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Efficacy of Florfenicol Premix in Weaning Pigs Experimentally Infected with *Actinobacillus pleuropneumoniae*

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ABSTRACT. The efficacy of a florfenicol premix was studied in weaning pigs experimentally inoculated with *Actinobacillus pleuropneumoniae*. Twenty five clinically healthy pigs were distributed into 3 groups; group A non-medicated, groups B and C orally medicated with 20 and 40 ppm of florfenicol respectively. The pigs were fed during 12 consecutive days and on day 5 all the groups were challenged with *A. pleuropneumoniae* serotype 1. All the animals in Group A developed clinical signs. Most of the pigs in the medicated groups maintained a good health status. Postmorten examination revealed severe pleuropneumonia in pigs from the control group and pneumonic lesions in 40% of the animals treated with 20 ppm of florfenicol. Development of pleuropneumonia was prevented in all the pigs medicated with 40 ppm of florfenicol. *Actinobacillus pleuropneumoniae* was recovered from the lungs of all control animals and from one pig of each of the medicated groups, however, the avidin biotin peroxidase (ABC-P) method detected the presence of the microorganism in all the animals. We demonstrated that medication with feed containing 40 ppm of florfenicol blocked efficiently the signs and lesions caused by *A. pleuropneumoniae* and increased the daily body weight gain.
Key words: *Actinobacillus pleuropneumoniae*, florfenicol.

RESUMEN. Veinticinco lechones clínicamente sanos fueron distribuidos en 3 grupos; Grupo A no medicados, grupos B y C oralmente medicados con 20 y 40 ppm de florfenicol incluido en el alimento, respectivamente. Los cerdos fueron alimentados con la premezcla durante 12 días consecutivos, en el día número 5 todos los grupos fueron desafiados con *Actinobacillus pleuropneumoniae* serotipo 1. Todos los animales del grupo A desarrollaron signos clínicos de pleuroneumonía porcina. La mayoría de los cerdos en los grupos medicados mantuvieron un buen estado de salud. Las lesiones encontradas a la necropsia fueron compatibles con una pleuroneumonía severa en cerdos del grupo control y zonas neumónicas en 40% de los animales tratados con 20 ppm de florfenicol. El desarrollo de pleuroneumonía fue totalmente prevenido en los cerdos medicados con 40 ppm de florfenicol. *A. pleuropneumoniae* se aisló de todos los pulmones de los animales del grupo control y de un cerdo de cada uno de los grupos tratados, sin embargo, la presencia de la bacteria fue detectado en todos los animales mediante inmunohistoquímica. Florfenicol administrado en forma de premezcla a una dosis de 40 ppm bloqueo eficientemente los signos clínicos y lesiones ocasionados por *A. pleuropneumoniae* y además incremento la ganancia diaria de peso.
Palabras clave: *Actinobacillus pleuropneumoniae*, florfenicol.

INTRODUCTION

Porcine pleuropneumonia (PP) caused by *Actinobacillus pleuropneumoniae* affects animals of all ages, however, is more frequently found in pigs from 12 to 16 weeks of age. The morbidity is generally high (0-40%) and the mortality (1-10%) depends on diverse factors such as herd immunity and environmental conditions.[6,21] Economic losses caused by this microorganism are enormous. Prevention and control of the disease are based on vaccination, early diagnosis, slaughter of healthy carriers and use of antimicrobial drugs.[7,12] Vaccination *against A. pleuropneumoniae* is commonly practiced. Bacterins containing antigens to the prevalent serotypes, as well as autogenous products, have been commercially available for several years. However, experimental data and field usage indicate that although vaccination with these products may reduce the clinical signs, the incidence of pneumonia and the mortality associated with acute infection, vaccinated pigs may still become subclinically or chronically infected.[6]

