

DOMESTIC RABBIT ENTEROPATHIES

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ABSTRACT

Digestive disorders are the main cause of morbidity and mortality, in fattening rabbit and are responsible for important economic losses in industrial rabbit farms. Among the specific causes of the intestinal pathology, parasites (coccidia) and some bacteria, mainly enteropathogenic *Escherichia coli*, are predominant. Since 1997, a new gastro-intestinal syndrome called epizootic rabbit enteropathy (ERE), close to another digestive pathology, the mucoid enteropathy, has appeared in Europe with a high incidence on mortality and morbidity. The etiological agent of this emergent disease was still not identified for the moment. During the last ten years, thanks to the rise of biotechnologies and molecular biology, considerable progress were obtained with regard to the identification of the virulence factors and to the knowledge of the mechanisms of pathogenicity of the rabbit *E coli*. In parallel, beside coccidiostats available for the treatment of coccidiosis, new prophylactic prospects based on vaccination were developed against the coccidia. In spite of difficulties, an experimental model to study ERE has been established and efforts are concentrated by some teams to identify and isolate the pathogenic agent(s) of this disease. Associated with the understanding of the physiopathology of the gut, at least in part under the influence of nutritional factors (both not developed here), the data obtained in these different areas would lead to new perspectives of control of the rabbit enteropathies.

Key words: rabbit, enteropathy, enteritis, diarrhea, coccidia, *Escherichia coli*, *Clostridium* spp.

INTRODUCTION

The digestive diseases are the main cause of morbidity and mortality, in growing rabbit and are those which are the most prejudicial to the rabbit farmers. In France, before 1997, the mortality in fattening rabbit was about 11-12% (KOEHL, 1997). It increased to 14-15% and sometimes reached more than 50%, in 1997 and 1998, at least in part because of the emergence of a new syndrome called epizootic rabbit enteropathy (ERE) (LEBAS *et al.*, 1997). Moreover, digestive disorders are responsible for significant morbidity characterized by growth depression and poor feed conversion and often induce more important economic losses than mortality. Etiology of the intestinal affections remains still difficult to establish because the causes are often multiple and symptoms, clinical signs and intestinal lesions are often comparable. But one of the clinical sign, the diarrhoea, is largely dominant. It can be encountered in more than 95%

of the cases. Thus, diarrhoea represents a serious economic importance, especially in young rabbits after weaning (4 to 10 weeks). Sometimes, diarrhoea can be present in suckling rabbits, rather during the pre-weaning period (3rd 4th week of age) or more rarely in adults where it generally represents the ultimate consequence of another affection. The causes of diarrhoea, and more generally of enteritis, are of two kinds: on the one hand they can be related to the direct intervention of pathogenic agent (virus, bacterium, parasite) or in the other hand related to a disorder of the digestive functions under the influence of favouring factors such as genetic predisposition of the animal, food, stresses. In industrial fattening rabbit farms, until the appearance of the ERE, the main pathogenic agents associated with these digestive pathologies were primarily of parasitic origin (*Eimeria* spp.) and/or bacterial origin (chiefly, enteropathogenic *Escherichia coli*; sometimes *Clostridium spiroforme* and *Klebsiella* and exceptionally, *Clostridium piliforme*). Some viruses can be observed (mainly rotavirus) but their role remains questionable. The remaining cases of diarrhoea which cannot be ascribed to a precise etiology are generally gathered under the term of "non specific enteritis" (BENNEGADI *et al.* 2000).

The purpose of this presentation is to deal with the main intestinal diseases encountered in industrial rabbit farms with special emphasis on coccidiosis, colibacillosis and ERE and on the acquired knowledges, in particular during the last decade.

PARASITES

There has not been a lot of new data on rabbit coccidia during the last years but we think it is important to recognize that coccidia are true pathogens that are always present in rabbit farms. Indeed, they are impossible to eliminate, in field conditions. In addition, their role in intestinal pathology have been recently amplified by the ERE syndrome (COUDERT *et al.* 2000a, 2003a). Besides, works are in process in order to obtain live attenuated strains that could be used as vaccine.

Coccidia

Coccidia of the genus *Eimeria* are common parasites in the digestive tract of the rabbit. They have an intra-cellular development and constitute a major etiology of the intestinal disorders and complications of parasitic origin in rational rabbit breeding units. Other genus like *Cryptosporidium* or *Toxoplasma*, which are also coccidia, can parasitize the rabbit but they are practically never seen in rational breeding. Thus, we will only focus here on the *Eimeria*.

