Proceedings 4th World Rabbit Congress, 10-14 October 1988, Budapest Hungary, Vol. 3, 325-333

PROTECTION OF RABBIT AGAINST EXPERIMENTAL *Escherichia coli* O-103 INTESTINAL INFECTION BY ORAL FORMALIN-KILLED VACCINE.

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Though coccidiosis is in regression, life-threatening digestive troubles are sometimes observed in weaned rabbits, during fattening period. The disease has been shown to be associated with the proliferation of *Escherichia coli* in the digestive tract of these animals. Several authors have described enteropathogenic strains, resembling the human enteropathogenic *E.coli* strains (E.P.E.C.), which could induce severe diarrhoea in weaned rabbits, after experimental oral administration (RENAULT L.*et al.*,1983; PEETERS J.E.*et al.*,1984; CAMGUILHEM R.,1985).

An epidemiologic survey realized in France from 1984 to 1987 in 119 industrial rabbit farms with diarrhoea problems showed that 53,5 p.100 of *E.coli* strains isolated belonged to O-103 serogroup.

Vaccination trials in weaned rabbits with formalin-killed O-103 whole bacteria, by intradermal route, did not protect the animals against oral challenge with pathogenic *E.coli* O-103 (CAMGUILHEM R.,1986a). Parenteral or oral vaccination of mothers could not protect young rabbits (CAMGUILHEM R. and MILON A.,unpublished). In this paper, we report successfull protection of weaned rabbits by formalin-killed vaccine given *per os* for ten consecutive days just after weaning.

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MATERIAL AND METHODS

1 - Animals.

32 New-Zealand male rabbits (INRA strain A 1077) from 8 different litters were used in this experiment. At 29 days of age (mean weight: 528 $\pm$ 70 g.),they were weaned and shared into three groups. Two groups, A and B (eight animals each) were vaccinated with two different vaccines. Group C (16 animals) remained unvaccinated as a control group. During vaccination period, vaccinated animals were housed in individual cages, while controls were kept in 4 cages. After challenge, all the rabbits were redistributed in cages of 2 animals. They were fed with a coccidiostatic supplemented feed (Robenidine) and water was given *ad libitum*.

2 - Vaccines.

Two different vaccine preparations were used :

Vaccine A : *E.coli* O-103/10 strain (CAMGUILHEM, 1986a, 1986b) was cultivated on Trypticase-Soy Agar, resuspended in RINGER buffer, inoculated to Trypticase-Soy Broth and incubated for 24 h. at 37°C. Culture was then treated with 4 p.1000 of formalin, and cell concentration was adjusted at 2.10^9 CFU/ml. Vaccine A was used to vaccinate group A.

Vaccine B : *E.coli* O-103/10 strain was cultured on Minca Agar + Polyvitex. Cultures were resuspended in phosphate-buffered saline, pH 7.4 (PBS) and formalin was added up to 4 p.1000. Concentration was 2.10^9 CFU/ml. It was given to group B.

3 - Vaccination and challenge protocols (figure 1).

Animals in groups A or B received *per os* daily administration of 2 ml of either vaccine A or B for ten consecutive days. Vaccine doses were given by oral canula. In the experimental schedule (fig.1), vaccination lasted from day -15 to day -6 before challenge. At day 0 (45 days of age),all the animals (vaccinated and controls)were challenged with 1.10^4 virulent *E.coli* O-103/10 in 2 ml of saline by oral route. After challenge,animals were controled daily for apparition

of diarrhoea and to record mortality. Individual weight was checked three times a week.

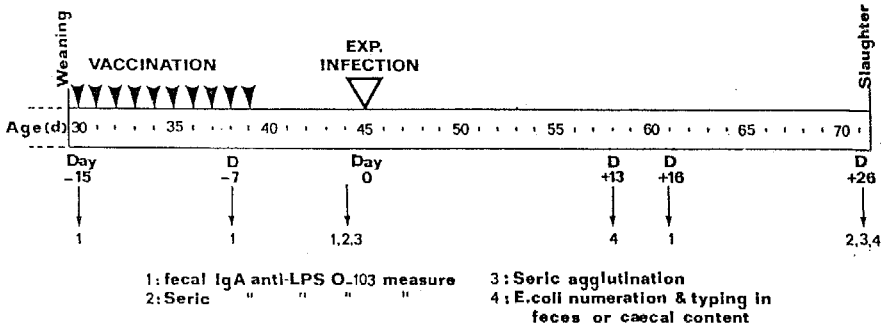

Figure 1 : Experimental protocol used to vaccinate and challenge the animals. Antibodies controls and bacteriological examinations were done as described on the figure.