Diagnosis of PP is made from clinical history and gross and histological appearance of the lesions. These proce-

dures are not definitive, therefore isolation and identification of the causative agent is required to confirm the presence of *A. pleuropneumoniae*. Failure of isolation occurs when only a few organisms are present in subclinical infected animals, when *A. pleuropneumoniae* is overgrown by other bacteria or when its presence is reduced by the use of antibiotics.[29] Serological tests are an essential tool to detect infection in clinically healthy pigs. Use of highly sensitive and specific assays determines the intensity and duration of maternal immunity, the time when animals are more susceptible and the time of infection. Therefore, these assays facilitate making subsequent decisions concerning herd management.[6] At the present time, antibiotic treatment is still a practical means of prophylaxis against disease caused by *A. pleuropneumoniae*. An efficient treatment should be instituted by providing medication in the feed or water during risk times, when infection is endemic, or as soon as possible after onset of the disease in the herd, however, in the later case the use of antibiotics is restricted to parental treatments due to the markedly reduction in water and feed consumption. Although a wide range of chemotherapeutic agents has been recommended for treatment of swine with pleuropneumonia, an increasing resistance to currently available drugs among isolates of *A. pleuropneumoniae* has been documented.[5,8,28,30,31] Florfenicol is a fluorinated derivative of thiamphenicol[1] that has been shown to have a spectrum of activity comparable to that of chloramphenicol, but does not have the p-nitro group that is associated with the irreversible aplastic anemia in man.[3,16] The mechanism of antibacterial activity of florfenicol is the same as that of thiamphenicol and chloramphenicol and involves inhibition of bacterial protein synthesis at the ribosome.[4,13] This activity is not affected by chloramphenicol acetyl transferase, the enzyme responsible for the majority of the plasmid mediated bacterial resistance to chloramphenicol and thiamphenicol.[20,22,25,27] Florfenicol was discovered in 1979 and currently is under development for use in veterinary medicine.[1,3] The objective of this study was to determine the efficacy of florfenicol in preventing clinical signs and lesions in weaning pigs exposed to an experimental *A. pleuropneumoniae* challenge.

MATERIAL AND METHODS

Animals. Twenty five, 6 weeks old, clinically healthy pigs with an average body weight of 10.5 kg were used in this experiment. All the animals were housed in an experimental farm and allowed to acclimatize for 7 days prior the start of the trial. Individual body weight and daily feed consumption were registered.

Experimental design. The pigs were distributed at random into 3 groups; Group A with five animals that were not medicated and groups B and C with ten animals orally medicated with 20 and 40 ppm of florfenicol respectively. Florfenicol at 2% (batch 24559) was received from Schering Plough and used in the trial. The antibiotic premix was mixed with the feed 1.0 to 2.0 kg per ton during 15 min to obtain a final proportion of 20 and 40 ppm respectively. Each medicated batch was individually sacked and identified. The feed used in this study was formulated with no antibiotics or promoters (Table 1). The pigs were fed *ad libitum* during 12 consecutive days and on day 5 all the groups were challenged with *A. pleuropneumoniae* serotype 1, strain isolated from an acute clinical case. The strain was cultured in brain-heart infusion broth during 8 h at 37°C in agitation. Final concentration was adjusted to $1x10^8$ UFC/ml using the Mc Farland nephelometer tube number 0.5. Five milliliters of the standarizated culture were delivered directly in the nose of each pig using an aerosol pump. Clinical signs were recorded every 4 h during the first 48 h post-challenge and then every 24 h until the end of the experiment. Identity of the groups was not known by the observers. All the animals were euthanized by stunning and exsanguination 7 days after challenge.

Table 1. . Composition of meal used to feed weaning pigs.

Constituent	Amount in kg
Corn	674.0
Soybean	272.0
Orthophosphate	18.0
Fat	10.0
Calcium carbonate	4.0
Caolin	4.0
Salt	3.2
Vitamins	3.0
Acidificant	2.5
Zinc oxide	2.0
Lysine	1.32
Threonine	1.18
Fungicide	1.0
Sweeten	1.0
Cooper sulfate	0.5

Pathological examination. At necropsy the macroscopic lesions of each animal were evaluated and scored subjectively from 0 to 100%. A schematic description from each lung was produced. Samples for histopathology and immunohistochemistry were removed and fixed in 10% formol-buffered saline. Microscopic lesions were analyzed by 2 different pathologists, samples were identified only by numerical labels to avoid bias.