From a taxonomic point of view, *Eimeria* belong to Protozoa (branch), Apicomplexa (phylum), Sporozoa (class) and Eimeriidae (family). The oocysts of the genus *Eimeria* are characterized by 4 sporocysts containing each one 2 sporozoites. The oocyst is the form of conservation of the parasite in the external medium. It is characterized by an extraordinary resistance, in particular in time and to the chemical agents. But it is very susceptible to heat and dessication.

Eimeria are monoxenes (only one host) and have a very strong specificity with respect to their host. They develop in the epithelial cells of the digestive system: intestine for intestinal coccidia and bile ducts for the hepatic coccidia *Eimeria stiedai*.

If synonymies are excluded, one can say that a dozen species of *Eimeria* parasitize rabbit. The description of these different species has been reported by ECKERT *et al.*, (1995). Identification of the various species is based primarily on the morphological criteria of the oocyst and it can be done effectively only on sporulated oocysts; however it is not always easy to perform because within the same species it exists a great variability regarding especially the size and the form of the oocyst and that can lead to certain confusions (between *E perforans* and *E media* or *E irresidua* and *E flavescens*, for example). Other characteristics make it possible to identify the coccidia (prepatent period, duration of sporulation, localization along the intestine (COUDERT *et al.*, 1995), and also genomic DNA profiles (CERÉ *et al.*, 1995)).

The life cycle of *Eimeria* includes distinct parts and lead to the production of a huge number of parasites. The multiplication factor for almost all the species is 1 to 3×10^6 oocysts produced for one inoculated oocyst. A rabbit can produce up to 1×10^9 oocysts of *Eimeria intestinalis* for example (LICOIS *et al.*, 1990).

Coccidiosis

The importance of the coccidiosis which affect especially the young rabbits after weaning, is due to several factors: these affections reach the digestive tract causing a stop or a brake of the growth; the great capacity to multiply, associated with the extraordinary resistance of the oocysts, ensure their persistence in the environment; it does not exist rabbits free of coccidia, apart from certain research laboratories; the coccidia persist always, in particular in the adult animals which constitute healthy carriers; the does transmit the coccidia to all their progeny; the contamination by a given species can be done by the introduction of the males.

As there are several species of *Eimeria*, one can say that there are several coccidiosis: the hepatic coccidiosis on the one hand and the intestinal coccidiosis on the other hand.

Hepatic coccidiosis, in rational breeding, causes economic losses only at the slaughter, when the liver is punctuated by whitish nodules. Indeed, under natural conditions of infestation the hepatic coccidiosis is never mortal and seldom involves reduction of performance. Moreover, this coccidiosis is relatively easy to eliminate by very strict medical and hygienic measures during a few weeks and by medical prevention of the disease. The traditional coccidiostats distributed in food on a purely preventive basis during 4 to 6 weeks practically make disappear this disease.

Intestinal coccidia are specific pathogenic agents because when inoculated to animals, they cause (for those which are pathogenic), in 100 percent of these animals, the same lesions and the same clinical signs and symptoms (diarrhoea, reduction of food and water consumption, growth depression, mortality).

Pathogenicity is not identical for all the species. On the basis of only the criteria of mortality and growth, intestinal coccidiosis of rabbit can be classified in 4 categories (COUDERT *et al.*, 1995).

- Non-pathogenic coccidia: *E. coecicola*.
- Little or very little pathogenic coccidia: *E. perforans*, *E. exigua* and *E. vej dovskyi*
- Pathogenic species: *E. irresidua*, *E. magna*, *E. piriformis* and *E. media*
- Highly pathogenic species: *E. intestinalis* and *E. flavescens*.

From the histological point of view, only an hypertrophy of the enterocytes can be noticed, the cellular structure remaining intact except at the time of release of the oocysts where the cells burst and desquamate.

Immunogenicity and immunity: although inoculation of coccidia involves the development of circulating antibodies, those are not protective. Thus the mother does not transmit any protection to her young rabbits (DROUET-VIARD *et al.*, 1996). Only cell-mediated immunity confers a real protection with respect to these parasites in the animals. But there is no cross immunity: rabbits contaminated by *E magna* will be protected against a challenge with the same species but not against a challenge with heterologous species. Immunogenicity varies from one species to another. With *E intestinalis*, we need only few oocysts (600) to observe a complete protection (COUDERT *et al.*, 1993). On the opposite *E perforans* (LICOIS *et al.*, 1992) or *E. flavescens* are not very immunogenic. In a general way, immunity settles quickly and is efficacious: for *E intestinalis* for example, protection is still complete, 6 months after the primary infection. Because of the acquired immunity with age, coccidiosis are thus a disease of the post weaning period. However a microbial infection or a stress can cause escape immunity.

