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Original article

Epizootic Rabbit Enteropathy: experimental transmission and clinical characterization

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Abstract – In late 1996 in France, a severe digestive disease appeared in fattening domestic rabbits. Named the Epizootic Rabbit Enteropathy (ERE), this digestive syndrome has become the main cause of mortality in rabbit farming. The diagnosis in field conditions is difficult because co-infection with other common rabbit pathogens is frequent. By using specific pathogenic free (SPF) rabbits and starting from a field sample of intestinal contents of diseased animals, a virulent material (inoculum) was obtained free of almost all known pathogens but reproduced the symptoms and lesions of ERE. Four hundred and seven SPF rabbits were used in five trials to describe the disease. ERE is characterized by a high contagiousness, 30 to 40% mortality in a few days and about 100% morbidity whatever the dose of the inoculum used. Clinical signs and lesions evolved acutely with the first sign (rambling noise) appearing one day after inoculation and the disease peaking 4 to 6 days later. Growth was strongly lowered from the second day to the end of the second week. Rambling noise and distended abdomen were frequent, mucus excretion and cecal impaction were frequent but not constant. ERE at necropsy was characterized by the absence of any inflammatory or congestive lesions on the gut or on other organs but with the typical presence of a stomach and/or duodenum dilated by liquid and gas and by the absence of specific histological lesions. The etiological agent has not been identified yet, but we demonstrate that the intestinal content was infectious as early as the second day. This work constitutes the experimental basis for studies on this emerging disease within the framework of etiological research led in different European laboratories working with the infectious material.

epizootic rabbit enteropathy / intestinal pathology / diarrhea / mucoid enteritis

1. INTRODUCTION

In late 1996 and early 1997, an emergent and severe gastrointestinal syndrome appeared in rabbit farms in the west of France. This pathology is characterized by a distended abdomen, emission of small quantities of watery diarrhea followed by a decrease in feed intake and by high mortality rates (30–80%) during this period. It spread very rapidly to other regions of France in 1997 and 1998 [11] and in Europe: Spain, Portugal, Hungary, Belgium, The Netherlands, Great Britain, etc. [9, 17, 20]. Nevertheless, to our knowledge, this disease has not been reported in other countries of the world except North Africa (Colin, personal communication). In France, it is currently estimated that over

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95% of farms, whatever the rabbit race and strain, are or have been affected by this intestinal affection [19]. Because of the rapid spreading of the disease, it was called Epizootic Rabbit Enteropathy (ERE). The disease mainly affects young fattening rabbits, between six and eight weeks of age. Problems usually occur after weaning but have also been observed in older rabbits and sometimes in adults or in suckling rabbits, just before weaning. Unlike other epizootic diseases (myxomatosis, viral haemorrhagic disease), wild rabbits do not seem to be affected. Nevertheless, ERE has been observed in wild rabbit breeding units [26].

Because of the outbreak of ERE on farms soon after the delivery of feed by a commercial factory, the feed was initially suspected and various hypotheses were studied (rate and nature of the raw materials, mycotoxins, pesticides, etc.) but all these assumptions have been eliminated [18]. Later it was demonstrated that the feed could be a passive vector [10, 16]. However, a disease was experimentally reproduced with the same symptomatology and/or lesions as those of ERE observed in the field, either with intestinal contents originating from ill or dead animals or by contact between animals or also by contact with contaminated breeding material [26]. All these observations associated with those from the field have demonstrated the contagious feature of the disease and therefore that a pathogenic agent is involved in the development of this intestinal pathology. However, no pathogenic organism has been identified and isolated for the moment.

Moreover, although most of ERE clinical signs could be reproduced successfully[1] [23, 24, 26], we encountered many difficulties in obtaining reproducible results, mainly because samples originating from the field are frequently contaminated by opportunistic pathogens (coccidia, *Escherichia coli*, *Klebsiella*, etc.). In order to reduce this variability, different methodologies were tried and several methods of experimental contamination were tested: immunosuppression of the animals [25], intubation via the oesophagus, spraying of the inoculum on the animal or the feed. Different potentially infectious materials were inoculated by the oral route (lung, mesenteric lymph nodes, blood, intestinal contents) without success, except with intestinal contents. This, associated to the fact that no etiological agent has yet been identified, led us to use this kind of biological material in our studies. Thus, an inoculum (TEC) constituted by virulent intestinal contents without opportunistic pathogens was obtained, as well as other inocula deriving from TEC.

This paper deals with the experimental transmission of ERE based on the use of these virulent materials and specific pathogen free (SPF) rabbits. The most constant results were obtained by contamination of animals with these inocula sprayed on the feed or directly given by the oral route. This made it possible to standardize experimental reproduction of ERE and precisely describe the disease.

