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EFFICACY STUDY OF SOLUBLE BACITRACIN (BACIVET S®) IN A CHRONICALLY INFECTED EPIZOOTIC RABBIT ENTEROPATHY ENVIRONMENT

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Abstract: The present study evaluated under field conditions the efficacy of bacitracin zinc soluble powder (Bacivet S®) for the treatment of Epizootic Rabbit Enteropathy (ERE). A blinded study was conducted in compliance with the "Good Clinical Practice" guidance. The trial site was known to be chronically infected with ERE. A total of 384 weaned rabbits (30 days of age) were enrolled in the study. They were randomly distributed over 6 rows of cages located in the same experimental room. Two rows of cages remained untreated (control group), another 2 rows were treated with Bacivet S® for 14 days (T14) and 2 rows were treated with Bacivet S® for 21 days (T21). The medication was administered per row via the drinking water and initiated when at least one animal, showing clinical signs of ERE, died. The dose was adjusted daily according to the weight of the rabbits to achieve the target dose of 420 IU/kg body weight in the two treatment groups. Animals were observed daily for mortality, diarrhoea, tympany, or any other abnormal sign for 42 days post enrollment. Weights of the animals and feed intake were recorded on a weekly base. On the second day after the set-up of the trial, mortality already occurred with clear signs of ERE and the medication was initiated. On the primary evaluation parameter, mortality, both treated groups showed significant (P<0.01) better results than the control group with an overall mortality reaching 13.5%, 12.6% and 26.6% in T14, T21 and control groups, respectively. Considering only ERE confirmed mortality, differences were even more pronounced; 4.0% (T14), 6.3% (T21) and 14.1% (control), respectively. The occurrence of clinical signs (tympany and diarrhoea) was also reduced in medicated rabbits but this reduction was markedly higher for T21 treated rabbits (P<0.001 compared to control) than in the T14 group. A few days after the termination of the medication period, moderate mortality and clinical symptoms were observed in both medicated groups. These isolated cases were probably due to a recontamination through the untreated groups. Data of daily weight gain and feed intake showed a good agreement with the observed mortality and clinical symptoms. In conclusion, Bacivet S® proved its efficacy against ERE in a chronically infected environment for both tested treatment regimens.

Key words: rabbit, Epizootic Rabbit Enteropathy, Bacivet S®, efficacy.

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INTRODUCTION

Epizootic Rabbit Enteropathy (ERE) is a disease whose aetiology is still unknown but characterised by a gut dysbiosis (LICOIS, 2004). This intestinal disease has been present in Europe from 1996 on and without antimicrobial treatment it may produce mortality rates of up to 60% during the growing period (COUDERT *et al*., 1997).

The main signs of the disease are anorexia, increased borborygmus, enlarged abdominal cavity, caecal impaction, diarrhoea and mortality. The first sign of the disease is a reduction in feed intake. In the post-mortem examination a non-specific enteropathy can be observed, with no inflammatory lesions in the small intestine, caecal impaction, non-specific reaction in the gut associated lymphoid tissues, and often mucus into the colon (LICOIS *et al*, 2000).

Bacitracin as feed additive (Albac®) at a dose of 100 mg/kg has proven to be efficient to control the ERE syndrome (DUPERRAY *et al*., 2000). However, from July 1999 off, because of the European ban (EU reg. 2821/98) Zn-bacitracin was no longer authorised as feed additive. First results with soluble bacitracin have shown the potential to prevent rabbits from ERE (BOISOT *et al*., 2004).

The objective of the present study was to obtain data on the use of Bacivet S® under field conditions and natural ERE infection at 420 IU/kg body weight and to evaluate the optimal duration of treatment with respect to mortality and clinical parameters.

MATERIALS AND METHODS

All aspects of the study were performed according to the VICH Guideline GL9 (2000) on Good Clinical Practice. The study was designed as a controlled, completely randomised, blinded study and was carried out between the 18th of February and the 31st of March 2004 at the Department of Animal Feeding and Husbandry.

Experimental buildings are located in Merelbeke, Belgium. In the last 3 years, this site has been chronically infected with ERE, characterised by a significant degree of mortality (20-30%) in the fattening period if no antibiotic was used.