Diagnosis is often particularly difficult to pose. It can be made only at the laboratory level, by making an examination of the digestive tract but also by enumerations of coccidia in the excreta, providing that several of them will be carried out, spaced by few days. But it is especially necessary to identify the species of *Eimeria* which are present.

Physiopathology: intestinal coccidiosis interferes with intestinal function. It causes villous atrophy (PEETERS *et al.*, 1984) and impairs intestinal motility (FIORAMONTI *et al.*, 1981) leading to bad feed conversion and growth depression. Peculiarities of rabbit's diarrhea have been first described for rabbit coccidiosis and confirmed later for other intestinal pathologies. It is mainly observed: hydration but reduction of the volume of faeces excreted by ill animals, severe hypokalemia and absence of acidosis and of extracellular dehydration. That distinguishes the rabbit diarrhea from that of the other mammals (LICOIS *et al.*, 1978a, b).

Treatments and prophylaxis *

Curative treatments: from this point of view, the sulphamides used in drinking water are always effective products against the coccidia. Tests carried out in our laboratory have shown that the sulfadimethoxine is very active at 0,8 ‰, that the sulphaquinoxaline must be used at least at 3 ‰, and finally that with 2 ‰, the sulfadimerazine is not very effective. Toltrazuril (Baycox), which has not a market approval for the moment in rabbit, is effective but, as for sulphamides, a treatment must last at least 3 days. Another molecule, the decoquinate, utilizable at 70 ppm by food, constitutes another alternative.

Medical prophylaxis

Chimioprévention: Since the beginning of the years 1980, the coccidiostat robenidine, very effective in particular against the very pathogenic *Eimeria*, was available for the rabbit and appreciably reduced the importance of the coccidia in rational breeding. During more than 20 years it was the only anti coccidial drug, used in supplementation in

food, on the market. More or less correct use of this product had made it possible the development of chimo-resistances (PEETERS and GEEROMS, 1992). The diffusion of chimo-resistant coccidia (*E. magna*, *E. media* and *E. perforans*) is now generalized. However the robenidine, currently commercialized under the name of Cycostat 66G, used in additive at 66 ppm, remains a molecule of choice with respect to all the other species and in particular against the most pathogenic (*E. intestinalis* and *E. flavescens*) (COUDERT and ZONNEKEYN, 2000). It is also the only one which is utilizable at the same time in growing rabbits and in maternity. Indeed, other molecules were tested in rabbit (COUDERT *et al.*, 2000b). Thus, salinomycin is a second molecule which is authorized in Europe, at 20ppm, but in fattening rabbits only.

Vaccination: If the only method of prevention of coccidiosis is the chimioprophylaxis, the disadvantages of this method are multiple, ie: very few molecules currently available for the rabbit; costs of the veterinary treatments, because all the rabbits, including the reproductive animals must receive a supplemented food with coccidiostat; acquisition of chimo-resistances; hesitation of the firms to invest in the search for new products owing to the fact that the market of rabbit is too narrow; and also the increasingly strong reserve of the consumers regarding the use of this type of livestock product. In an other hand, efforts of diversification of the rabbit breeding multiply now in France, especially in the areas of traditional production. These attempts raise against the problem of coccidiosis because the supplemented food cannot be used any more during all the cycle of production. This means that in the medium term for the intensive or short-term for the breeding of diversification, vaccination seems the only solution in the future.

To date only inoculation with live coccidia has been found to provide sufficient protection against coccidiosis. Precocious lines deriving from wild strain and obtained by selection for early development of oocysts have been extensively studied in poultry and proposed as vaccine candidates. These lines, less pathogenic than the wild strains remain immunogenic and are able to induce a good protection. Two vaccines (Livacox and Paracox) comprising several precocious lines corresponding to different avian species are commercialized in Europe and used with efficacy (SHIRLEY *et al.*, 1995, WILLIAM *et al.*, 1999). Similarly we have obtained several precocious lines of rabbit *Eimeria* (LICOIS *et al.*, 1990, 1994, 1995). These lines are drastically less pathogenic than wild parental strains (by 500 to 1000 times) and have been proven to be stable and to totally protect animals against challenge with the corresponding wild strains. Modalities of utilization have been tested with *E. magna*: age at vaccination, doses, methods... (DROUET-VIARD *et al.*, 1997a, b, c), the better way being spraying the nest box, when rabbits are 25-day-old, with less 3500 oocysts.

Obtention of other precocious attenuated lines, at least for the more pathogenic species (*E. flavescens*, *E. irrisidua*, *E. piriformis*...) is necessary to the development of such a vaccine against rabbit coccidia utilizable in the field. This is now in hand and we hope that this will succeed in a near future.

