

EPIZOOTIC RABBIT ENTEROPATHY. STUDY OF EARLY PHENOMENA WITH FRESH INOCULUM AND ATTEMPT AT INACTIVATION

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ABSTRACT: Using 180 35-day-old SPF rabbits, this study used the effectiveness of bacitracin as a tool for acquiring more information on the various phases of ERE, in particular during the hours following inoculation. Five groups of animals were used, including 3 treatments with Bacivet S[®] (bacitracin) at different times from inoculation, with the standard inoculum TEC3. Three parameters were studied: growth, mortality and stomach noises (borborygmi). A significant fall in growth rate was observed during the first 18 hours following the inoculation in all the inoculated groups, both medicated and not medicated. Treatment with bacitracin eliminated mortality and borborygmi, but not the initial fall in growth rate. Treatment starting 18 hours after inoculation is less effective during the acute phase than the preventive treatment. With a preventive treatment interrupted as soon as 18 hours after inoculation, a delay of several days was observed before the appearance of the disease (fall in growth rate, manifestation of borborygmi) and total mortality was reduced. Very few pathogens can explain this early fall in growth rate. Bacitracin is an antibiotic which offers good control of the disease, and probably of the pathogen but not of the physio-pathological disturbances in the first few hours. The intervention of an exogenous toxin in the first hours of contamination seems likely. Borborygmi are important criteria. The intensity and/or frequency could be used as semi-quantitative criteria to characterize the disease and for the prognosis. In a simultaneous trial, a group was contaminated with the same inoculum, heated for 10 min at 55°C, in order to obtain more information on the type of pathogen involved in the etiology of ERE. This treatment did not modify the virulence of the inoculum.

Key words: rabbit, epizootic enteropathy, enterocolitis, bacitracin, physiopathogeny.

INTRODUCTION

Epizootic Rabbit Enteropathy (ERE) can be systematically reproduced with an inoculum composed of an extract of the intestinal contents of sick rabbits (TEC3;

LICOIS and COUDERT, 2001). The disease can be reproduced in the laboratory (LICOIS *et al.*, 2005) as well as under field conditions (BOISOT *et al.*, 2003). In a previous work, COUDERT and LICOIS (2004) described ERE as an acute disease, the first symptoms appearing as early as the first day post-inoculation. The results seemed to indicate two successive and independent periods during the evolution of the disease: a very acute phase during the first hours following the inoculation and an acute phase, four or five days later. Moreover, it has been shown that an antibiotic treatment completely controls the acute phase but not the symptoms of the very acute phase. The aim of this work was to study the daily reactions (weight gain, mortality, borborygmi) of the inoculated animal and the modulation of these reactions by an antibiotic. We also report an attempt to modify the virulence of the inoculum by heating.

MATERIAL AND METHODS

Experimental design

Seven groups of animals were used including 4 treatments with Bacivet S[®], at different times, being day 0 the inoculation day (Table 1):

Room A: standard inoculum (TEC3). Study of the early phenomena after inoculation with the standard inoculum (TEC3):

- Group A1: uninoculated and continuously treated from day -3 to day +15 (Control A; Uninoculated Treated A).
- Group A2: inoculated and never treated (Positive Control A; Inoculated Untreated A).
- Group A3: inoculated and treated from day -3 to day +15 (Inoculated Treated A from D-3 to D+15).
- Group A4: inoculated and treated from day -3 to 18 hours after inoculation (Inoculated Treated A from D-3 to D+1).
- Group A5: inoculated and treated from the 18 hours after inoculation to day +15 (Inoculated Treated A from D+1 to D+15).

Table 1: Experimental design with 5 animals per cage for all groups.

Room	Inoculation	Treatments (Bacitracin)			
		No treatment	From D-3 to D+15	From D-3 to D+1	From D+1 to D+15
A	Uninoculated (Control A)	-	A1 (20)	-	-
A	Inoculated TEC3	A2 (25)	A3 (25)	A4 (25)	A5 (25)
B	Uninoculated (Control B)	-	B1 (20)	-	-
B	Inoculated TEC3H ¹	B2 (40)	-	-	-

¹ Heated 55°C 10 min. No. of animals per treatment in brackets.

Room B: inoculum TEC3 after heating (TEC3H). Study of the virulence of this inoculum after heating at 55°C, 10 min (bain-marie). This temperature is low but was selected in order to inhibit the action of certain bacterial toxins such as *Clostridium perfringens* enterotoxin, while preserving the integrity of the vegetative forms. *Clostridium perfringens* is a bacterium present in the inoculum TEC (LICOIS *et al.*, 2005).

- Group B1: uninoculated and continuously treated from day -3 to day +15 (Control B; Uninoculated Treated B).
- Group B2: inoculated with TEC3H and never treated (Positive Control B; Inoculated Untreated B).