Animals and housing

In total 384 rabbits belonging to the final cross of the Institute's lines and showing no symptoms of disease or abnormalities were enrolled in the study (Maertens, 1992). At weaning (30 days of age), litters were homogeneously distributed over the different treatments and all rabbits were individually ear tagged. Animals were housed in cages of 4 rabbits each. Cages were placed in 6 rows of 16 cages in one room of an experimental rabbit building. Each row had a separate water reservoir and a distribution line with one nipple per cage. The wire cages used for the study were flat-deck cages measuring 71 cm (length) × 47 cm (width) × 52 cm (height) equipped with a feeder with 2 feeding places.

The building central heating system and the forced ventilation (over- and under-pressure) allowed for optimal environmental conditions. The room ambient temperature was registered daily using a minimum-maximum thermometer and amounted between 14 °C (minimum) and 22°C (maximum) during the whole study period.

Feed

The feed was manufactured in the experimental plant of the Institute located at the trial site. Common raw materials were used and the diet was formulated to contain 16.1 % crude protein, 17.5% crude fibre, 5.0 % ADL and 9.55 MJ DE/kg. The normal anticoccidial (Robenidine, 66 mg/kg) was included in the diet. No other concomitant therapy was used during the trial.

The same diet was always offered *ad libitum* to all rabbits during the entire experimental period.

Treatment groups

There were three treatment groups, each group being composed of 2 rows of 16 cages (i.e. 128 rabbits). One group was untreated (negative control) while the two other groups were treated with Bacivet S® at a dose of 420 IU bacitracin /kg body weight (BW) per day via the drinking water during 14 days in one group (T14) and during 21 days (T21) in the other group. The applied dose had been selected based on the results obtained in pharmacodynamic, pharmakokinetic studies as well as in previous *in-vivo* studies. Treatments were assigned randomly to the rows of cages.

Medication was intended to start when at least one animal with clinical signs of ERE died. The daily dose administered per row was calculated based on the weaning weight, the number of rabbits alive and a daily gain/rabbit of 40 g. Every week the dose was adjusted to the measured weight of the animals so that the daily target dose would be as accurate as possible. The calculated dose was pre-diluted in a concentrate (bottle) and then further diluted in a volume equal to 70% of the expected daily water intake.

This fresh medicated water was placed daily in the water reservoir at approximately 3:00 pm. Once all the medicated water had been consumed, unmedicated water was provided *ad libitum* until the next day till 3:00 pm.

In order to avoid any bias this procedure was applied to all groups (treated / non-treated) for 21 days after the start of treatment period.

Variables measured

During the medication period, the consumption of medicated water per row was monitored daily in order to check that the rabbits drunk all the medicated water. Feed consumption (per cage) and body weight (individual) were weekly measured.

Daily mortality was recorded during the entire study period. Visual observation of the animals for general health condition, diarrhoea (soiling of the hind quarters) and tympany were conducted daily. The clinical observations were conducted by a person who was not aware that treatment had been administered.

During the study period, all dead animals were transported as soon as possible to the analytical lab where they were necropsied (Animal Health Care Flanders, Deinse Horsweg 1, 9031 Drongen, Belgium). The presence of pathology consistent with ERE was checked and the cause of death was stated in the autopsy report. Lesions due to an overfilled stomach and small intestine, mucus in the small intestine or in the caecum and desiccation of the caecal content were considered as pathology consistent with ERE.

Data treatment and statistical analysis

Analyses of mortality and clinical signs were performed using PROC FREQ of the SAS 8.2 System®. Pearsons chi-square test was used to judge mortality and clinical symptoms. Each medicated group was compared to the negative control group using an α=0.05 significance level (one-sided) in a test of the null hypothesis. The 2 medicated groups were compared to each other using an α=0.05 significance level (two-sided). Assuming the animal response is independent of cage and row, each treatment can be represented as a binomial random variable and pairwise treatment comparisons can be made. However, there was evidence of within-cage correlation for animals with clinical signs associated with ERE. In the latter case, the pairwise comparisons were tested using both an animal level binary measure (presence/absence of at least one clinical sign) and a cage level binary measure (one or fewer animals with signs versus multiple animals with signs).