2. MATERIALS AND METHODS

2.1. Animals and experimental conditions

All the rabbits used were SPF animals originating from the Experimental Unit "Pathologie Aviaire et Parasitologie" (INRA, Nouzilly, France). These SPF rabbits were notably free of Rotavirus, *Eimeria* spp., *Passalurus ambiguus, Pasteurella multocida*, *Clostridium spiroforme* and enteropathogenic *Escherichia coli* belonging to the serogroups O_2, O_{15}, O_{26}, O_{49}, O_{85}, O_{103}, O_{109}, O_{128}, and O_{132}. They are also regularly monitored according to the recommendations of Federation of European

[1] Licois D., Lebas F., Coudert P., Le Gall G., Note d'information N° 10 sur les travaux de recherche conduits sur l'Entérocolite Épizootique du Lapin, [on line], 1999. http://www.tours.inra.fr/urbase/internet/resultats/enterocolite/info10.htm [consulted January 20, 2005].

Laboratory Animal Science Association (FELASA) [30]. They were reared in highly protected facilities (air filtered at 10 µm, specific staff) according to the methods previously described[2]. At weaning (30 days), they were transferred to experimental facilities. The experimental conditions have been previously described [6]. Briefly, the rooms and material were disinfected twice before each experiment (steam under pressure followed by gaseous formalin). All the equipment used for water supply was autoclaved before the experiments. The experimental rooms were in depression (200 Pa) and the air was filtered (1 µm input–0.3 µm output). The animals were distributed with 2 or 3 per cage according to their original litter and weight. They received water and laboratory feed free of antibiotic and anticoccidial drugs ad libitum (UAR 110, Villemoison/Orge, France). They were inoculated between 33 and 36 days of age depending on the trial.

2.2. Inocula and inoculation

The first sample used as the inoculum, denominated TEC, was kindly provided by P. Hervouet (Biovac, Beaucouzé, France) and P. Robart (Techna, Coueron, France), in late 1997. It was obtained from rabbits affected by ERE on a commercial farm. The crude intestinal content (small intestine plus cecum-colon) was diluted to 1/3 in sterile PBS (phosphate buffered saline), filtered (0.5 mm) to eliminate large vegetable particles, then centrifuged at 1000 *g* for 15 min. This centrifugation does not eliminate bacteria nor viruses, but does eliminate parasites (coccidia). The raw supernatant thus obtained constituted the first inoculum, which was stored at –20 °C until use. One millilitre of this inoculum corresponds to about 1 mL of fresh intestinal content. Regarding the trials carried out in 2001–2002, different inocula originating from TEC were used. They were treated as described above except that PBS was replaced by sterile water, that was proven not to modify the infectivity of the inocula and subsequently the response of the animals.

In April 2001, an inoculum (TEC1) was produced by mixing several selected samples originating from 5 trials carried out on SPF animals inoculated with TEC. Some of these intestinal contents were obtained after about 10 successive passages in rabbits. TEC1 came from the intestinal contents of 19 sick or dead animals chosen between day 3 and day 8 post inoculation (d PI) in order to cover the acute period of the disease as largely as possible. A second inoculum (TEC2) was composed of the intestinal contents of 27 moribund or dead rabbits infected with TEC1, between d3 and d7 PI, and then a third inoculum (TEC3) was obtained from animals inoculated with TEC2, dead or sick between d2 and d11 PI. TEC3 was stored for over 2 years at –20 °C without losing its infectivity.

Different doses of the inocula expressed in the text for the individual rabbit, were sprayed on 100 g of pelleted feed, considered as a daily ration for one rabbit, then let to dry a dozen hours before being distributed in the mangers.

All the control animals were inoculated in the same way, with intestinal contents obtained from healthy SPF rabbits.

2.3. Microbiological characterization of the inocula

TEC1, TEC2 and TEC3 inocula were partly characterized at the virological, bacteriological and parasitical level. Coccidia were sought for according to the method described by Coudert et al. [7]. The search for rotavirus was performed by an enzyme immunoassay and PCR according to the

[2] Coudert P., Licois D., Besnard J., Establishment of a Specified Pathogen Free breeding colony (SPF) without hysterectomy and hand-rearing procedures, in: Holdas S. (Ed.), Proc. 4th Congress of the World Rabbit Science Association, Budapest, RCPAN, Herceghalom, Hungary, 1988, pp.137–148.