Immunohistochemistry. Lung samples were processed through the avidin biotin peroxidase complex (ABC-P Vectastain kit, Vector, Burlingame, California, USA) method, to detect *A. pleuropneumoniae* antigens using rab-

bit polyclonal antibodies produced in our laboratory against a reference strain of serotype 1, received from Dr K. R. Mittal of the Faculty of Veterinary Medicine, University of Montreal.

Bacteriology. All the lungs were removed aseptically after the necropsy and cultured on blood agar with a cross streak of *Staphylococcus aureus* as a source of NAD. All suspected colonies were identified by standard bacteriological techniques including; Gram stain, oxidase, urease, satellitism beside a *S. aureus* streak and CAMP factor production.[2]

Statistical Analyses. Data were analysed using the general linear models procedures of SAS.[19] Analysis of variance was used to evaluate treatment effects on body temperature and body weight gain. Further mean separation was accomplished using Duncan's multiple range test. A P value of 0.05 or less was considered statistically significant.

RESULTS

Clinical observations. All the animals in Group A developed clinical signs. Four out of five pigs had cough, dyspnea, anorexia and depression ranging from moderate to severe. Clinical signs started 4 h postchallenge.
Most of the pigs in the medicated groups maintained a good health status, however, 3 out of 10 animals in Group B developed cough, dyspnea or depression in a moderate form at 8 h post challenge, and slight depression was detected in three animals of Group C after 12 h of infection (Table 2).

An increase in the body temperature was registered in all animals. A significantly greater mean body temperature ($P<0.05$) was found in Group A with temperatures ranging from 39 to 41.4°C. The febrile response started 8 h after challenge and lasted for 36 h. Groups B and C had a slight and transient increase ($T<40$°C), but there was not a significant difference among them ($P>0.05$). Temperature differences were evaluated statistically every 24 h, however, the significant difference was present only at 24 h after challenge (Fig. 1).

The body weight gain and feed conversion efficiency in Group C were superior than in Groups A and B, however, only the body weight gain difference with Group A reached statistical significance ($P<0.05$) (Table 2). The daily average feed intake per pig was 0.788 kg, therefore the approximate drug intake was 1 and 2 mg/kg of body weight in groups B and C respectively.

Pathological findings. At postmortem examination pigs from Group A exhibited a severe pleuropneumonia with necrosis and abscess formation, tissue was affected in a range from 10% to 50% (Fig. 2). Respiratory tracts from 4 out of 10 animals in Group B had pneumonic lesions, in addition to moderate oedema and congestion with hemorrhages. Pigs in the Group C did not have pneumonic lesions, however, all developed slight hemorrhages and congestion. Microscopic examination of the lungs from pigs in group A revealed increased pleural thickness with severe hemorrhages and alveolar oedema. The affected tissue was congested and infiltrated by large numbers of polymorphonuclear cells and lymphocytes. In group B diffuse moderate congestion and incipient hemorrhages were found, whereas, in group C the findings were congestion and discrete interstitial oedema.

Immunohistochemistry. *A. pleuropneumoniae* was

Table 2. Clinical and pathological parameters of weaning pigs experimentally inoculated with *Actinobacillus pleuropneumoniae* serotype 1. Group A; non-medicated, Group B; orally medicated with a feed containing 20ppm of florfenicol, and Group C; orally medicated with a feed containing 40ppm of florfenicol.

	Groups		
	A	B	C
Animals per group	5	10	10
Mortality	0/5	0/10	0/10
Clinical signs	5/5	3/10	0/10
Lung lesions	5/5	4/10	0/10
A. pleuropneumoniae[1]	5/5	1/10	1/10
Feed conversion (kg)	3.59	2.29	2.01
Body weight gain (kg)[2]	2.2 (±0.53)[a]	4.35 (±0.37)[b]	5.20 (±0.37)[b]

[1]App (*Actinobacillus pleuropneumoniae)*

[2]Mean± SEM. [a-b]Means with no common superscript differ significantly ($P \leq 0.05$)