* **NB:** the molecules available and their use in the rabbit can change according to the rules of each country.

BACTERIA

Colibacillosis

Colibacilli (*Escherichia coli*), are normal inhabitants of the intestinal tract of numerous animal. They can also represent one of the most important etiologic agents in animals and, in developing countries, in childhood diarrhea. Diarrheagenic *E. coli* have been classified into at least five categories (NATARO and KAPER, 1998), including enteropathogenic (having *eae* gene but not *stx* (encoding shiga-like toxins): EPEC), enteroinvasive *E. coli* (having invasive plasmid genes: EIEC), enterotoxigenic *E. coli* (having *est* and/or *elt* (encoding thermostable or thermolabile toxins): ETEC), enterohemorrhagic *E. coli* (having *stx*: EHEC) and enteroaggregative *E. coli* (producing specific adhesion to Hep-2 cells and having a 60 MDa specific plasmid: EAEC). Because of the inhibitory influence of the caecal volatile fatty acids (PROHASKA, 1980), the gut of healthy weaned rabbits contains low levels of *E.coli*. Some of the animals even harbor no colibacilli flora (PADILHA *et al*, 1996). EPEC is the only known class of *E. coli* in rabbits which induces acute intestinal pathology marked by inflammatory lesions of the distal part of the gut where these *E. coli* are strictly located (ileum, caecum and colon). Thus, they cause important losses after weaning and sometimes before weaning.

EPEC (as well as EHEC) induce a specific attaching and effacing (A/E) lesions (MOON *et al*, 1983) on brush border surface of enterocytes characterized by effacement of microvilli, intimate attachment of bacteria and formation of an actin-rich pedestal beneath intimately attached bacteria (PEETERS *et al*, 1985, LICOIS *et al*, 1991).

It is not our purpose here to do a long development about rabbit EPEC. For that you can refer to the review of Milon made in 1996 at the time of 6th WRC (MILON 1996) and of MILON *et al* (1999) and those more devoted to human EPEC strains (DONNENBERG and KAPER, 1992, FRANKEL *et al.*, 1998). I only would to remember in the broad outline, the knowledges acquired since the beginning of the nineties and the progress made concerning the virulence factors associated with the mechanisms of pathogenicity. They have been intensively studied, particularly by mean of molecular biology, greatly also on the human reference EPEC strain E2346/69 (O127:H6). This has allowed to better characterize the EPEC strains and to develop new strategies of vaccination.

Virulence factors and pathogeny

The understanding of mechanisms of EPEC involving bacterial factors and host responses mainly results of *in vivo* studies and *in vitro* models (cultured epithelial cells). A three steps model was first described by DONNENBERG and KAPER (1992) which involves initial attachment of the bacteria to the host epithelial cells, then, signal transduction and phosphorylation of host proteins and finally, intimate attachment of the bacteria to the enterocyte apical surface.

The first stage corresponds to a loose adhesion mediated by specific attachment factors which are plasmid encoded fimbrial proteins called adhesins. They allow the bacteria to colonize the gut and to oppose to non-specific resistance mechanisms such as peristalsis. In the rabbit, adhesins AF/R1, described in O15 *E. coli* RDEC1 strain (INMAN *et al*, 1986) or AF/R2 in O103 serogroups (FIEDERLING *et al.*, 1997) are concerned. The type IV-like fimbriae such as bundle forming pili (BPF) described in human EPEC strains have never been identified in rabbit EPEC strains (ROBINS-BROWNE

et al., 1994). Detailed adhesion of EPEC to host cells has been reviewed by NOUGAYRÈDE *et al.* (2003).

The second and third stages lead to a close contact between bacteria and the target enterocytes. This needs a set of proteins encoded by chromosomal genes responsible for A/E lesions located in a pathogenicity island, the Locus of Enterocyte Effacement (LEE) (MC DANIEL *et al.*, 1995). The LEE encodes several proteins with a wide range of function, most of them being secreted *via* a type III secretion system (TTSS). Various secreted effector proteins and their chaperones are also encoded by genes of the LEE (DONNENBERG *et al.*, 1997, ELLIOTT *et al.*, 1998). In the central region of the LEE, the *eae* gene encodes an outer membrane protein called intimin which allow the close contact between bacteria and the target cells, thanks to the Tir, the translocated intimin receptor (MARCHÈS *et al.*, 2000). Tir is secreted by the TTSS and translocated through the cell membrane by a bacterial syringe comprising several effectors: EspA, EspB and EspD, (for *E. coli* secreted proteins); all of them are needed for signal transduction and to induce the A/E lesions.