procedures previously described [5]. Calicivirus was searched for using RT-PCR according to the method described by Capucci et al. [3]. The search for other enterotropic viruses (pestivirus, circovirus, adenovirus, coronavirus, parvovirus) was performed by G. Le Gall (AFSSA, Ploufragan, France) using PCR or RT-PCR. The search for coliforms and *Clostridium spiroforme* was carried out by Professor A. Milon (UMR 960, INRA-ENV Toulouse, France). Detection of *C. spiroforme* was performed by light microscopic examination on Gram stained smears of 10^{-1} dilution of the inocula, according to Carman and Borriello [4]. The presence of *E. coli* was evaluated after culture at 37 °C for 24 h of 10-fold dilutions of inocula in EMB (Eosine Methyleneblue) agar [21]. The search for other *Clostridium* and *Bacteroides fragilis* was carried out by Doctor M.R. Popoff (Centre National de Référence des Bactéries Anaérobies, Institut Pasteur, Paris, France). PCR detection of toxin genes of *C. perfringens* (toxins α, β1 β2 and enterotoxin), *C. sordelli* (toxin LT) and *Bacteroides fragilis* (enterotoxin), was carried out according to the method previously described [31] after 24 h liquid culture in anaerobic conditions, at 37 °C in tryptose-glucose yeast (TGY) extract, followed by DNA extraction (Instagen, Biorad).

2.4. Experimental design (Table I)

The aim of the three trials (A, B and C) was to describe the disease and test the virulence of the inocula at different doses and with different methods of inoculation. Trial D was conducted to analyze the first lesions at autopsy and trial E to detect the early appearance of virulent material in the intestinal content. All the experiments followed the guidelines stated in the Guide for the Care and Use of Laboratory Animals [13].

2.4.1. Study of ERE clinical signs and lesions

Trial A: Fifty-five rabbits were distributed 2 per cage in 2 different rooms with respectively 23 animals inoculated with 10 mL TEC1 and 32 control animals. They were observed for 7 days post inoculation. In addition, 8 rabbits were used for routine histological examination of the intestinal mucosa. Four inoculated rabbits were successively slaughtered at d5 and d7 PI.

Trial B: Two groups of 25 and 36 animals were inoculated with 10 mL and 1 mL TEC2, respectively. One group of 12 rabbits was used as the control. Each group was in a separate room, with 3 animals per cage observed for 9 days PI. Thirty additional rabbits were also used for gross lesion observation and histological study. Two diseased animals and one control animal were slaughtered from d1 to d10 PI.

Trial C: In this third trial, we used 6 batches of animals (3 per cage) placed in 3 separate rooms: 4 inoculated groups and 2 control groups. Three batches of 24 rabbits each were inoculated via contaminated feed with 10 mL, 1 mL and 0.2 mL inoculum TEC3, respectively. A fourth batch of 24 rabbits was inoculated by oral route by means of a micropipette with 0.2 mL TEC3. One of the two control groups was in a room without contaminated animals. The animals were studied for 17 days.

Trial D: In this trial, 45 rabbits were inoculated with 1 mL of TEC2 and 12 were used as the control. There were 3 rabbits per cage in the same room. On days 1, 2 and 3 PI, 15 infected and 3 control rabbits per day were euthanized for autopsy.

2.4.2. Detection of early virulent material in the intestinal content (Trial E)

Five rabbits showing signs of disease (rambling noise for 2 animals and/or weak DWG) and 6 rabbits also with signs of disease (rambling noise for 5 animals, diarrhea for 1 animal and/or weak DWG), originating from inoculated animals of trial D, euthanized respectively on d1 and d2 PI, were used. Their intestinal contents were used as fresh material for infecting two groups of 15 rabbits (3 per cage). These rabbits were

Table I. Experimental design of the different trials (A, B, C, D and E).

Groups	Room No.	No. of animals	Inoculum	Dose (mL of inoculum)
Trial A (7 days)				
Control	1	32	HIC[b]	10
Inoculated	2	23 (+ 8[a])	TEC1	10
Trial B (9 days)				
Control	1	12 (+10[a])	HIC	10
Inoculated, 10 mL	2	25 (+10[a])	TEC2	10
Inoculated, 1 mL	3	36 (+10[a])	TEC2	1
Trial C (17 days)				
Control 1	1	18	HIC	10
Control 2	3	18	HIC	10
Inoculated, 10 mL	1	24	TEC3	10
Inoculated, 1 mL	1	24	TEC3	1
Inoculated, 0.2 mL	2	24	TEC3	0.2
Inoculated, 0.2 mL (OR)[c]	2	24	TEC3	0.2
Trial D (3 days)				
Control	1	12	HIC	10
Inoculated	1	45	TEC2	10
Trial E (6 days)				
Control	2	18	HIC	10
Inoculated IC d1[d]	2	15	IC d1	10
Inoculated IC d2[d]	2	15	IC d2	10