Note: the uninoculated control groups were continuously treated with Bacivet S[®], as otherwise these animals could have been rapidly contaminated because of the very high contagiousness of this disease.

Animals and experimental facilities

At weaning (35 days) 180 SPF rabbits were selected on the basis of their weight ($\bar{x} \pm 2SD$) from a contemporary population of 206 young rabbits. The distribution in each group was randomly made in a block structure (weight). The mean weight was 895 ± 129 g.

The cages of metal netting were laid out in batteries on 2 levels. The buildings were disinfected by gaseous formalin before and after each experiment. The temperature and the ventilation were controlled. Rooms A and B had the same layout.

Inoculation and treatments

The animals were inoculated by oral route with 0,5 ml of the TEC3 inoculum (LICOIS *et al.*, 2005). The control groups (referred to as “uninoculated” in the text) also received 0,5 ml of intestinal contents taken from SPF rabbits. The drinking water of the treated groups contained 0,7 g per litre of Bacivet S[®], which corresponds on average to a consumption of 420 IU of bacitracin per kg of body weight.

Measured parameters

Animal were weighed at distribution in the cages (D-6), then at the beginning of the treatment (D-3), at inoculation (D0), 18 hours after inoculation (D+1) and D+2, D+3, D+4, D+5 during the acute period, and finally three times during the period of convalescence (D+7, D+10 and D+15).

Detection and characterization (+, ++, +++) of borborygmi (bowel rumbling noises) were carried out at each weighing. Mortality was noted every day and all animals were necropsied.

Statistical Analysis

The daily weight gains (DWG) were analyzed by variance analysis with 2 factors (Treatment (5) and block (2)) and Tukey Test, (Systat). Mortality was analyzed by the test of χ^2 .

RESULTS

Mortality

As can be seen in Table 2, no mortality was observed in the 2 uninoculated control groups (A1 and B1). There was no mortality in group A3 (inoculated and continuously treated) and one animal died (4%) in the group where the treatment began one day after contamination (A4). In the group in which the treatment was stopped the day after inoculation (A5), the mortality was similar but delayed as compared with the inoculated untreated group (A2). Mortality in the inoculated groups with the heated inoculum (B2) or with the standard inoculum (A2) was similar.

Table 2 : Number of animals and percentage of mortality at different groups.

Groups	No. of animals	Mortality	Days of death
A1: Uninoculated Treated A	20	0%	
A2: Inoculated Untreated A	25	20%	4 - 5 - 9 - 11- 12
A3: Inoculated Treated A from D-3 to D+15	25	0%	
A4: Inoculated Treated A from D+1 to D+15	25	4%	5
A5: Inoculated Treated A from D-3 to D+1	25	20%	7 - 10 - 13 - 14 - 15
B1: Uninoculated Treated B	20	0%	
B2: Inoculated Untreated B ¹	40	13%	6 - 11 - 13 - 13- 13

¹ animals inoculated with TEC3, heated at 55°C, 10 min.

Growth

The two uninoculated groups (A1 and B1) showed a similar growth rate ($P=0.30$) in whatever room they were placed (Table 3).

The results referring to the effect of the different treatments on the DWG were analyzed separately for the two experiments:

1. Study of the early phenomena after inoculation with the standard inoculum (TEC3; room A; Figure 1). The inoculated and untreated group (A2) showed the expected evolution in growth. The disease showed the characteristic evolution of ERE with an early fall in growth rate followed by a peak of morbidity toward D+4, D+5 and a re-establishment starting from D+10. In all the inoculated groups the growth rate fell significantly before the 18th hour (D+1) ($P<0.01$). This fall lasted only one or two days for the treated groups, but as early as D+3 the growth became not significantly different ($P=0.20$) to that of the uninoculated control group (A1). In the group treated shortly after inoculation (A4), the evolution of

Table 3: Comparison of the mean daily weight gain of the two control groups.

	Mean Daily Weight Gain (g) for each period								
	D-6 to D-3	D-3 to D0	D0 to D1	D1 to D3	D3 to D5	D5 to D7	D7 to D10	D10 to D15	D-3 to D15
Control Room A	25	43	31	38	27	46	32	27	32
Control Room B	28	36	28	45	26	35	45	33	36
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS

