USE OF A 3-/O15 D*eae* ENTEROPATHOGENIC *ESCHERICHIA COLI* VACCINE IN A RABBITRY WITH MIXED ENTEROPATHY PROBLEMS: SPREADING CHARACTERISTICS AND PROTECTIVE EFFECT

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ABSTRACT

In a rabbitry affected by colibacillosis, complicated by epizootic enteropathy, preliminary screenings detected the presence of enteropathogenic *Escherichia coli* (EPEC) pathotypes 1+/O109 and 2+/O128. A trial was performed involving 108 newly weaned rabbits, in order to evaluate the capacity of an attenuated EPEC strain of pathotype 3-/O15, deleted in the *eae* gene, to spread between cages, and to assess its protective effect. Litters of six were distributed homogenously over a vaccination and a control group, housed in a separate building but under comparable environmental conditions. Rabbits were caged per three. In the vaccination group, the rabbits of three cages were vaccinated *per os* with 10^9 CFU of the vaccine strain. The other cages had side, corner or no direct contact with the vaccinated rabbits. The experiment lasted for five weeks. Rabbits were assessed for clinical symptoms, *E. coli* excretion, serum antibodies, feed intake and body weight. Rabbits that died during the experiment were necropsied. The vaccine strain did not spread on a detectable level between the cages, indicating that all rabbits in a rabbitry need to be vaccinated. The wildtype *E. coli* strains isolated belonged to pathotypes 1+/O109 and 2+/O132. No significant differences were found between the control and the vaccination group for diarrhoea, mortality, necropsy lesions, coprological or bacteriological results. Nevertheless, the serological results of an EspB-based ELISA yielded a significant difference between the vaccinated and control rabbits. This might indicate that the vaccine strain effectively limits wildtype strains in their possibilities to colonise and thus immunostimulate the rabbits. The production parameters of the vaccinated rabbits and the other rabbits were similar, indicating that the vaccine strain has no adverse effect in this respect.

Key words: EPEC, vaccine, spread, serology, production parameters.

INTRODUCTION

Colibacillosis in meat rabbits, caused by enteropathogenic *Escherichia coli* (EPEC), is an important source of economic loss in Belgium and other countries (PEETERS *et al.*, 1984). In diarrhoeic weaned rabbits, the pathotypes 3-/O15, 4+/O26 and 8+/O103 are

the most important, whereas pathotypes 2+/O128 and 2+/O132 are of variable virulence (PEETERS *et al.*, 1988). Pathotype 1+/O109 mainly causes problems in young rabbits before weaning. In Belgium and the Netherlands the pathotype 3-/O15 is the most prevalent, while in France, Spain and Italy EPEC of the 8+/O103 and 4+/O26 types are detected. In view of the economic importance of this disease, the high cost of antibiotic treatment, the development of antibiotic resistance in the field strains and the observation that even after succesful antibiotic treatment, 3 - 7% of the animals may remain asymptomatic EPEC carriers (PEETERS, 1989), there is a need for an efficacious vaccine. We have previously created an attenuated strain by deletion of the *eae* gene of a 3-/O15 strain (97/241.6 Δ*eae*) (STAKENBORG *et al.*, 2001). The rabbits vaccinated *per os* with this live attenuated strain were protected against a homologous infection, and the strain persisted long enough to induce an immunological response. Under laboratory conditions, protection against heterologous challenge strains was limited. The 3-/O15 Δ*eae* strain needed to be tested under field conditions as well, to assess its ability to spread between cages and its potential for protection against EPEC of other pathotypes under field conditions. The results of this field trial are presented here.

MATERIAL AND METHODS

Vaccine strain

The attenuated 3-/O15 Δ*eae* strain (STAKENBORG *et al.*, 2001) was kept at -80°C in Luria Bertani broth (LB) containing 50% glycerol, and cultured in Penassay Broth (PAB) at 37°C. A PCR test covering the deleted *eae* fragment was available to differentiate the wildtype EPEC strain from the mutant.

Rabbitry

The rabbitry where the field trial was performed was selected based on the biosecurity measures that could be applied on the premises, in view of the stringent standards set by the Belgian government. This rabbitry produced an own selected strain of New Zealand White rabbits. Over a period of four months before the start of this field trial, the does of the rabbitry and their available offspring were screened twice for presence of EPEC by means of rectal swabs, as described by PEETERS *et al.* (1988). Necropsy and a parasitological, bacteriological and virological analysis as described by PEETERS *et al.* (1986) and VAN OPDENBOSCH *et al.* (1981) were performed on 5 and 7 diseased animals.