Weight gain and feed intake data (averaged by cage) were analysed as a completely randomised block design with treatment as the main source of variation and litter as block effect. All data of animals surviving at the end of the period considered were used in the analysis of weight gain. Cage feed intake data were adapted when mortality occurred. Average daily feed intake in such a cage was calculated with the assumption that no feed was consumed during the last 2 days preceding the death. The estimated intake of the dead rabbit was than subtracted from the cage feed consumption. The economical feed efficiency was calculated as the total feed consumed (including the amount of the dead rabbits) / (total finishing weight – start weight).

RESULTS

Three mortality cases were already recorded on the second day after the set up of the trial. The autopsy of the 3 cases revealed clinical signs of ERE. The medication was initiated immediately according to the experimental design. Further results do not include the mortality observed before initiation of the treatment (n=3) since these mortalities occurred before any treatment was installed and therefore have no relevance to the aims of the study.

The daily administered quantity of medicated water was always totally consumed. Therefore the target dose of 420 IU of bacitracin/ kg of BW was achieved.

Mortality

During the study a considerable level of mortality (17.6%) was reached in the entire group of animals with clear distinctions between the different test groups. The overall mortality reached 26.6% in the untreated control group, 13.5% in T14 and 12.6% in T21 (Table 1). Statistically both treated groups performed significantly better than the control group (P=0.007 and P=0.004 for T14 and T21, respectively). No statistically significant difference was observed between the two treated groups.

In order to focus the assessment on the impact of the test treatment on ERE, a distinction was made between mortality with signs of ERE confirmed at necropsy and mortality with no signs of ERE at necropsy. ERE was confirmed in 52.9% of the mortality in the untreated control group, in 29.4% of the mortality in T14 and in 50% in T21. This resulted in an absolute ERE confirmed mortality of 14.1% in the untreated control group and of 4.0% and 6.3 % in T14 and T21, respectively (Table 1). Statistical analysis showed that for both medicated groups the difference with the control group was significant (P=0.004 and P=0.032 for T14 and T21, respectively). However, the difference between the two medicated groups was again not significant.

Cumulative ERE confirmed mortality over the entire observation period is shown in Figure 1. It appears that mortality in the unmedicated control group occurred

Table 1: Total and ERE related mortality in controls and Bacivet S® treated rabbits.

Classification at Necropsy	Treatment	Deaths[1]	Pair-wise Comparisons	
			vs Control[2]	14 *vs* 21[3]
All[4]	14 Days	17/126 = 13.5%	0.007	0.854
	21 Days	16/127 = 12.6%	0.004	
	Control	34/128 = 26.6%		
ERE[5]	14 Days	5/126 = 4.0%	0.004	0.571
	21 Days	8/127 = 6.3%	0.032	
	Control	18/128 = 14.1%		

[1] Animals dead on day 0 prior to treatment (n=3) are excluded, [2] *P*-value for one-sided exact Pearson χ^2 test, [3] *P*-value for two-sided exact Pearson χ^2 test, [4] All dead animals, [5] Animals with signs of ERE confirmed at necropsy.

mainly in the earlier part of the trial while mortality was higher in the later part of the trial for the medicated groups, when the actual treatment had ended.

Clinical signs

The percentage of animals showing at least one occurrence of diarrhoea was significantly lower in treated rabbits (23.8% and 11.0% for T14 and T21, respectively) compared to controls (35.9%) (Table 2). The difference between both treatment groups was significant ($P<0.01$) in favour of T21. For tympany, the results were 32.8%, 19.8% and 11.8% in the control, T14 and T21 groups, respectively.

The incidence of rabbits showing at least one occurrence of either diarrhoea or tympany during the entire observation period were 37.5%, 27.0%, and 15.0% in the control, T14 and T21 groups, respectively (Table 3). Both treatment groups performed significantly better than the untreated control group ($P=0.049$ and $P<0.001$ for T14 and T21, respectively). Furthermore T21 performed significantly better than T14 ($P=0.021$) since the T14 had almost twice as many affected animals as T21.

