

APRAMYCIN IN THE CONTROL OF ENTERITIS ASSOCIATED WITH *ESCHERICHIA COLI* IN RABBITS

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ABSTRACT

The digestive disease is the main cause of mortality in industrial fattening rabbit farms. The economical impact of an episode of digestive disease has been assessed to be 0.78 € by produced rabbit. For some years, apramycin has been used by rabbit producers as an option to control digestive syndrome associated with *Escherichia coli*. The results of two clinical studies confirm that the optimum dose regimen of apramycin in feed for the reduction of mortality and clinical signs in case of enterocolitis outbreak associated with *E. coli* in rabbit is 7.5 mg apramycin/kg of body weight/day (i.e. 100 ppm in feed) for 21 consecutive days.

Key words: Apramycin, Rabbit, Enterocolitis, Enteritis, *Escherichia coli*.

INTRODUCTION

The digestive disease is the main cause of mortality in industrial fattening rabbit farms. Two main digestive syndromes are generally identified: colibacillosis and Epizootic Rabbit Enteropathy (ERE). Digestive diseases are rarely caused by one single pathogen. It is quite frequent that there is an overlapping of different causative agents that work together to develop the digestive syndrome (Marlier *et al.*, 2006). In a recent publication, Morel-Saives *et al.* (2007) indicate that the economical impact of an episode of digestive disease was evaluated to be 0.78 € by produced rabbit. This cost allowed us to look for a therapeutic strategy. Apramycin is an aminoglycoside with a bactericidal activity concentration dependant against *Escherichia coli*. It has been registered in Spain and Italy to control clinical signs associated with *E. coli*.

The objective of this paper is to present the results of two clinical studies performed to define the optimal dose regimen for apramycin distributed in feed for the reduction of mortality and clinical signs in case of enterocolitis outbreak associated with *E. coli* in rabbit.

MATERIALS AND METHODS

Animals and experimental design

Two clinical studies were performed under natural conditions in 2 different French experimental rabbit farms. Both studies were randomised, blinded, controlled studies and performed according to VICH GCPV VICH GL9. The key elements of the design of each study is summarised in Table 1.

Animals were treated via feed medicated with either apramycin or nothing (negative control group).

Animal follow-up consisted in regular individual clinical examination over the study period (focused on digestive tract: diarrhoea, borborygmi, abdominal distension) and a necropsy with tissue samples for bacteriological analyses in case of death. During the necropsy of each dead animal, some criteria (liquid in stomach, paresia, diarrhea, intestinal inflammation, ulceration) were noticed by the

veterinary to classify the origin of the death (colibacillosis, enterocolitis or both). It was called Diagnosis Plan with scoring system.

Table 1: Design of the two clinical studies

	Study No. 1	Study No. 2
Objective	Dose titration	Confirmation
Infection	Natural	Natural
Number of animals in total	700	600
Number of animals per cage	7	6
Number of groups	4 groups of 175 animals	2 groups of 300 animals
Animal strain	Hyplus	Hyplus
Age on study start = day 0	Weaned - 31 days	Weaned - 33 days
Observation period	41 days	33 days
Treatment start	When mortality due to digestive disorders reached 2%	
	On Day 11	On Day 6
Treatment duration	21 days	21 days
Mean apramycin dose (mg/kg bw/d)	0 - 2.5 - 7.5 - 23.3	0 - 7.9

Data collected were: presence of digestive clinical signs (diarrhoea, borborygmi, abdominal distension), mortality and cause of death, body weight at least on arrival, treatment end and study end and feed consumption per cage during treatment and post-treatment periods.

For both studies the primary efficacy parameter was the percentage of mortality due to digestive disorders at the end of treatment (Day 21).

Statistical Analysis

For study No.1 (dose titration study), mortality rates were compared by using Chi-Square test for a global comparison and then a multiple comparison with Bonferroni correction for pair comparisons. Mean daily weight gain (DWG) and feed conversion ratio (FCR) calculated for each cage were compared using an ANOVA with effects for treatment. In case of treatment effect, a Tuckey multiple comparison test was performed to analyse the treatment effect. For study No.2 (confirmation study), mortality rates were compared by using Chi-Square test. Mean DWG and FCR calculated for each cage were compared using an ANOVA with effects for block and treatment. In all cases, the degree of significance was set at P=0.05.