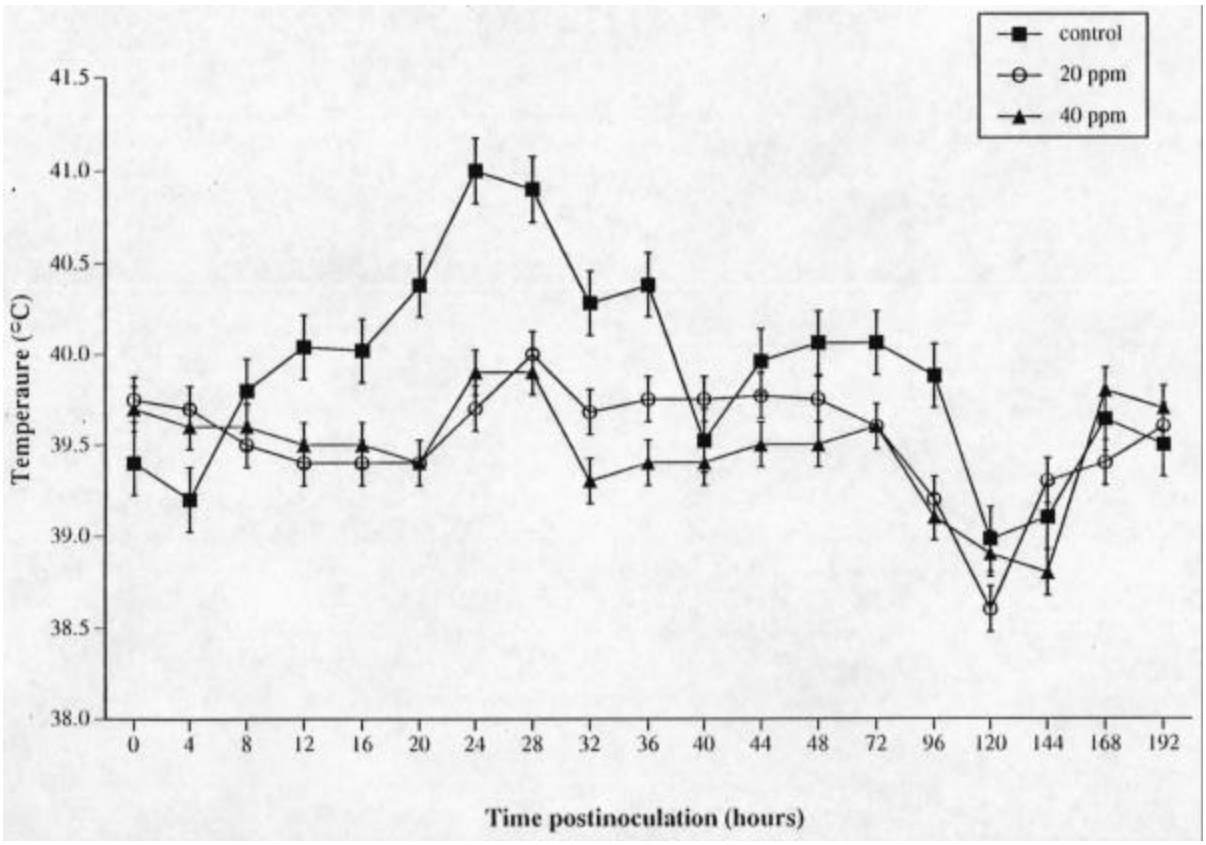

Figure 1. Average temperature (± SEM) of pigs experimentally infected with App serotype 1. Control group infected and non-medicated (squares); infected and medicated with 20 ppm of florfenicol (circles), infected and medicated with 40 ppm of florfenicol (triangles).