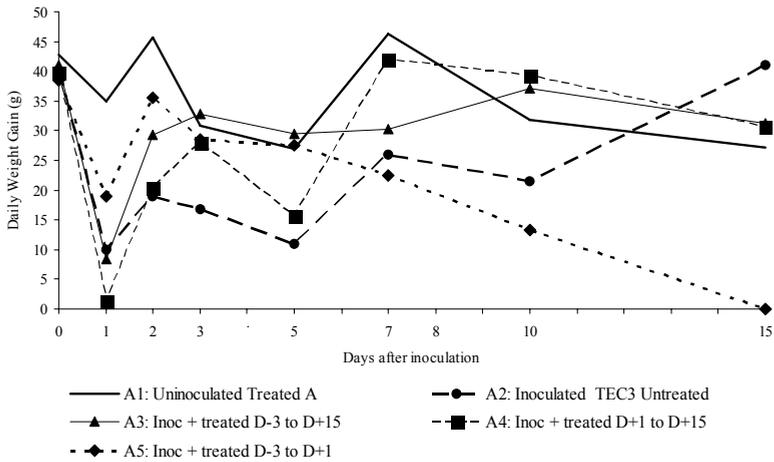


Figure 1: Epizootic Rabbit Enteropathy - Study of early fall of daily weight gain.

growth rate was similar to that of the inoculated non-treated group (A2), for a few days (D0 to D+5). Subsequently, growth rate was not significantly different from that of the uninoculated control group (A1). No immediate effect was observed when the treatment was stopped 18 hours after inoculation (A5). The growth rate was similar to that of the uninoculated control group (A1) for several days, before falling significantly after D+5 ($P < 0.01$).

2. Study of the virulence of the TEC3 inoculum after heating at 55°C, 10 min (TECH; room B; Figure 2). The uninoculated control groups (A1, B1) of both rooms had the same DWG between D0 and D+15 (A: 32g; B: 36g; $P = 0.30$). The daily fluctuations of growth rate were also similar. The group inoculated with the heated inoculum (B2) had the same DWG as the group inoculated with the unheated inoculum (A2), (D0 to D+15: A2: 24g; B2: 22g; $P = 0.30$).

Diarrhea and stomach noises (borborygmi).

Diarrhea was observed in only one animal (inoculated and untreated group). No borborygmus was heard either before inoculation in all animals, or on the uninoculated groups (A1, B1). As can be seen in Figure 3, the first borborygmus was heard on D+3 in the inoculated and untreated group (A2). In this group borborygmus increased rapidly and persisted in 20 to 40% of the animals until D+10. The inoculated but treated groups (A3, A4) had a slight peak of this clinical sign at D+5. The group

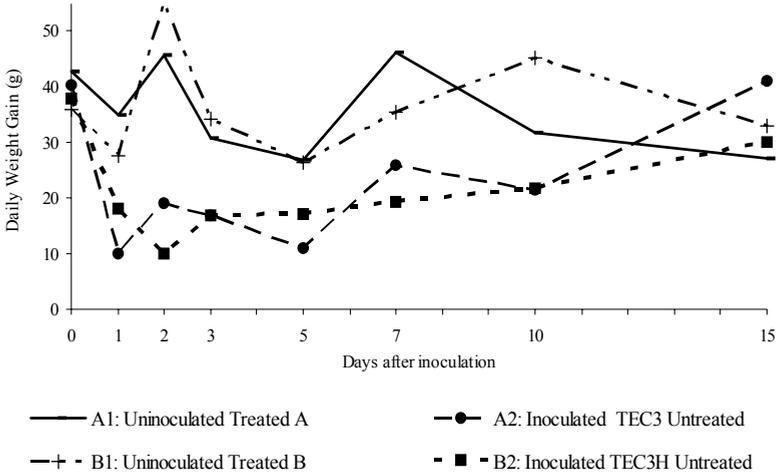


Figure 2: Effect of the heated inoculum on daily weight gain.

treated until one day after inoculation (A5) had practically no clinical signs until the 7th day, but the frequency of borborygmus increased later simultaneously with a decrease in the growth rate (see Figure 1).

Relationship between borborygmi, weight gain and mortality.

For this analysis we used only the inoculated unmedicated groups (with the uninoculated groups as reference). The data of the two uninoculated groups (A1+B1) and of the two inoculated groups were pooled (A2+B2).

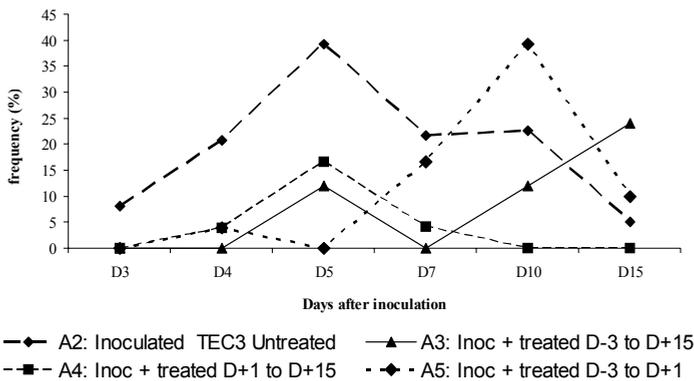


Figure 3: Epizootic Rabbit Enteropathy – Study of a clinical symptom: frequency of borborygmus.