From a pathogeny point of view, it is important to mention that a new toxin called Cif (for Cycle Inhibiting Factor) has been described in EPEC (and EHEC) strains (MARCHÈS *et al.*, 2003). This toxin encoded by gene not located in the LEE mediates cytoplasmic stress fibers accumulation responsible for cytopathic effect on epithelial cells and blocks the cell cycle at G2/M transition (DE RYCKE *et al.* 1997).

Characterization and Epidemiological data

The strain of rabbit *E. coli* where first identified by their O antigen (serogroup). But it appeared rapidly that this criterion to define a pathogenic strain was not sufficient. Indeed within some serogroup like O103:H2:K- for example, we can have pathogenic and non pathogenic strains. The biotype (ability of a strain to ferment certain sugars) proposed by OKERMAN and DEVRIESE (1985) and CAMGUILHEM and MILON (1989) offers the possibility to better identify highly pathogenic strains. The most important pathotype is 8+/O103 (rhamnose négative), in France and 3-/O15, in Belgium, which are highly virulent, whereas the pathovars 2+/O128 and 2+/O132 are less pathogenic (PEETERS *et al.*, 1988). Now new molecular targets are available. Thus, BLANCO *et al.* (1996, 1997) have added the search for the *eae* gene to better identify virulent pathovars and to carry out epidemiological studies. In this congress some papers are concerned by this type of approach (AGNOLETTI *et al.*, 2004; CAMARDA *et al.*, 2004; D'INCAU *et al.*, 2004).

Treatments and Prevention

Antibiotherapy to treat or prevent EPEC diarrrhea in the rabbit is not the best solution for a lot of reasons: the peculiarities of the digestive physiology and of the normal flora; the high susceptibility of the rabbit to some of antibiotics (certain macrolides, or β -lactames are toxic) (LICOIS, 1996); the difficulties to solve the problems on the field; the high costs of these treatments; the acquired multiresistance of *E. coli* strains and the potential diffusion of antibio-resistance in other bacteria from the gut; the fact that even after a successful treatment a proportion of animals are always healthy carriers.

Therefore, the better way to prevent the colibacillosis remains to have an efficacious vaccine. Early studies carried out by A. Milon and his team against O103 strains have

given encouraging results. Formaldehyde-killed bacteria orally inoculated at high doses protect the rabbit against a challenge (CAMGUILHEM and MILON, 1990), but the method is difficult to apply in the field (heaviness of schedules, doses and protocols). Inoculation of live vaccine using the strain C6 of serogroup O128, less pathogenic than O103 B10 strain, but both sharing the LEE and AF/R2 adhesin, induces partial protection against the B10 strain (MILON *et al.*, 1992).

The better knowledge of the virulence factors, in particular those mediated by the genes of the LEE and the development of the molecular engineering have made it possible to propose new strategies of oral vaccination. STAKENBORG *et al.*, (2001) have shown that the construction of an avirulent strain of the pathovar 3-/O15, deleted in the *eae* gene resulted in a complete protection against homologous virulent strain. In addition, ELISA test using EspB has shown that the deleted strain persists a long time in the gut inducing a specific immune response. Nevertheless this mutant offers insufficient protection against other pathovars (4+/O26, 8+/O103 and 2+/O132). In another experiment BOHEZ *et al.* (2004: this congress) have demonstrated that a 2+/O132 strain deleted in the *Tir* gene is able to protect against an heterologous challenge with a 3-/O15 strain but to partially protect against an heterologous challenge with a 8+/O103 strain. Using a double mutant (inactivation of genes *Tir* and *EspB*) of the pathogenic O103 strain E22 as live vaccine, BOULLIER *et al.* (2004: this congress) have reported that rabbits challenged by the parental E22 strain were resistant to colonization by E22 strain and totally protected. Moreover the mutant induces a specific humoral response against the adhesin AF/R2 but cross protection against other pathotypes has not been tested. These authors agree with the fact that the virulence factors encoded by the LEE are not sufficient to induce a protective immune response and that others factors like lipopolysaccharides (LPS) or colonization factors like fimbriae could be involved. Anyway it can be claim that a lot of progress has been performed on the knowledge of the pathogenicity factors of the rabbit EPEC and that should lead to propose in a near future an efficacious vaccine that could be an association between different avirulent mutants.

RABBIT EPIZOOTIC ENTEROPATHY

Since the last congress in Valence in 2000, several works have been carried out in different directions: better characterization of the disease for diagnosis purpose, research of the etiological agent which remains still unknown for the moment, better control of the pathology by mean of treatments or preventive measures...