[a] Additional rabbits used for histology.
[b] HIC: Intestinal content from healthy SPF rabbits, administered to animals from a control group by spraying on feed.
[c] Group of rabbits inoculated by the oral route with a pipette. All the other animals in the different trials were inoculated by spraying the inoculum on feed. Doses are given for the individual animal.
[d] Groups of rabbits inoculated with fresh intestinal content from affected rabbits of trial D, slaughtered on day1 (IC d1) and day 2 (IC d2) post inoculation.

contaminated (via feed) at their arrival in the experimental room with 10 mL of the fresh intestinal material. Another group of 18 rabbits was used as the control animals inoculated with fresh samples from SPF animals.

2.5. Histological examination

Diseased and control rabbits were euthanized by pentobarbital anesthesia and then exsanguinated. The abdomens were opened and samples of the intestine (1-cm long) and different organs (5 mm^3) were collected and fixed in 10% buffered formalin. In the first experiment, 8 inoculated rabbits were used for only ileum histological examination. In the second experiment, the stomach, duodenum, jejunum, ileum, cecum, vermiform appendix, proximal colon, lung, mesenteric lymph nodes, liver, spleen, kidney and heart were sampled in 10 control animals and

20 inoculated rabbits. In the inoculated group, the animals with the most obvious clinical signs (11 rabbits) were the only cases submitted to histological examination. The tissue specimens were embedded in paraffin wax, cut at 4 μm and stained with Hematoxylin eosin and Saffran (HES) to be examined under light microscopy.

2.6. Parameters recorded and statistical analysis

A daily examination of the clinical signs was performed: diarrhea, bloat, rumbling noise when the animals were grasped and slightly shaken (borborygmus), and cecal impaction on abdominal palpation. The animals were weighed three times during the first week after inoculation and twice later. Weight gains were compared by variance analysis of two factors with a mean comparison by means of the Tukey test using the Systat statistical software package [33] and mortality was analyzed by $\chi 2$. All dead rabbits were autopsied for examination of the digestive tract and vital organs (lung, liver, heart, kidney, etc.).

3. RESULTS

3.1. Microbiological characteristics of the inocula

On direct examination, TEC1, TEC2 and TEC3 were controlled free of coliforms (10^{-1} dilution); the flora was poor and unbalanced, Gram positive bacteria like *Clostridium* were dominant; however, *Clostridium spiroforme* was not detected. On the contrary, *Clostridium perfringens* belonging to types Alpha or Beta2 were identified. In each of the inocula, the search for rotavirus was negative by an ELISA test but positive by PCR. The search for other enterotropic viruses (calicivirus, pestivirus, circovirus, adenovirus, coronavirus, parvovirus) was negative. No intestinal parasites were detected.

3.2. Description of the disease (trials A, B, C)

3.2.1. Mortality and clinical signs

Neither mortality nor clinical sign of disease were observed in the control groups of any of the three trials, except for the control animals of trial C which were located in the same room as the inoculated rabbits. In this control group no animal died during the 17 days of observation, but clinical signs (rambling noise and/or diarrhea) could be detected after the 5th day PI in 28% ($n = 5/18$) of the animals.

After the experimental contamination (day 0) in the three trials A, B and C, mortality began on day 3 or 4 with a peak on day 5 or 6. For the first cases of mortality, the rabbits had no diarrhea. At the end of the second week, 30 to 50% of the rabbits were dead or moribund. Regarding morbidity, all the rabbits showed at least one sign of disease: rambling noise (which was the most constant clinical sign, appeared as soon as d2 PI), cecal impaction (which could be detected as soon as d3 on abdominal palpation), diarrhea, distended abdomen and excretion of mucus (which could mainly be observed from d4). On days 4, 5 and 6, most of these criteria were associated, but none of them was constant. Diarrhea was very aqueous and of low intensity at its onset.

3.2.2. Gross lesions

At autopsy, no macroscopic sign of inflammation or congestion was detectable in any part of the intestine or other organs (liver, spleen, kidney, lung, heart). This particularity concerned all the inoculated rabbits, whatever the trial. Cecal impaction which affected 20 to 30% of the dead animals according to the trial, was either total or partial (Fig. 1); in the latter case, impaction was generally located on the side of the appendix vermiform and the content of the other side was very liquid. The distal colon was generally empty and free of droppings.

The most constant feature, encountered in 50 to 80% according to the trial, was the dilatation of the stomach filled with liquid and gas (Fig. 2) and the distension of the small intestine, particularly the duodenum, by a large amount of liquid and sometimes gas.