Inoculated animals were grouped in four classes according to the intensity (+, ++, +++) and the frequency of the borborygmus between D+3 and D+15: Class 1: no borborygmus; Class 2: slight borborygmus (+), low frequency; Class 3: borborygmus ++, one or two times; and Class 4: borborygmus +++, several times.

As can be seen in Figure 4, from D0 to D+1 the fall in growth rate of inoculated animals was not related to the subsequent appearance of borborygmi. As early as D+1 inoculated animals which later had no borborygmus at all (class 1) had a better weight gain than those which obtained the highest borborygmus score (class 3 and 4). Mortality was only observed in the sub group of classes 3 (++) and 4 (+++).

Necropsy

All dead animals presented the typical signs of ERE and no lesion of intercurrent pathology was observed.

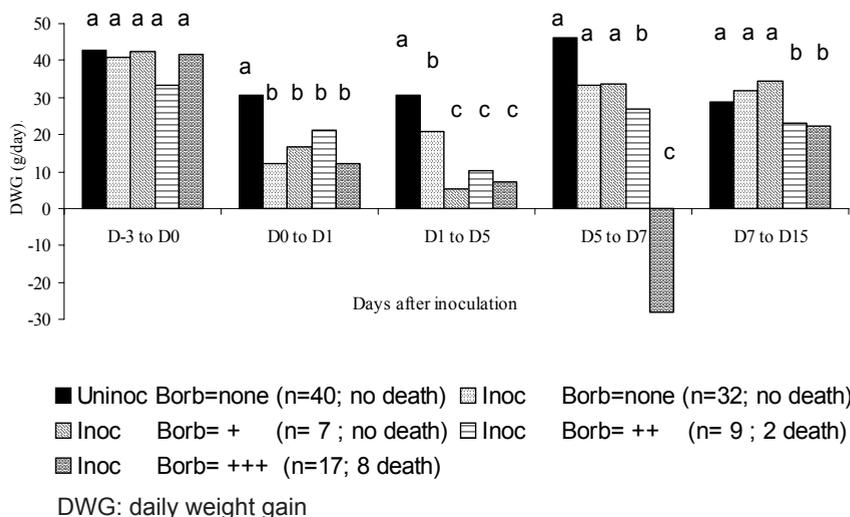


Figure 4: Relationship between borborygmi, weight gain and mortality. Borborygmus = rumbling sound made by gas and fluids moving through the stomach + intestines; Inoc = Inoculated with the inoculum TEC3, for each period, weight gains indexed with the same letter are not different (variance analysis and Tukey test: $P < 0,01$).

DISCUSSION

A significant fall in growth rate was observed less than 24 hours after inoculation in all the inoculated medicated and non-medicated groups. This early adverse reaction lasted one or two days and animals recovered between D+2 and D+3. Untreated animals had a second peak of the disease around D+5, which is in agreement with the development of ERE (LICOIS *et al.*, 2005; KÜHN, 2005). Further research is needed to identify with greater precision the rapidity and the origin of this early adverse reaction of the animal. The aetiology of the two peaks of the disease may be different. Very few pathogens can explain this early fall in growth rate. Bacitracin is an antibiotic which makes good control of the disease possible (COUDERT and LICOIS, 2004; MAERTENS *et al.*, 2005) and thus probably of the pathogen as well, but not the physiopathological disturbances of the first few hours. The intervention of an exogenic toxin, as suggested by MARLIER *et al.* (2003), as early as the moment of contamination seems likely.

The preventive and unbroken treatment with bacitracin allowed mortality to be eliminated and improved the weight gain of inoculated animals, but some borborygmi appeared at the peak of the disease (D+5). When the preventive treatment was stopped as early as 18 hours after inoculation, a delay of several days was observed before the disease appeared (fall in growth rate, manifestation of borborygmi) and total mortality was reduced. As in our previous work (COUDERT and LICOIS, 2004), the treatment starting as early as 18 hours after inoculation was less effective during the acute phase of the disease (D0 to D+5) but it reduced mortality and improved growth after the 5th day.

Borborygmi are an important criterion since they are representative of the seriousness of the disease. There are few early symptoms for ERE: diarrhoea is not constant, caecal compaction appears later and is also inconstant. The intensity and/or frequency of borborygmi could be used as a semi-quantitative criterion to study the disease and for prognosis.

The inefficacy of heating to destroy an eventual toxin and /or the pathogen itself, by heating the inoculum for 10 min at 55°C, is demonstrated. Further research is necessary with higher temperatures or by fractioning of the inoculum, in order to get more information and to better characterize the unknown pathogen.

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