4 - Bacterias and parasites controls.

Animals that had died were autopsied. Caecal content was examined for number of *E.coli* using Mac Conkey(without crystal violet) agar dilution technique. At day +13 and day +26, *E.coli* were numerated in feces or in caecal content of survivors by the same way (see fig.1).Coccidia were also numerated each time. For each rabbit, after *E.coli* numeration, 3 colonies were subcultured and tested with anti-O-103 antiserum by slide agglutination.

5 - Antibodies monitoring.

a - Fecal IgA anti-LPS O-103 evaluation.

Local IgA antibody response in feces was evaluated using enzyme-linked-immunosorbent assay (ELISA), with LPS O-103 as antigen. The technique is derived from that described by VOLLER *et al.*(1978). LPS was extracted from the cell wall of *E.coli* O-103/10 by hot phenol-water method (ADAMS G.A.,1972) and adsorbed in excess in the wells of microtitration plates (Dynatech,MicroELISA) by overnight incubation at +4°C in carbonate 0.02M pH9.6. Fresh feces samples were diluted 1/5 (w/v) in PBS plus sodium azide 2 p.10000. After trituration and centrifugation, the supernatants were conserved at -20°C until use. When tested, fecal supernatants were diluted 1/4 in PBS plus Tween-20:2p.1000. All washings were done with PBS plus Tween-20 : 0.5 p.1000. IgA anti-LPS O-103 were revealed by combina-

tion of goat anti-rabbit IgA antibodies (alpha chain specific)(MILES Scientific,Naperville,IL) and alcaline phosphatase conjugated rabbit anti-goat IgG (H+L specific) antibodies (BIOSYS S.A.,Compiegne,France). Paranitrophenylphosphate (SIGMA 104 tablets), at 1 mg/ml in diethanolamine/HCl ,pH 9,6 was used as enzyme substrate. Optical density was recorded with a multi-channel spectrophotometer (Multiskan, Flow Laboratories). Results were expressed as milliunits of specific optical density (= mean of OD in two wells coated with LPS - OD of a well without antigen).

b - Seric antibodies determination.

The same ELISA test was used to quantify IgA antibodies against LPS O-103 in sera of animals. The sera were diluted 1/1000 in PBS plus Tween-20 : 0.5 p. 1000.

Agglutinating antibodies were titrated using suspension of 5.10^8 *E.coli* O-103/10 per ml of saline plus formalin : 4p.1000 as antigen. Serial two-fold dilutions of sera were tested, starting at 1/20. Tubes were read after overnight incubation at 56°C.

RESULTS

1 - Mortality and morbidity.

Figure 2 shows weight losses, diarrhoea and mortality that happened to the animals after challenge,as well as date of apparition and lasting of the symptoms. No symptoms were noted in group A.

2 - Growth parameters of survivors following challenge (figure 3).

Growth in group A was excellent, with a mean weight gain of 1046 g. recorded at slaughter, 26 days after challenge, and a mean weight gain per day of 40.2 grams. Growth of survivors in control group C was significantly less (776 g. at day +26; 29.8 g. per day). Survivors in group B gave an intermediate feature (908 g. at day +26)which did not differ significantly from control group.

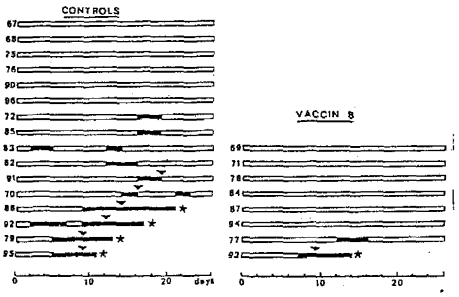

Figure 2 : Weight losses (▬),onset of diarroea (▼) and death (★)in rabbits of groups B and C (controls) after challenge with oral 1.10^4 *E.coli* O-103/10. No symptoms were noted in vaccinated group A.

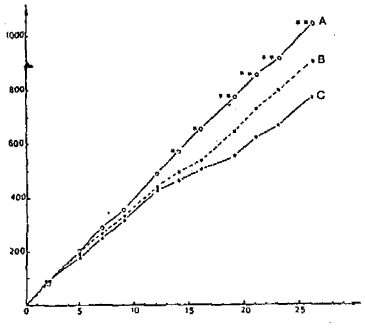

Figure 3 : Mean weight gain of survivors after experimental infection (grams vs days after challenge).Growth in group A is significantly better than in group C (★:$p<0.01$ ★★: $p<0.001$. Group B vs group C : not significant.

3 - Numeration of caecal or fecal *E.coli*.