Whatever the doses (10 mL, 1 mL or 0.2 mL) or the method (feed contamination or oral route), no differences in mortality, clinical signs or lesions were observed.

3.2.3. Evolution of the daily weight gain

In all trials, the daily weight gain (DWG) of all uninoculated control groups was around 40 g/day during the first week (Fig. 3). When the uninoculated control group was placed in the same room as the inoculated animals, a significant decrease in the DWG appeared at the end of the first week ($P < 0.01$).

In all groups inoculated with TEC inocula (trials A, B, C as well as trial D), the DWG began to decrease between d0 and d2 PI (Fig. 3). This early DWG decrease was significant in each trial ($P < 0.01$). The lowest DWG in all inoculated groups was reached 5 to 7 days after inoculation and no difference was observed according to the doses used. During this peak of the disease, in each trial (A, B and C), the DWG was significantly different from that of the uninoculated groups located in a separate room ($P < 0.01$). Between d7 and d9 (trial B) or d17 (trial C), the surviving animals slowly recovered a DWG comparable to that of the uninoculated (and non contaminated) groups.

3.2.4. Histological examination

In trial A, the histological examination (of the ileum) of d5 PI inoculated rabbits ($n = 4$) did not reveal any degenerative changes of the epithelium nor inflammatory lesions of the *lamina propria.* In d7 PI inoculated rabbits ($n = 4$), a mild and focal infiltration by a polymorphonuclear heterophil was observed in the *lamina propria* of all the inoculated animals with limited figures of transepithelial exocytosis. The size and aspect of the villi were normal without any epithelial changes.

In trial B, none of the 12 control rabbits presented any histological changes of the different parts of their digestive tract (Fig. 4a), nor of their other examined organs. In the 11 inoculated animals examined, histological lesions were totally absent in 2 rabbits respectively sampled at d7 and d10 PI, in spite of marked gross changes observed in both animals. The stomach as well as the liver, spleen, lung and heart, were devoid of lesions in all the examined rabbits. The different parts of the intestinal tract presented mild to moderate lesions in all the other 9 inoculated rabbits. The lesions were characterized in 6 of them by a mild to moderate atrophy of the villi with focal images of villous fusion (Fig. 4b). The villi changes were associated in two animals with a moderate epithelial exocytosis of heterophil cells. No changes in lesion intensity were noted according to time PI. In one rabbit slaughtered on d2 PI, only a moderate hyperhemia located in the duodenum was observed. The jejuno ileal part of the intestine of one rabbit slaughtered at 7d PI and the duodenal part of another rabbit sampled at 5d PI exhibited focal limited acute necroticohemorrhagic and ulcerative lesions of the mucosa associated with numerous bacterial colonies. Moderate diffuse infiltration by polymorphous inflammatory cells associated with a moderate crypt hyperplasia were the only lesions observed in one rabbit at d6 PI. Aplasia of the lymphoid follicles of the vermiform appendix was noted in three animals at d5, d7 and d10 PI.

3.2.5. Detection of early infectious material derived from affected animals (trial E)

In rabbits inoculated with intestinal contents coming from animals of trial D euthanized at d1 PI, no significant difference was observed between the uninoculated and inoculated groups from d1 to d6: no death,

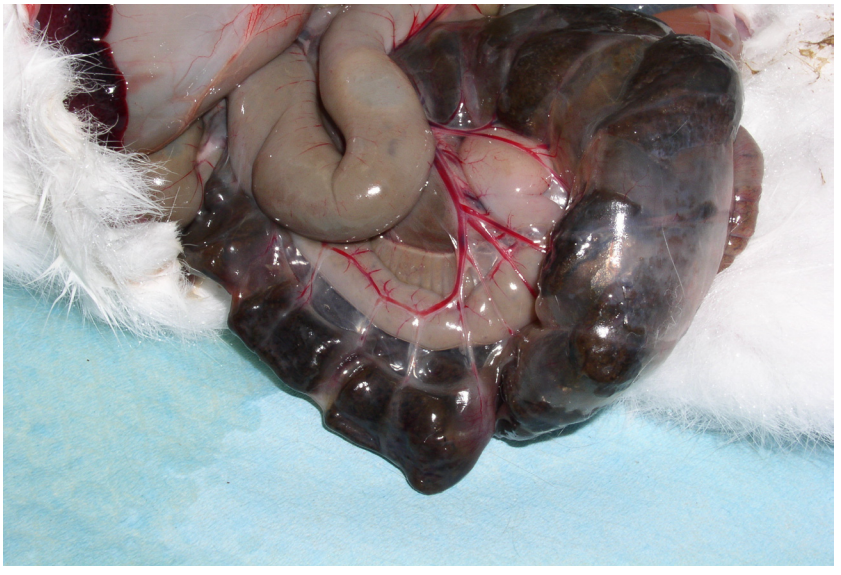

Figure 1. Six-week-old rabbit, dead 5 days after experimental reproduction of ERE with inoculum TEC3, showing total caecal impaction, one of the main gross lesions of the disease. There are no inflammation or congestion visible on the caecum wall.