Development of an experimental model and study of the disease

Up to 2001, although experimental reproduction of the REE succeeded from a qualitative point of view since 1997 (LEBAS *et al.* 1997-2001, LICOIS *et al.*, 2000), we encountered many difficulties to obtain constant results from a quantitative point of view. From one trial to another, intensity of the disease (mortality, morbidity, importance of the gross lesions) varied in a significant way. This variability was also found for the delay of appearance of the disease after inoculation: 2 to 12 days. To reduce this variability, different methodologies were used and several methods of experimental contamination were tested: immunosuppression of the animals (LICOIS *et al.*, 1998), intubation via the oesophagus, and pulverization of the inoculum on the animal or on the food. Inoculums

of different nature were tested (lung, ganglia, mesenteric lymph node, blood), without constant success except with intestinal contents. Finally infectious material obtained from various breedings suspected to be concerned by ERE were tested but they were generally contaminated with other pathogenic agents. Thus one of the main objectives was to develop an experimental reliable and reproducible model, based on the use of specific pathogen free (SPF) rabbits and on a virulent material originating from intestinal content of contaminated animals. This was performed in mid 2001 with the constitution of an inoculum of reference (TEC1), starting from several samples originating from several protocols carried out with the first sample used as inoculum, denominated TEC, kindly provided by P. HERVOUET and P. ROBART, at the end of 1997. These samples came from intestinal contents of about twenty sick or died animals, between day 3 and day 8 post inoculation (D PI), in order to cover most largely possible the duration of the disease (LICOIS and COUDERT, 2001). Then, other inoculums (TEC2, TEC3 and now TEC4) deriving each time from the precedent one were obtained.

Using SPF rabbits it was possible to confirm the specific clinical and lesion features of ERE, which distinguishes it from the symptoms and lesions of other known intestinal affections. They are the same as those observed on the field. Briefly remember that animals are bloated with watery diarrhea of low intensity. Gross lesions are mainly a distension of the whole intestinal tract including the stomach which is fill with gas and fluid. No inflammation or congestion of the intestine is visible. These symptoms are sometimes associated with caecal paresis and the presence of mucus, especially in the colon. The earliest clinical sign detected as soon as D2 PI are borborygmus ("rumbling noise"). To better define the precocious events that occur at the onset of the disease, several works are in process in our lab (COUDERT and LICOIS, 2004: this congress).

In addition, in order to enlarge the representativity of samples that could reproduce the disease, two inoculums originating from the Nederland and from Belgium were prepared in 2002, at the veterinary medicine of Liège (Belgium). They were characterized at the virological, bacteriological and parasitic level (MARLIER *et al.*, 2003). They were tested on SPF animals in our experimental fittings and have been proven to reproduce the ERE (LICOIS *et al.* 2003a). These samples originating from the field and obtained out of France, 5 to 6 years after the appearance of the ERE in Europe, constitutes an additional biological material for the studies on this pathology, in particular within the framework of the search for the etiological agent.

Histological study

According to the fact that it was difficult to obtain clear data on histological lesions, greatly due to the use of field animals, an histological research on ERE has been proceeded with M. Wyers (Ecole Nationale Vétérinaire de Nantes, France), two years ago, in comparison with colibacillosis (EPEC strain O103:H2:K- Rh-) and coccidiosis (*Eimeria media* and *E. magna*), all in SPF animals.

The two last infectious models (*E. coli* and *Eimeria*) induce almost identical lesions of diffuse, acute to subacute, atrophying, erosive and regenerative enteritis localised to the intestinal part colonized by these microorganisms. These lesions are distinguished thanks to the presence of the pathogenic agent, protozoa or Gram- bacilli. In the case of *E. coli* infection, intestinal lesions are accompagnied by a severe lymphoid depletion of the mesenteric lymph nodes, of the vermiform appendix, spleen and thymus, being able to be responsible for a transient immunodepression. Coccidia and *E. coli* O103 are also

responsible for lesions whose kinetics is similar, with a peak of severity of the lesions, then reduction in intensity and finally persistence of "sequelas", even in absence of the implicated pathogen. These similarities underline, in the absence of the pathogenic agent, the non-specific character of these lesions.

In the case of ERE, it was noted in the majority of rabbits, the presence of generalized lesions throughout the intestine, with atrophy and fusion of the villy, transepithelial infiltration and migration of viable or pycnotic inflammatory cells. These lesions are regarded as non-specific. Moreover, it has not been observed a lesional kinetic which is characteristic of the development of a pathogenic agent. Lastly, it is important to underline the fact that, although rabbits were carefully selected according to precise symptoms evoking ERE, several animals did not present any intestinal lesion. Under these conditions, it is thus difficult to conclude on the implication of a precise kind of pathogenic agent (bacterium, parasite or virus) in the development of ERE. Neither macroscopic nor histological lesion has been observed on the lungs, heart, liver, spleen or kidneys but an atrophy of the vermiform appendix was sometimes noted.