Caecal content of the 5 dead animals in groups B and C was very liquid but did not show any blood. There was no evidence of haemorragic lesions of the caecal mucosa. *E.coli* flora reached 7.10^8 to 1.10^{10} per gram. All colonies tested belonged to O-103 serogroup. Two animals out of 5 had coccidia in their caecal content (respectively 4.10^2 and 5.10^3 per gram).

E.coli rates in feces (day +13) and caecal content (day +26)of survivors are shown in table I. In all groups, the mean rate diminished approximatively of 2 log between days +13 and +26. The mean rate was two $\log_{10}$ higher in group C than in group A. It is worthy of note that O-103 strain had disappeared as soon as day +13 in group A and at slaughter in group B. Two animals in the group B and three in the group C had low rates of coccidia in their feces (2.10^2 to 5.10^3).

Table I : Mean rate of *E.coli* and O-103 serotyping in feces (d +13) or caecal content (d +26) of survivors. ★★: A<C, $p<0.001$; ★: A or B<C, $p<0.05$.

GROUPS	Day + 13		Day +26	
	Mean *E.coli* rate	O-103/ Nr tested	Mean *E.coli* rate	O-103/ Nr tested
A	$1.3\ 10^4$	0/12★★	$8.7\ 10^2$	0/18★
B	$5.4\ 10^5$	9/12	$1.2\ 10^3$	0/15★
C	$1.8\ 10^6$	20/24	$2\ 10^4$	9/33

4 - Antibody responses.

IgA anti-LPS O-103 were found in feces of some animals of all groups before vaccination, i.e. one day after weaning. These antibodies were probably transfered from mother through milk. We found IgA anti-LPS O-103 in lactoserum and in blood of several non vaccinated mothers (A.MILON and R.CAMGUILHEM, unpublished). After 8 days of vaccination, these antibodies had almost completely disappeared, and, just before challenge, they were irregularly detected at very low level in control group (1 positive reaction; SOD=69) and in group A (range 0-167mU SOD) (see table II). Responses were higher in group B (range 2-728 mU SOD). After challenge, feces from most cages showed IgA anti-O-103, except those of group A, where 2 cages out of 4 were negative.

Table II : Antibody responses in feces and serum of animals before and after vaccination, and after challenge with *E.coli* O-103/10 (see fig.1 for schedule).

	GROUPS	Day -15	Day -7	Day -1	Day +16	Day +26
	ELISA Mean IgA anti-LPS O-103 (mU of SOD) (SEM)					
FECES	A	198 (235)	2 (3)	24 (37)	76 (93)	
	B	107 (126)	18 (3)	153 (156)	228 (320)	
	C	257 (231)	0 (0)	17 (30)	281 (266)	
SERUM	A			59 (70)		22 (13)
	B			111 (58)		89 (126)
	C			4 (3)		134 (78)
	Mean $\log_2$ of agglutination titers/10 (SEM)					
SERUM	A			2,875 (1.75)		0.75 (0.96)
	B			5.000 (1.60)		2.14 (1.16)
	C			0.187 (0.37)		2.25 (1.32)

Oral vaccination elicited seric antibodies (table II),which could be detected by agglutination technique using homologous strain as antigen. These antibodies werenot found in control animals before challenge.The mean titer elicited by vaccination was higher in group B than in group A.These agglutinating antibodies were presumably not of the IgA class since IgA anti-O-103 anti-

bodies were scarcely detected in the serum (table II). After challenge, most of control animals became serologicaly positive whereas animals in group A showed decreasing titers.

DISCUSSION

We undoubtedly reproduced *E.coli* O-103 enteritis on control group C. Diarrhoeas, mortality of 25p.100 of the tested animals, as well as colonisation of the caecum by *E.coli* O-103 with few coccidial contaminations allow us to conclude that the enteritis were due to the challenge dose. Mortality rate is similar to that seen in french industrial farms with *E.coli* diarrhoea problems. However, this rate is lower than that we described in a previous experiment (50 p.100 : CAMGUILHEM R.,1986b). This difference may be due to the frequence of overcontamination which occur from fecal sources of diarrhoeic animals held in the same cage : in the experiment described herein, rabbits were only two per cage, against 7 to 8 per cage in the previous one.

Results given by group A show an excellent efficiency of vaccine A,in the described schedule, to prevent illness. No symptoms were observed and growth remained normal during the observation period. Furthermore, no *E.coli* O-103 were detected in these animals, suggesting that the challenge dose did not colonize their digestive tract at all. In group B, results were not significantly different from in control group, though mortality and diarrhoea rates appeared somewhat lower and growth rate slightly higher.It may be considered that vaccine B has brought a partial protection. O-103 strain was still found in feces 13 days after challenge.