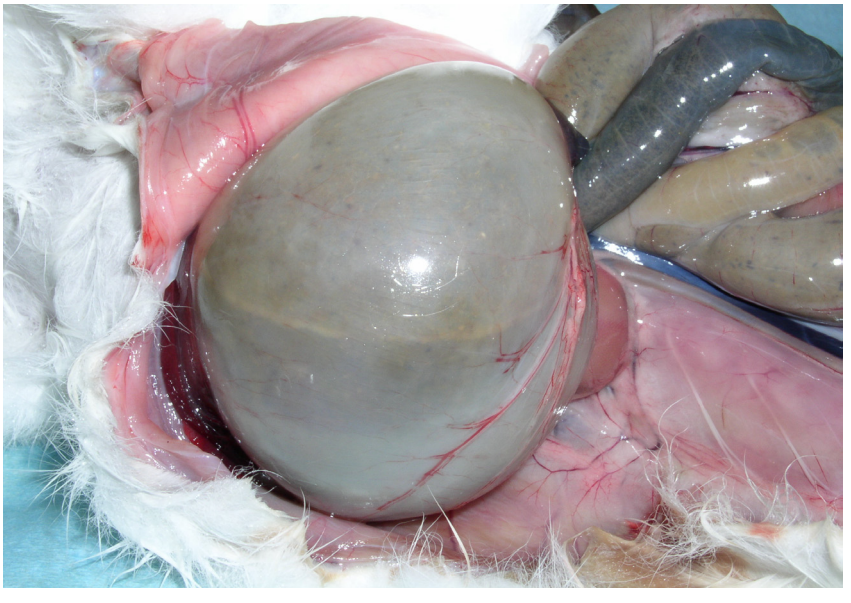

Figure 2. Six-week-old rabbit, dead 5 days after experimental reproduction of ERE with inoculum TEC3. The stomach and small intestin are distended and filled with liquid and gas and are responsible for the bloated abdomen. These lesions associated with the absence of visible inflammation of the intestinal tract can be considered as being pathognomonic of the disease.

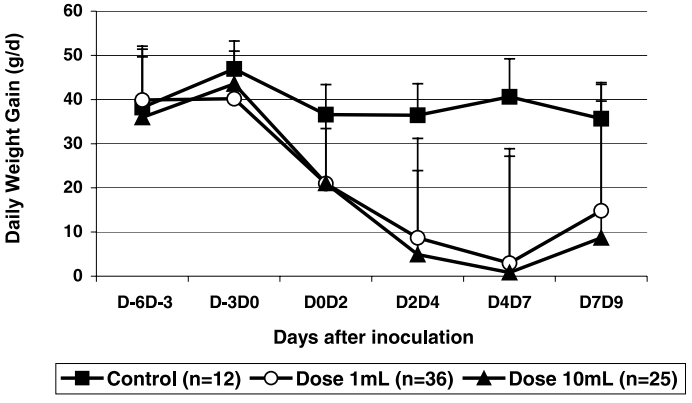

Figure 3. Description of the ERE (Trial B). Daily weight gain of control animals and rabbits inoculated with 1 mL and 10 mL of inoculum TEC2. The results are expressed as the mean ± standard deviation.