Therefore, it can be conclude that histology does not offer a satisfactory mean of diagnosis. Only some evident criteria of gross lesions, such as the distension of the stomach or of the small intestine, with a liquid content, observed on a sufficient number of animals, associated with parasitologic and bacteriologic analysis, allow to establish the diagnosis of ERE.

Search for pathogenic agent

Virus

At the onset of the ERE, several arguments supported a viral hypothesis, particularly the epizootic nature and the diffusion of the pathogen, the transmissibility of the disease and the existence of histologically-proven lung lesions suggesting a viral infection (WYERS, 1998). In addition there was the ineffectiveness of the majority of common antibiotics used on the field at the onset of the disease. Thus, up to the end of 2000 the search for a virus as the etiological agent of the ERE has been carried on. All the methods classically involved in virology and used at INRA (Institut National de la Recherche Agronomique, Tours, France), at AFSSA (Agence Française de la Sécurité Sanitaire des Aliments, Ploufragan, France) and in private firms failed to assure that a virus was involved in ERE. The only viruses sometimes found on field samples were rotavirus (CERÉ *et al.*, 2000; MARLIER *et al.*, 2003) but it was not possible to reproduce the disease with rotavirus strains isolated from animals with ERE. In the same way the potential role of bacteriophages was considered. Indeed bacteriophages, in particular the caudal bacteriophages were found in sick rabbits. An experimentation led at AFSSA has not allowed to reproduce the ERE with this type of phages (LE GALL-RECOLÉ *et al.* 2002 <http://www.tours.inra.fr/urbase/internet/resultats/enterocolite/entero1.htm>)

The search for other enterotropic viruses were all negative: calicivirus, pestivirus, circovirus, adenovirus, coronavirus, parvovirus (G. LE GALL, AFFSSA, 22440 Ploufragan, France). Consequently, at this time, in the absence of new element, it was decided to suspend the research in virology.

Bacteria:

The role of enteropathogenic strains of *E. coli* has been excluded since the beginning of the studies on ERE. Indeed this bacteria was sometimes found in rabbits with ERE but

the serotypes of the isolated strains were not constant. Moreover, most of the field samples we have obtained from sick rabbits with symptomatology of ERE do not harbour any *E. coli*. In addition, *E. coli* are absent from our inoculums. It is interesting to note that in direct examination the microflora of these inoculums is poor and unbalanced with dominant Gram+ and *Clostridium spiroforme* was never detected (Milon, personal communication). In the opposite, the implication of other *Clostridium* and particularly of *C. perfringens* in the genesis of the disease is suspected for a long time. Several reasons are invoked: ie similarity of the symptoms with those of enterotoxemia (dilatation due to gas in the intestine), bacteria often encountered in field rabbits (DEWRÉE *et al.*, 2003, LE NORMAND *et al.*, 2003), bacteria present in the inoculum TEC2, TEC3 (LICOIS *et al.*, 2003a; MARLIER *et al.*, 2003), efficacy in rabbit farms of some of antibiotics directed against Gram+ bacteria.

A large-scale bacteriological study has been carried out in collaboration with the team of D. Marlier in Belgium starting from their field samples and from our inoculum TEC3. A lot of strains have been isolated after aerobic or anaerobic culture, among them various strains of *Clostridium* including *C. perfringens* (DEWRÉE *et al.* 2003, MARLIER *et al.* 2003). But, none of the bacterial strains used alone or in mixture has been able to reproduce a pathology (LICOIS *et al.* 2003). Thus, if the role of bacteria is the good assumption, either a non-cultivable bacterium may be concerned, or, this one does not express its virulence factors.

Nevertheless, strains of *C. perfringens* were isolated in approximately 80% of rabbits suffering of ERE originating from rabbit farms of Belgium and The Nederland (MARLIER *et al.*, 2003). These strains have been characterized (DEWRÉE *et al.*, 2003). Broadly, 66% and 34% of them respectively belong to the A and C toxinotypes, whereas it is classically admitted that only type E is involved in rabbit intestinal pathology (PERCY *et al.*, 1993). The gene encoding the enterotoxine was identified in 73% of the studied strains. The search by PCR for α , β and $\beta 2$ toxin has revealed that on 86 dead rabbits (32 with symptoms of ERE and 54 of other intestinal pathology), 69% were positive for α toxin among dead rabbits with ERE instead of 16% among the other dead animals. The gene encoding $\beta 2$ toxin was only detected in one strain isolated from a dead rabbit with ERE. LE NORMAND *et al.* (2003), confirms the prevalence of the α toxin gene among strains (20/38 strains) isolated from dead rabbits with ERE. They indicate also that 17/38 strains were simultaneously $\alpha\beta 2$ toxin positive while none was β positive. These same authors establish a relation between the toxinotype and the clinical aspect of the intestinal lesions. The α toxin positive strains are more frequent in rabbits having liquid caecal content whereas the strains $\alpha\beta 2$ dominate in those having compacted caecal content. This observation has to be confirmed on large-scale field studies.