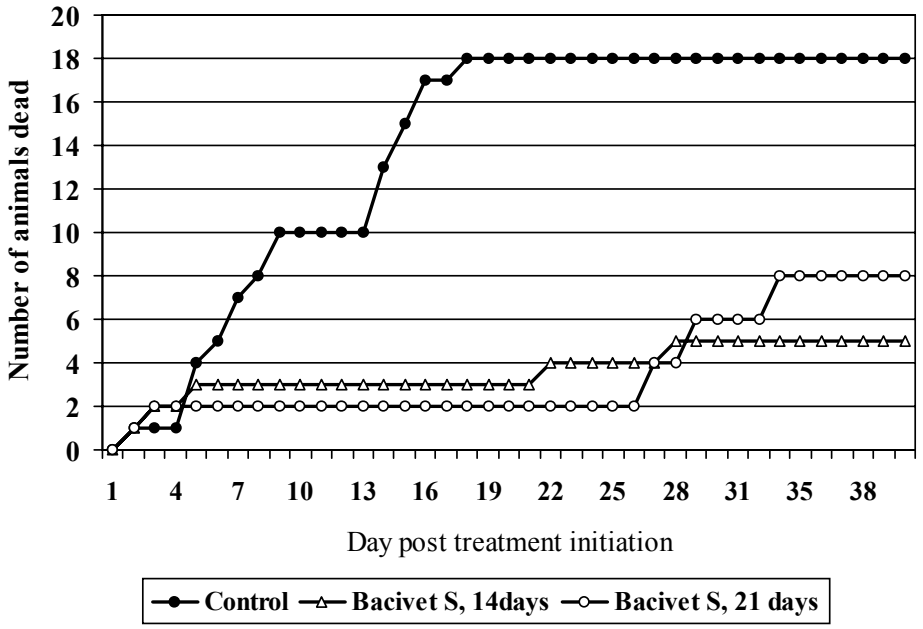

Figure 1: Cumulative ERE related mortality per treatment group.

An analysis of the clinical signs of the survivors per group demonstrated the same tendency as the clinical observations in the total group, with 20.2% of the survivors affected in the control group, 16.5% in T14 and 8.1% in T21 (Table 4). Statistically, T14 did not perform better than the control group (P=0.308). T21 treatment group, however, demonstrated a significantly better result than the control group (P=0.010) and also performed better than T14 (P=0.066).

Table 2: Diarrhoea and tympany per treatment group[1].

	Treatment Group		
	Control	Bacivet S® – 14 Days	Bacivet S® – 21 Days
Diarrhoea, %	35.9[a]	23.8[b]	11.0[c]
Tympany, %	32.8[a]	19.8[b]	11.8[c]

[1] % of animals showing at least one occurrence of diarrhoea post treatment initiation (i.e., between day 1 and 40). Animals dead on day 0 prior to treatment (n=3) were excluded.
Means within a row with different superscripts differ (P<0.05).

Table 3: Occurrence of clinical signs in all rabbit[1].

			Pair-wise Comparisons	
Unit of Measure	Treatment	Percent Affected[2]	*vs* Control[3]	14 *vs* 21[4]
Animal	14 Days	34/126 = 27.0%	0.049	0.021
	21 Days	19/127 = 15.0%	< 0.001	
	Control	48/128 = 37.5%		
Cage	14 Days	8/32 = 25.0%	0.059	0.082
	21 Days	2/32 = 6.2%	<0.001	
	Control	15/32 = 46.9%		

[1] Three animals dead on day 0 prior to treatment initiation are excluded, [2] For Animal: Percent of animals with at least one clinical occurrence of diarrhoea or tympany post treatment initiation. For Cage: Percent of cages with more than one animal affected post treatment initiation, [3] *P*-value for one-sided exact Pearson χ^2 test, [4] *P*-value for two-sided exact Pearson χ^2 test.