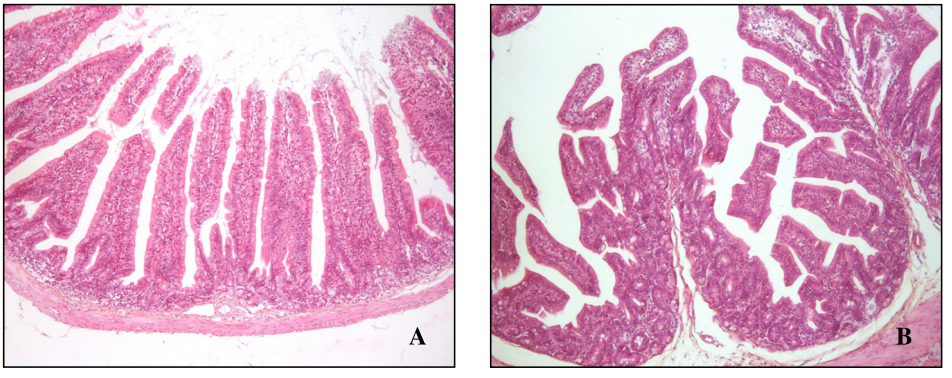

Figure 4. Histological examination of the jejunum of six-week-old rabbits, slaughtered 10 days after experimental reproduction of ERE with inoculum TEC2 (HES x10). (**A**) healthy non inoculated rabbit; (**B**) disease inoculated rabbit. Compared with the control, at the same magnification, the villi of the jejunum part of the small intestine appear shortened with irregular shape and size. The superficial epithelium of the villi, the crypts and the *lamina propria* are normal without any degenerative, hyperplastic or inflammatory visible changes. The lesions are mild and devoid of etiological specificity.

no clinical signs, no decrease in the DWG. Between d0 and d1, the DWG of the uninoculated group was unusually low and therefore no conclusion could be drawn.

In the rabbits inoculated with intestinal contents coming from animals of trial D and euthanized at d2, a significant lower DWG ($P < 0.05$) was observed in the inoculated group on day 2 PI (Fig. 5), associated with signs of rambling noise in 40% of the rabbits. No mortality was observed until the end of the experiment (D6).

4. DISCUSSION

Under field conditions, very few criteria enable a precise diagnosis of the ERE and none of them are specific: increased mortality, presence of mucus under the cages, cecal impaction, inefficacy of usual antibiotics are the most common anatomo-clinical signs usually noticed. Associated or primary pathologies generally mask the specific lesions. Moreover, ERE itself apparently promoted the development of germs rarely

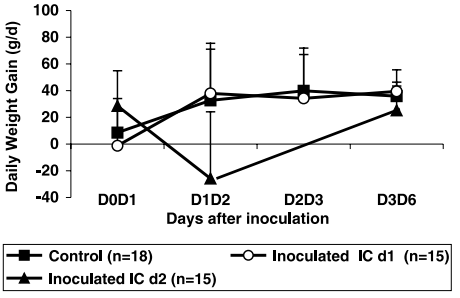

Figure 5. Detection of early virulent material (Trial E). Daily weight gain of control animals and rabbits inoculated with intestinal content (IC) from affected rabbits slaughtered on day 1 (IC d1) and day 2 (IC d2) post inoculation. The results are expressed as the mean ± standard deviation.

isolated before 1997 (*Klebsiella*, *E. coli* O2, *Clostridium*, etc.) [1] and even parasites like coccidia [8]. In the field, the diagnosis is all the more dubious as the general hygienic conditions of the farm are precarious. In the literature, denominations such as "mucoid enteritis", "mucoid enteropathy", etc., are misleading because mucus excretion as well as cecal impaction in rabbits are physiopathological reactions common to several diseases.

The aim of this work was to demonstrate the transmission of the disease and to identify some specific criteria for diagnosing ERE and describing its development. We developed an experimental model based on the use of SPF animals and of an inoculum of reference. SPF rabbits are an experimental tool of choice to overcome interference with all known pathogens. Starting from a field sample (TEC) of intestinal content from diseased rabbits obtained in 1997 during the highest period of contagion in France, the daughter inocula TEC1, 2 and 3 reproduce the same disease. Thus, at least part of Koch's postulate is respected (nevertheless isolation of the etiological agent in pure culture is missing). This material is stable throughtout the constitution of the different inoculum. In the five trials reported, not only ERE was reproduced on a qualitative level, but the evolution of the disease and morbidity were also closely identical. Similar results were obtained with our inocula in more than 2000 conventional rabbits [2, 32]. In the whole trial, morbidity was close to 100%. ERE is an acute disease and the first signs characterized by rambling noise begin one day after contamination. The decrease in the DWG was observed as early as the second day. The peak of the disease was around 5 to 7 days after inoculation. Generally, recovery began one week after contamination, but the cure was slow to settle. Mortality appeared as soon as day 3 with a peak on day 5 and in our experimental conditions, it varied from 20 to 50%. The earliest clinical signs were rambling noise, abdominal bloating and cecal impaction detected on palpation. Diarrhea and the presence of mucus under the cages were not constant and observed a little later. ERE is contagious and when control rabbits are placed in the same room as the inoculated animals they develop clinical signs of the disease, probably because of contamination of the environment by the inoculated groups but this hypothesis cannot be demonstrated as long as the etiological agent has not been found.