RESULTS AND DISCUSSION

Determination of the optimum dose

The efficacy analysis was done on 677 animals only. Fifteen animals died due to digestive disorders before treatment start, and 8 animals died after study start due to disorders other than digestive disorder. Over the whole study period, among the 133 rabbits dead with clinical signs of digestive disorders, an *E.coli* infection (alone or associated) was diagnosed on 128 (96.2%). Details are:

- 17.3% *E. coli* alone,
- 71.4% *E. coli* and ERE,
- 0.7% *E. coli* and caecal paresia,
- 6.8% *E. coli*, ERE and caecal paresia,
- 2.3% ERE and caecal paresia,
- 1.5% caecal paresia.

Analysis of tissues (intestine and liver) from dead animals isolated 36 *E. coli* strains:

- 12 from serotype O2,
- 11 from serotype O85,
- 2 from serotype O132,
- 11 seronegative (to O2, O15, O49, O85, O103, O128, O132).

Mortality rates were significantly different between the 4 treatment groups ($P < 0.001$) (Table 2). More precisely, mortality rates were significantly lower in groups No.2 and 3 compared to the negative control group ($P < 0.05$ and $P < 0.001$, respectively). No significant difference was detected between other pair groups.

For mean DMG, it was not possible to detect statistical difference between groups. In those randomised and blinded studies, no moving of animals was done during the period of observation for equalize population in each cage following the mortality. So after the peak of mortality and morbidity, a compensating growth allowed animals of negative control group to catch their delay again. For mean FCR, no statistical difference was detected between groups except between group No.2 and the negative control group ($P < 0.05$). In summary, the apramycin dose of 7.5 mg/kg of body weight (bw)/d for 21 days in feed (i.e. approximately 100 ppm in feed) is the optimum dose in this study to reduce the mortality rate due to digestive disorder.

Table 2: Study No. 1: dose titration study - Results

	Group No. 1 Apramycin 2.5 mg/kg/d	Group No. 2 Apramycin 7.5 mg/kg/d	Group No. 3 Apramycin 23.3 mg/kg/d	Group No. 4 Negative control product
Mortality rate (%)	17.9 ^{ab}	12.5 ^b	9.1 ^b	24.1 ^a
Mean DMG \pm sd (g/day)	42.1 \pm 0.6 ^a	41.9 \pm 0.6 ^a	42.7 \pm 0.6 ^a	41.4 \pm 0.6 ^a
Mean FCR \pm sd	3.07 \pm 0.03 ^{ab}	3.05 \pm 0.03 ^b	3.06 \pm 0.03 ^{ab}	3.16 \pm 0.03 ^a

DMG: Daily Mean Gain; FCR: Food Conversion Ratio; sd: standard deviation
a, b, values with different letters are significantly different at least at $P < 0.05$

Confirmation of the dose regimen

The efficacy analysis was done on 579 animals only (289 in apramycin group and 290 in negative control group). Fourteen animals died due to digestive disorders before treatment start, and 7 animals died after study start due to disorders other than digestive disorder (pneumonia or origin unknown). Over the whole study period, among the 90 rabbits dead with clinical signs of digestive disorders, an *E. coli* infection (alone or associated) was diagnosed on 87 (96.7%). Details are:

- 3.3% *E. coli* alone,
- 56.8% *E. coli* and ERE,
- 3.3% *E. coli* and caecal paresia,
- 33.3% *E. coli*, ERE and caecal paresia,
- 3.3% ERE and caecal paresia.

Analysis of tissues (intestine and liver) from dead animals isolated 19 *E. coli* strains:

- 1 from serotype O 103,
- 18 seronegative (to O2, O15, O49, O85, O103, O128, O132).

Mortality rate was significantly lower in apramycin group than in the negative control group ($P < 0.05$) (Table 3). Mean DMG or FCR values were not significantly different between groups ($P > 0.05$).

Table 3: Study No. 2: confirmation study - Results

Group	Apramycin	Negative control group	Signification
Morbidity rate (%)	13.5	20.3	$P < 0.05$
Mean DMG \pm sd (g/day)	40.1 \pm 0.6	38.6 \pm 0.6	$P > 0.05$
Mean FCR \pm sd	3.19 \pm 0.03	3.23 \pm 0.3	$P > 0.05$

DMG: Daily Mean Gain; FCR: Food Conversion Ratio; sd: standard deviation

CONCLUSIONS

The results of two clinical studies confirm that the optimum dose regimen of apramycin in feed for the reduction of mortality in case of enterocolitis outbreak associated with *E. coli* in rabbit is 7.5 mg apramycin/kg bw/day (i.e. 100 ppm in feed) for 21 consecutive days.

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