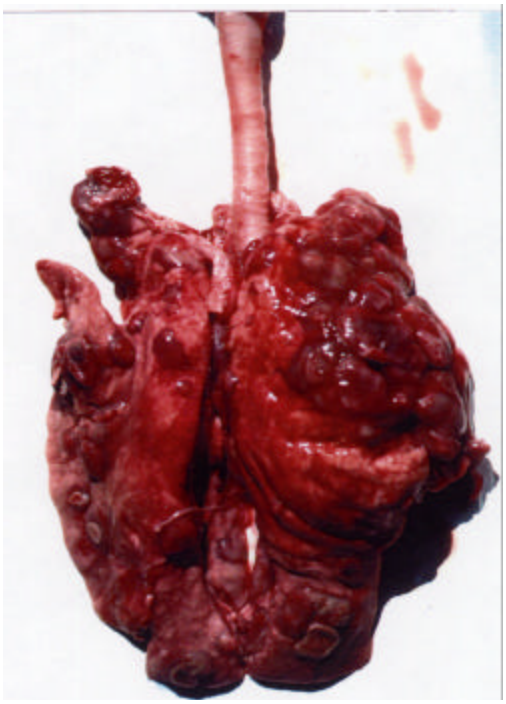

Figure 2. Severe pleuropneumonia and abscess formation caused by *Actinobacillus pleuropneumoniae* in a pig experimentally infected and non-medicated.

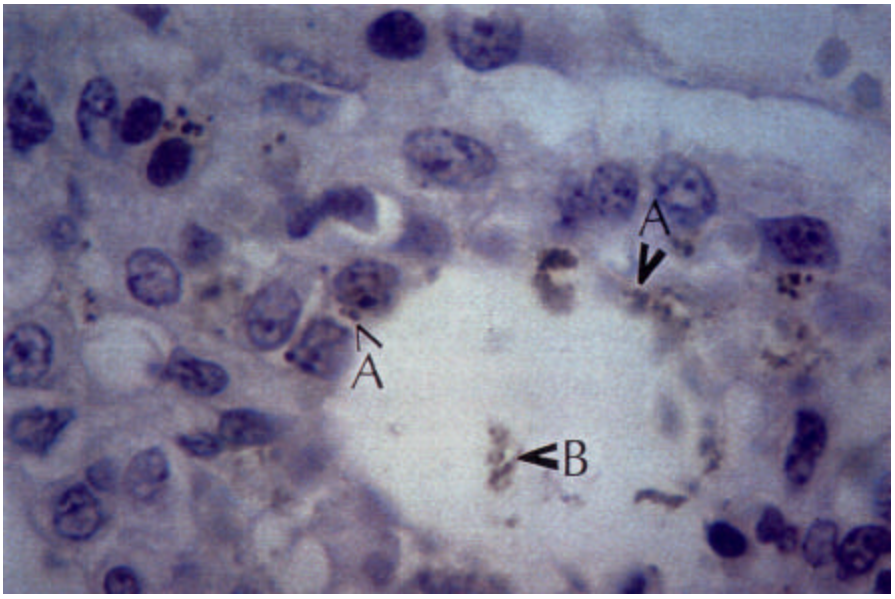

Figure 3. Histological section of lung of a pig experimentally infected with *Actinobacillus pleuropneumoniae*. The bacterial antigen was detected in the cytoplasm of alveolar macrophages (A) and in the alveolar lumen (B) by the avidin biotin peroxidase method.

detected in 100% of the samples. Brownish coccobacilli occurring in clusters or individually were observed inside of blood vessels, bronchi, bronchioles, septa and alveolar spaces, alveolar lumen and in the cytoplasm of alveolar macrophages (Fig. 3).

Bacteriological findings. *A. pleuropneumoniae* was isolated from all the animals in Group A and from one animal of each of the medicated groups. All strains recovered belonged to serotype 1, as determined by the coagglutination test.

DISCUSSION

Clinical protection against PP using antibiotics has been shown to give good results when they are provided opportunely. Parenteral treatments are suggested when clinical disease emerges in the herd, whereas the oral route is proposed as a preventive measure during weaning and later around the time of infection, previously determined by serology.[6] In most cases antibiotics do not eliminate the microorganisms completely. However, if they are used at the appropriate time they may decrease the impact of the pneumonic process and offer an economic benefit.[14,18]