At necropsy, the main features were the absence of obvious gross lesions of the mucosa of the different parts of the digestive tract, more precisely an acute inflammatory process or congestion of the small intestine, cecum and large intestine. During the first 4–5 days, distension of the stomach and/or duodenum by a very liquid and gaseous content was almost the only pathognomonical changes. These main features, associated with the distension of the abdomen observed on live animals or before autopsy, should be taken into consideration for field diagnosis of ERE. The frequency of caecal impaction was very variable: generally 20 to 30% of dead animals but it can vary from 0 to 70% [26]. The distal colon was generally empty. In fact, the digestive changes observed at necropsy in the inoculated rabbits obviously indicate physiopathological alterations of the digestive

functions. Everything occurs as if the intestinal transit was stopped for several days, leading to the dilatation of the stomach and small intestine filled with large amounts of liquid and gas and caecal impaction prolonged paresia of the whole digestive tract. This could also explain the multiplication of opportunistic germs under field conditions (*Escherichia coli* O2, *Klebsiella*, coccidia, etc.).

Histological lesions of the intestinal mucosa, characterized by mild to moderate atrophy and fusion of the villi, associated with moderate transepithelial migration of heterophilic cells and mucosal infiltration of inflammatory cells, are mostly observed in some inoculated rabbits sampled for histological examination. Nevertheless the intestinal lesions appear non constant, with some inoculated rabbits being totally devoid of histological intestinal changes. Moreover, the lesions always remain moderate and clearly devoid of etiological specificity and more particularly without lesional kinetics which usually characterizes the development of infectious pathogenic agents. The focal ulcerative lesions described in two inoculated animals appeared to be associated with probably secondary bacterial proliferation emphasized by the digestive paresia. As previously mentioned[3], the histological lesions of ERE appeared mainly non repetitively observed in all affected rabbits and devoid of enough specific aspects to be precisely assigned to an identified pathogenic agent. The macroscopic digestive changes can be considered as the consequences of a physiopathological dysfunctioning and this fact probably explains the obvious discrepancy observed between the gross and histological lesions. Regarding the results of our various trials, we can conclude that gross changes appear as a more reliable tool for diagnostic purposes than histological findings. Nevertheless, it is noteworthy that only light microscopy was used and further examinations by electron microscopy should be made to complete our study.

It has also been shown elsewhere that the disease does not induce any fever [16].

ERE can be characterized by the absence of any inflammatory phenomena, which distinguishes this affection from the other usual rabbit intestinal diseases [22]. However, many similarities exist with a disease previously described as mucoid enteritis or mucoid enteropathy [14, 15, 29, 35]. According to these authors, this disease has decimated large numbers of colonies in Great Britain, the United States and Hungary, the main countries where this disease has been described. The absence of macroscopic inflammation and histological lesions, apart from hyperplasia of mucous cells throughout the small intestine, is also a feature emphasized by Van Kruiningen and Williams [34]. This is why the term mucoid enteropathy was used for mucoid enteritis because there was no visible inflammation of the intestine [12]. As for ERE, none of the authors mentioned above were able to identify a pathogen responsible for the mucoid enteropathy.

During these trials, we were unable to detect a dose effect in the range of 10 to 0.2 mL and further experiments are needed to find the limit of a working dilution. Nevertheless, in trial E, we demonstrate that the unknown pathogen is present in the intestinal content as early as the second day after contamination of animals but we are not stating that it is absent from the intestinal content on day 1 PI. In this trial, with the inoculum from day 2, we could observe a clear but moderate disease without any mortality. Consequently, early samples may be very useful. On a practical level, we first eliminated a part of the flora coming from intestinal fermentation generated by the

[3] Coudert P., Jehl N., Gidenne T., Guittet M., Larour G., Licois D., Persillon C., De Rochambeau H., Note d'information N° 15 sur les travaux de recherche conduits sur l'Entérocolite Épizootique du Lapin, [on line] (2003) http://www.tours.inra.fr/urbase/internet/resultats/enterocolite/noteEntero_2003-15.pdf [consulted January 20, 2005].

intestinal stasis and then limited the multiplication of a certain flora introduced with the inoculum.

The search for a possible pathogenic agent has only revealed the presence of rotavirus and *Clostridium perfringens* in all our inocula, but their role remains questionable [5, 9, 27, 28]. Nevertheless, further etiological investigations are necessary, particularly in bacteriology. Several collaborations are now underway, especially for etiological research. In this way, further characterization of the early events of the disease may be very useful for a better knowledge of the type of agent responsible for ERE.

In conclusion, this work constitutes the experimental basis for studies on this emerging disease within the framework of etiological research led in different European laboratories working with the infectious material.

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