Even if all these studies show the interest in studying *C. perfringens*, it remains that the role of this bacteria as primary pathogen is still questionable. Indeed, no experimental reproduction of ERE has been obtained after inoculation with strains of *C. perfringens* (LICOIS *et al.*, 2003a, MARLIER *et al.*, 2003). Moreover, this bacteria as well as its main toxin were absent of numerous experimental samples originating from acute phase of successful reproduction of the disease (MARLIER *et al.*, 2003).

Molecular approach:

As we have seen above, works aiming at identifying a virus or a cultivable aerobic or anaerobic bacterium have not yet succeeded. Thus, other trails must be explored. That is why a molecular approach based on analysis of nucleic acids originating from sick vs healthy rabbits, was engaged by a French private company. In order to avoid the use of intestinal DNA and RNA rich in genomes other than that of the organism responsible for the disease, it was decided to carry out sampling of air in the rooms where the animals were placed (even if the transmission of ERE by air has never been demonstrated). Samples obtained at various times throughout the experiment were tested for virulence (LICOIS *et al.*, 2003b) and then chosen for molecular studies which are still in process.

Treatment and prevention

Very few efficacious antibiotics can be used to fight ERE. Only one, tiamulin has to date, a market approval for a 32 ppm use in food, according to French rules. Another molecule, bacitracin was available since 1998. The effectiveness of Zinc-bacitracin at 100ppm in food has been demonstrated (DUPERRAY *et al.*, 2000, 2003). Approval for Bacivet S® (soluble bacitracin) is in hand. This last molecule was studied after experimental reproduction of ERE at 0.657g/l of drinking water, before (preventive use) or after (curative use) contamination of animals with TEC3. The results indicate that the preventive use of Bacivet S® is as effective as 100ppm of bacitracin in food used during the acute period of the disease (BOISOT *et al.*, 2003a). The curative use of Bacivet S® during 14 days after the onset of symptoms reduces mortality and morbidity compared to control animals but is less effective than the preventive use. A field study has also shown that the tylosine (Gram+ spectrum macrolide) could be used as an alternative antibiotic to control ERE (BOSTVIRONNOIS and MOREL SAIVES, 2003). The better results have been obtained when tylosine is associated with apramycin (Gram-spectrum) confirming previous results on apramycin of BADIOLA *et al.* (2000). Indeed the development of secondary infections and incidence of complication are frequent in field situation. It is true for bacteria but also for coccidia (COUDERT *et al.*, 2000). In a recent epidemiological survey in France it has been shown that a high mortality rate in growing rabbit, due to ERE was associated with the highest level of parasitism (COUDERT *et al.*, 2003a). In addition COUDERT *et al.* (2003b) have shown that two molecules: salinomycin (against coccidia) and tiamulin (against ERE), can be used simultaneously, without incompatibility, in growing rabbits.

However, for the same reasons invoked to fight coccidiosis or colibacillosis, other methods than antibiotherapy should be used. That is why nutritional approaches constitute other strategies to prevent the health of the rabbits (GIDENNE *et al.* 2003). BOISOT *et al.* (2003b) have shown that in rabbit inoculated with TEC3, a feed restriction, at least of - 20% of the *ad libitum* level of the consumption of the control animals, leads to a reduction of the mortality and of the morbidity due to ERE. In an other hand, an attempt to use genetic variability of animals to select rabbits resistant to ERE was intended by DE ROCHAMBEAU *et al.* (2004, this congress).

CONCLUSION

Noticeable progresses were made these last years on the knowledge of the pathogenic organisms concerning the gastrointestinal tract of rabbit, in particular concerning the EPEC. It probably remains very much to do from a fundamental point of view. Promising vaccine solutions against the EPEC strains and also against the pathogenic coccidia should succeed in the future. Efforts must be continued to improve diagnostic, to identify the etiological agent of ERE and about epidemiology without forgetting the rules of hygiene and the management of the rabbit farms. All this should contribute to minimize the utilization of drugs (antibiotic, coccidiostats...), which is important for the consumers, but also for the rabbit farmers and for the rabbit farming.

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