Further statistical analysis of the clinical results indicated that a cage effect could not be excluded. If it is assumed that a cage is affected when it contains more than one diseased animal, the infection levels were 46.9%, 25.0% and 6.2% for the untreated control group, T14 and T21, respectively (Table 3). In this approach, T21 performed significantly better than the control group (P<0.001) and T14 (P=0.082, two-sided). T14 however did not perform significantly better than the untreated control group.

Table 4: Occurrence of clinical signs in survivors[1].

			Pair-wise Comparisons	
Subgroup	Treatment	Percent Affected[2]	*vs* Control[3]	14 *vs* 21[4]
	14 Days	18/109 = 16.5%	0.308	0.066
Survivors	21 Days	9/111 = 8.1%	0.010	
	Control	19/94 = 20.2%		

[1] Animals that completed the entire observation period. [2] Animals with at least one clinical occurrence of diarrhoea or tympany post treatment initiation, [3] *P*-value for one-sided exact Pearson χ^2 test, [4] *P*-value for two-sided exact Pearson χ^2 test.

Performance data

Body weight gain showed significant differences between treatment groups and according to the fattening week (Table 5). In the first week a significantly lower daily weight gain (DWG) was already observed in the control group. This difference was even more pronounced in the second week. DWG was not significantly different between Bacivet S® treated groups. However in the 4th week, controls had a significantly higher DWG than T14 rabbits. This can be considered as compensatory growth after a period with moderate growth. During the last 2 weeks, T14 performed significantly better than controls and T21. This resulted in an overall DWG for the 6 week period which was 2.2 g higher ($P<0.05$) in both Bacivet S® treated groups.

Daily feed intake data (Table 6) generally followed the same tendencies as DWG. However, the intake data did not differ significantly between the 3 treatments when the total period is considered. Moreover, differences in the overall technical feed efficiency were small and not significant. However, values for the economical feed conversion were 11% and 10% more favourable for T14 and T21, respectively.

Table 5: Daily weight gain (g/d) during the trial[1].

	Treatment Group				
	Control	Bacivet S® – 14 Days	Bacivet S® – 21 Days	SEM	Significance
0-7 days	37.7[a]	43.2[b]	44.1[b]	0.77	<0.01
7-14 days	30.9[a]	48.9[b]	50.1[b]	1.26	<0.001
14-21 days	44.6	45.7	47.9	0.67	NS
21-28 days	48.1[a]	34.6[b]	42.3[c]	1.04	<0.001
28-42 days	44.0[a]	47.0[b]	42.5[a]	0.57	<0.01
0-42 days	43.1[a]	45.3[b]	45.3[b]	0.36	<0.05

[1] Cage mean of average per animal (total cage gain/survivors).
Means within a row with different superscripts differ. ($P<0.05$).

Table 6: Daily feed intake and feed efficiency during the trial.

	Treatment Group				
	Control	Bacivet S® – 14 Days	Bacivet S® – 21 Days	SEM	Significance
Feed intake[1] (g/d)					
0-7 days	76.0[a]	82.4[b]	84.3[b]	1.0	<0.01
7-14 days	89.1[a]	113.0[b]	115.3[b]	1.8	<0.001
14-21 days	123.7[a]	130.6[ab]	133.6[b]	1.4	<0.05
21-28 days	150.1[a]	129.7[b]	145.1[a]	1.8	<0.001
28-42 days	167.9	168.9	163.8	1.8	NS
0-42 days	129.4	131.5	134.3	1.1	NS
Feed efficiency[2]					
0-42 days	3.01	2.91	2.98	0.02	<0.10
Economical feed efficiency[3]					
0-42 days	3.77	3.35	3.38		

[1] Cage mean of average per animal (total feed intake/survivors, corrected for dead rabbits), [2] Cage feed intake/cage weight gain, [3] Total feed intake/total final weight - start weight.
Means within a row with different superscripts differ. (P<0.05).

DISCUSSION

The objective of the present study was to assess the efficacy of bacitracin zinc soluble powder in the early treatment of ERE in rabbits reared under normal field conditions. Because of the early appearance of ERE in the experimental unit and the high mortality observed in untreated rabbits (26.6%), this objective could be verified. The fact that the aetiology of this disease is still not clear implies that the data gathering must be focussed on clinical aspects applied to necropsies on all mortality cases in the study. The choice of mortality as the primary parameter is in this case justified since high mortality levels are always associated with an outbreak of ERE (LICOIS *et al.*, 2000).