Stephano-Hornedo et al. determined that enrofloxacin intramuscular at 2.5, 5.0 and 10 mg/kg of body weight was effective against acute pleuropneumonia.[24] Smith et al. showed that administration of 150 ppm of enrofloxacin with the feed produced a marked control of the infection in terms of reduced average severity of thoracic lesions and much reduced prevalence of the organism in the lung at necropsy.[23]

Medicated feed containing 400 or 800 mg/kg of oxytetracycline failed to prevent clinical signs of PP after challenge, but medication with 1600 mg/kg resulted in 100 per cent clinical protection. In addition, doses of 400, 800 or 1200 mg/kg did not prevent shedding and transmission of the infection to seronegative animals.[10] Feed medicated with 125 ppm of sulfamethoxazole in combination with 25 ppm of trimethoprim provided a prophylactic effect against an *A. pleuropneumoniae* challenge.[15]

Mortality in *A. pleuropneumoniae* experimentally infected pigs is not always a common feature. Hunneman et al. reported mortality rates ranging from 0 to 20% in different experimental groups.[10] Whereas, Smith and coworkers documented 12% of death animals after exposure to infection. In this study, it was intended to use an aerosol-based model to mimic the natural route of infection.[23] Although, the concentration of the inoculum was similar as the one reported by other researchers,[10,23,24] there was no mortality. However, animals from the control group developed severe clinical signs. Feed medicated with 20 ppm of florfenicol prevented the development of clinical signs in 7 out of 10 animals, whereas a dose of 40 ppm efficiently prevented the classical signs of PP, and only 3 pigs showed mild depression.

It is known that infection by *A. pleuropneumoniae* produces anorexia, however, pigs from both medicated groups did not develop any degree of anorexia, which would have impaired antibiotic uptake. A febrile response of the con-

trol group was registered at 8 h postinfection and lasted for at least 36 h, this result is in agreement with previous reports.[15,17] Florfenicol administered at a dose of 20 and 40 ppm in the feed eliminated completely the presence of fever in the pigs (T<40°C).

Postmortem examination revealed severe pleuropneumonia in pigs from Group A and pneumonic lesions in 4 out of 10 animals treated with 20 ppm of florfenicol. Development of pleuropneumonia was prevented in all the pigs medicated with 40 ppm of florfenicol. The presence of lesions in animals from group B might be related with variation of individual susceptibility to the infection.

A. pleuropneumoniae was recovered from the lungs of all control animals and from one animal of each of the medicated groups, however, the ABC-P technique detected the presence of *A. pleuropneumoniae* in all the animals. This is not surprising, because the ABC-P method detects antigens from viable and non-viable bacteria, whereas, isolation always requires living bacteria in the sample to identify their presence.[9,11] These results suggest that florfenicol at 20 and 40 ppm did not completely eliminate the microorganism, however, decreased its presence to a minimum expression that was not detected by isolation. Herd immunity under these circumstances is developed minimizing the potential economic consequences associated with an outbreak of clinical pleuroneumonia.[6] When the herd is already endemically infected with *A. pleuropneumoniae* and management procedures and environmental factors are adequate, the chances of a clinical disease outbreak are greatly reduced. It is important to consider that worldwide most swine herds are endemically infected with *A. pleuropneumoniae* and producers have just two options: a) live with the disease minimizing its economic impact, or b) have an *A. pleuropneumoniae*-free herd.[6]

Florfenicol has been reported to have an excellent bioavailability after oral administration to veal calves,[1,26] and it penetrates into most tissues and body fluids at concentrations equal to or greater than the concurrent serum concentration.[1] Florfenicol concentration in tissues decreases over time, however, presence of residues is always a potential risk that must be considered as a very important issue in public health. Appropriate withdrawal time must be instituted prior to slaughter.

Results from this study suggest that florfenicol at 20 ppm provides some protection against an *A. pleuropneumoniae* challenge, however, this concentration is not enough to eliminate clinical signs and lesions from all the animals. On the other hand, 40 ppm blocks efficiently the signs and lesions caused by *A. pleuropneumoniae* and increases the daily body weight gain, however, it does not eliminate the microorganisms from the lungs. Further research should be conducted to determine the optimal dosage that completely eliminates the bacteria from tissues and does not represent a hazard in public health.

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