of abnormal respiration or clinical demeanor (ie, scores  $> 0$ ) was numerically lower in the surviving pigs that had received CCFA-SS on Days -1, -4, and -7 than in the Day -10 and -13 groups. The percent of controls with abnormal respiration or demeanor was similar to that seen in the Day -1, -4 and -7 groups, but this was based on data from only four surviving control pigs. For the period Day -4 to Day 9, average feed consumption was numerically lower as the time between treatment and challenge increased, ranging from 0.99 kg per pig per day in the Day -1 group to less than half that value in the Day -10 and -13 groups.

Eight of 11 surviving pigs from the Day -13, -10, and control groups (approximately 73%) were seropositive for antibodies to *A pleuropneumoniae*. In contrast, 52 of the 80 survivors in the Day -1, -4, and -7 groups (65%) were seronegative, with S:P ratios  $< 0.40$ . Four of the nine surviving sentinels (approximately 44%) had positive or sus-

pect ELISA results. The range of S:P ratios across all animals was 0.04 to 1.47.

The mean concentration of ceftiofur and related metabolites at challenge ranged from  $3.96 \mu\text{g CE per mL}$  in pigs treated with CCFA-SS on Day -1 to below the limit of detection ( $0.04 \mu\text{g CE per mL}$ ) in the Day -13 group (Table 3).

## Discussion

This challenge model caused peracute, severe pleuropneumonia, resulting in nearly 90% mortality or removal of pigs in the control group within 24 hours. The most likely cause of this peracute response was endotoxin release from the *A pleuropneumoniae* in the lung.<sup>4</sup>

The challenge administered to pigs in this study was more severe than would be expected under natural field conditions. Not only was the challenge dose very high and administered directly into the trachea, but all pigs were housed in the same room with pens close to each other, increasing the possibility of lateral exposure. The death of one of the sentinel pigs, as well as the positive or suspect ELISA results for *A pleuropneumoniae* antibodies in four of the nine surviving sentinels, suggests transmission of infection between pens.

The available data for ancillary variables suggest a pattern of less severe clinical signs of disease and greater feed consumption among the Day -1, -4, and -7 treatment groups. These findings are consistent with the lower mortality observed for these three treatment groups, suggesting a milder form of clinical disease compared with other groups.

ELISA results suggest lower seroconversion among the Day -1, -4, and -7 treatment groups than the other groups, consistent with previous reports that effective treatment blunts seroconversion.<sup>9</sup> However, the 9-day observation period may have been insufficient for maximal seroconversion. In addition, seronegative animals may harbor subclinical *A pleuropneumoniae* infection (carrier state), which often goes undetected by serological testing.<sup>14</sup>

Plasma pharmacokinetic results for the Day -1, -4, and -7 treatment groups validate pharmacodynamic-pharmacokinetic analyses<sup>15</sup> which suggested that plasma levels of ceftiofur and desfuoylceftiofur metabolites  $> 0.2 \mu\text{g per mL}$  subsequent to a single dose of CCFA-SS provide efficacy against respiratory disease caused by sensitive

**Table 3:** Impact of treatment with ceftiofur crystalline free acid sterile suspension (CCFA-SS) on mortality or euthanasia\* after challenge with *Actinobacillus pleuropneumoniae* (APP)

Treatment group†	n	Treatment interval (days)	Mean plasma ceftiofur (SD) ‡	No. died or euthanized (%)
1	29	13	< LOD§	25 (86.4)
2	29	10	0.05 (0.04)	27 (96.4)
3	29	7	0.21 (0.12)	7 (23.3) <sup>a</sup>
4	29	4	0.92 (0.17)	0 (0) <sup>a</sup>
5	29	1	3.96 (0.80)	0 (0) <sup>a</sup>
6	29	NA	NA	25 (89.3)
Sentinel¶	10	NA	NA	1 (10)

\* Pigs were observed twice daily after challenge with APP and euthanized if they exhibited either a depression score of 3 (Table 2) or a respiratory index score of 3 (Table 2) on two consecutive observations, or a score of 3 for both signs concurrently on any single observation.

† Treatment groups are described in Table 1.

‡ Concentrations of ceftiofur and related metabolites presented as ceftiofur equivalents (CE) per mL.

§ Below the limit of detection (0.04 µg CE/mL).

¶ No treatment given, not challenged with *A pleuropneumoniae*.

<sup>a</sup> Percentage differed from that of the controls (Group 6) (ANOVA;  $P < .001$ ).

NA = not applicable.

bacterial pathogens. *Actinobacillus pleuropneumoniae* is highly sensitive to ceftiofur.<sup>5-7,9,10,12</sup> Multiple daily doses of either the sodium or hydrochloride formulation of ceftiofur significantly reduce mortality caused by swine respiratory disease associated with this pathogen.<sup>9,16-18</sup> However, the current study used only a single dose of CCFA-SS, suggesting obvious economic advantages in terms of reduced labor and treatment costs, as well as improved animal welfare and treatment compliance.

## Implications

- A single IM dose of CCFA-SS (5 mg CE per kg BW) can provide 7 days of therapeutic activity against respiratory disease caused by *A pleuropneumoniae* in feeder pigs.
- Use of a single dose of CCFA-SS rather than multiple daily doses of other ceftiofur formulations would lower treatment costs, improve treatment compliance, and enhance animal welfare.

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