For this primary parameter, both total mortality and mortality associated with ERE were assessed. Both approaches yielded almost identical results and conclusions. Significantly better statistical results were obtained in both approaches in favour of the medicated groups compared to the untreated group. However no difference was observed between the medicated groups.

The infection was rapidly controlled in both medicated groups while the mortality continued for approximately 2.5 weeks in the untreated controls. This implied that medication of the T14 group stopped when mortality was still continuing in the control group. The ERE linked mortality in the T14 group from 22 to 28 days has probably to be linked with a recontamination in the environment from the control group. Results of the T21 group confirmed the risk of recontamination under such conditions. There was some mortality observed from days 26 to 34. However, the mortality level in the two medicated groups never reached the same level as that attained in the control group. The same observation was performed in the comparable study of BOISOT *et al.* (2004) using an experimental reproduction of ERE. Clearly, the housing of a non-medicated but infected control group has to be considered as an additional continuous infectious burden on the total group, which does not normally occur under good husbandry conditions.

A correct analysis of the effect of the studied treatment on the syndrome requires that parameters other than mortality, which is to be considered as an endpoint rather than a symptom, have to be taken into consideration. Tympany and diarrhoea were chosen for this in view of their connection to the disease and the good conditions for consistent observations of these symptoms. Discrimination by ERE confirmed or non-ERE confirmed by necropsies, as applied for the mortality parameter, is obviously impossible since not all the affected animals died. On the other hand a distinction has been made between all animals and the animals that survived the entire 40-day observation period.

A statistical evaluation of both ancillary variables together (tympany and diarrhoea) provides additional information. Taking into account all the affected animals, T14 performs significantly better than the unmedicated control group, but

when only the surviving animals are considered this is no longer the case. T21 however demonstrates a statistically significant better effect than both the control group and T14 in both approaches. Although wire cages were used and a good interaction between the animals from the different groups was possible, it appears from the statistical analysis that a cage related effect cannot be excluded. This observation however does not change the above conclusion. On the contrary, it even strengthens the observed difference between T14 and T21, with P=0.059 and P<0.001 for T14 and T21, respectively when compared to the untreated control group.

Furthermore, it is remarkable that the morbidity occurring after treatment in the 14 day group was nearly double that of the 21 day group, and even quadruple if the cage effect is taken into account. It should also be noted that in both treatment groups the morbidity reaches its peak a few days after the end of the treatment. As has already been stated, this can be attributed to a recontamination from the unmedicated group.

The efficacy observed under field conditions (present study) and those after experimental infection (Boisot *et al.*, 2004) showed a good agreement. Mortality due to ERE was less than 1/3 of the control group in both studies. Under normal rearing conditions, when good husbandry practices (no unmedicated rabbits in the same house) are applied, an even more marked efficacy against ERE can be expected.

Both DWG and feed intake data showed a good agreement with the observed mortality and clinical symptoms. The significantly higher performance obtained during the first 2 weeks in both medicated groups demonstrated that the medication immediately controlled the infection. Moreover, the drop in DWG, observed during the 4th week in T14 and in the last period in T21, coincided with the mortality and clinical signs in medicated groups. However, surviving medicated rabbits had on average a 2.2 g higher DWG and a 92.4 g higher slaughter weight than controls (P<0.05). This has to be attributed to the lower mortality rate, which was less than 50% of the unmedicated group. Both factors together reduced the feeding cost by 10-11% in treated rabbits.

CONCLUSION

Both a 14 day and a 21 day treatment with Bacivet S® were capable of controlling ERE under field conditions. Mortality, clinical symptoms as well as performance data were significantly improved compared to the untreated control group. Some days after the termination of the medication period, moderate mortality and clinical symptoms were observed in both medicated groups.

Because of the remarkable reduction in clinical symptoms in T21 compared to T14, a 21 day treatment period could be considered as the treatment of choice in order to effectively control ERE under current field conditions.

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