The discrepancies found between both vaccinated groups are only due to the way of preparation of vaccines A and B. Vaccine B contains only bacterial cells, whereas Vaccine A contains liquid medium, where the strain has been cultivated. Thus, vaccine A may contain antigenic substances, excreted during the growth of bacteria, which may be of importance in the protection. Strain O-103/10 does not produce thermostable or thermolabile enterotoxins and is probably close to EPEC strains

found in humans. It may produce cytotoxin (or"Shiga-like"toxin)involved in the hemorragic lesions which are found in acute forms of the illness (OKERMAN,1987). Local antibodies against this toxin might then be implicated in protective mechanisms. Vaccine A may include this excreted toxin, while vaccine B may not.

E.coli O-103/10 strain has been shown to be piliated by electron microscopy (CAMGUILHEM *et al.*,1986b).It has been shown that it could adhere *in vivo* to the apex of rabbit enterocytes. So, pathogenesis of this enteritis may include adhesion of the bacteria to the digestive mucosa, as it has been shown by CANTEY and BLAKE (1977) for RDEC-1 strain. Expression of *E.coli* adhesins *in vitro* is known to depend on culture conditions, and it is possible that culture conditions used to prepare vaccine A allow it, whereas those used for vaccine B do not. Consequently, local anti-adhesin antibodies may be elicited only by vaccine A administration.

Anyway, protection against challenge is not correlated to local or general antibody responses against bacterial cell or against O-103 LPS. O-103 LPS has been used to check local response because *E.coli* is a normal host of digestive trad in rabbits. For this reason, antibodies against resident strains of the normal flora would have been detected when using whole cell antigen. Protected animals from group A always show lower amounts of local or general antibodies than those found in partially protected rabbits of group B. After challenge as well, local response is higher in unprotected groups. This may be due to absence of colonization of the gut by the challenge strain. It is known that larges doses of antigen and repeated or continuous contacts are required for an effective s-IgA response in orally immunized individuals (MESTECKY, 1987).

There is now a need for identification of antigenic substances responsible for protective responses obtained in group A. Antigenic analysis and comparison of both vaccines are now in progress.

BIBLIOGRAPHY

1 - ADAMS G.A.,1972. Lipopolysaccharides.Preparation from Gram-negative bacteria. In: Methods in carbohydrate chemistry, vol.VI,WHISTLER R. and BEMILLER J.Edit. Academic Press,pp.157-162.

2 - CAMGUILHEM R., 1985. Isolement d'une souche d'*Escherichia coli* (serogroupe O-103) responsable d'enterite colibacillaire du lapin en engraissement. Mise en évidence de son pouvoir pathogène. Revue.Med.Vet.136,51-68.

3 - CAMGUILHEM R., 1986a. Essai de vaccination des lapins par voie intradermique contre l'entérite colibacillaire à *E.coli* O-103. 4èmes Journées de la Recherche cunicole.INRA-ITAVI, dec.1986,comm.N°35.

4 - CAMGUILHEM R.,LEBAS F.,LABIE C.,1986b. Reproduction experimentale chez le lapin en engraissement d'une diarrhée provoquée par une souche d'*Escherichia coli* de sérogroupe 0-103. Ann.Rech.Vet. 17,4,409-424.
5 - CANTEY J.R.,BLAKE R.K.,1977. Diarrhoea due to *Escherichia coli* in the rabbit: a novel mechanism. J.Infect.Dis. 135,454-462.
6 - MESTECKY J., 1987. The common mucosal immune system and current strategies for induction of immune responses in external secretions. J.Clin.Immunol. 7,265-274.
7 - OKERMAN L.,1987. Enteric infections caused by non enterotoxigenic *Escherichia coli* in animals : occurence and pathogenicity mechanisms. A review. Vet. Microbiol. 14, 33-46.
8 - PEETERS J.E.,POHL P.,OKERMAN L.,DEVRIESE L.A.,1984. Pathogenic properties of *Escherichia coli* strains isolated from diarrheic commercial rabbits. J. Clin. Microbiol. 20, 34-39.
9 - RENAULT L.,ROUX J.,LEBOURHIS E.,COUDERT P.,LICOIS D.GUILLOT F.,1983. Description d'un serogroupe 0-103 d'*Escherichia coli* enteropathogène chez le lapin au sevrage. Bull. Acad. Vet. Fr.56, 387-400.
10 - VOLLER A.,BIDWELL D.E.,BARTLETT A.,1978. Enzyme immunoassays in diagnostic medicine. Theory and practice. J.Clin.Pathol. 31,507-520.

NOTE of the Editor : The authors failed to provide any summary or abstract