Animals

A total of 108 newly weaned New Zealand White rabbits, aged 29 days, were used for the trial. Litters of six were divided homogenously over the control and the vaccination group, which were situated in separate houses with access control. The rabbits were individually ear tagged. They were housed per three in 18 flat-deck cages, keeping the three litter mates per group together. The cages were numbered, and the cages with the same numbers in the control and vaccination group contained rabbits from the same litter. At sampling, the order of the cage numbers was respected to ascertain that the vaccine strain could not spread through the manipulations. Standard pelleted rabbit feed

(containing no medication except for robenidine at 66 ppm) and water were available *ad libitum*. Feed intake was determined weekly per cage. The rabbits were individually weighed at the start and the end of the trial. Observations for presence of diarrhoea and rectal swabbing for detection of EPEC and the vaccine strain were performed once per week in the control group and three times per week in the vaccination group. In the vaccination group, four subclasses of cages were present (Figure 1).

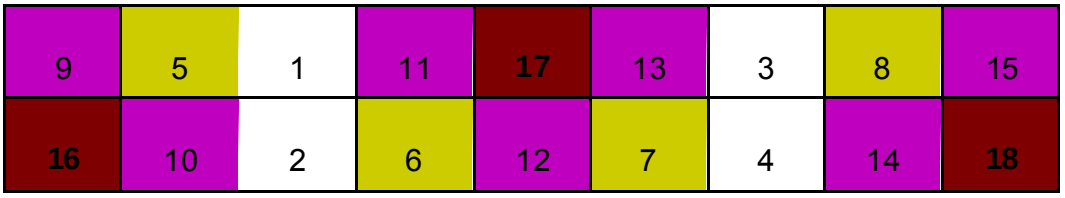

Figure 1. Cage subclasses in the vaccination group. Subclass 1 is shown in red (cages 16 to18), subclass 2 in orange (cages 9 to 15), subclass 3 in yellow (cages 5 to 8) and subclass 4 in white (cages 1-4)

Subclass 1 contained the three vaccinated cages, of which the rabbits had received 10^9 colony forming units of the vaccine strain. The non-vaccinated rabbits were housed in the other cages. Subclass 2 shared sides with the vaccinated cages. Subclass 3 had corner-contact and subclass 4 had no direct contact with the vaccinated cages. Blood samplings were performed on a weekly basis on 28 rabbits per group, i.e. 7 rabbits per subclass, and their 28 litter mates in the control group. If a rabbit died, the subsequent blood samples were taken from a surviving cage mate. After five weeks, all rabbits were euthanatised and destroyed, together with all biological material and protective wear.

Serological analysis

The EspB-based ELISA was described by STAKENBORG *et al.* (2001).

Statistical analysis

Groups were compared using Fisher's exact test, Pearson's chi-square, ANOVA (Statistix 1.0) and repeated measures analysis (SAS 8.02)

RESULTS AND DISCUSSION

During the preliminary screenings, EPEC pathotypes 1+/O109 and 2+/O128 were detected. At necropsy, diarrhoea was present in 6/12 rabbits, and epizootic enteropathy lesions (bloated abdomen, a lot of gas and liquid in the gastro-intestinal tract, partial or complete desiccation of the caecal content) in 9/12 rabbits. Coprology of the necropsied rabbits revealed the presence of low numbers of *Eimeria spp.* in 5/12 rabbits, EPEC 1+/O109 in 1/12, *Clostridium spiroforme* in 4/12, and rotavirus in 3/12 rabbits.
The results of the vaccination trial are presented in Table 1. There were no significant differences between the two groups. Even though the rabbits used in the experiment originated from the same breeding unit as those sampled during the initial screenings, they yielded EPEC strains of pathotypes 1+/O109 and 2+/O132. This can be explained

by the fact that per animal and per sampling only three colonies were selected for typing purposes, which limits the detection possibilities of the different pathotypes present. Only 3/9 vaccinated rabbits excreted the vaccine strain on a detectable level. Since none of the other rabbits in the vaccination group became excretor of the vaccine strain, it does not seem able to spread on a detectable level between the cages. Therefore individual vaccination should be applied in rabbitries.

Table 1. Clinical, necropsy and bacteriological results of the field trial.

	Vaccination group	Control group
Nr of animals at onset	54	54
Clinical symptoms		
diarrhoea	11	18
overall mortality	8	11
mortality in cages 16-18	0	3
Necropsy results of deceased rabbits		
lesions of enteritis	3	6
lesions of epizootic enteropathy	5	5
Coprology of necropsied rabbits		
Eimeria spp.	6	7
Worms	1	0
C. spiroforme	7	8
EPEC (2+/O132)	1	3
rotavirus	0	0
Bacteriological analyses		
nr E. coli excretor (minimum -maximum)	22 - 50	30 - 41
nr EPEC excretor (minimum -maximum)	2 - 8	1 - 3
EPEC pathotypes detected	1+/O109, 2+/O132	2+/O132
nr excreting vaccine strain in cages 1-15	0	0
nr excreting vaccine strain in cages 16-18	3	0
duration of vaccine excretion	day 9 - 26	-

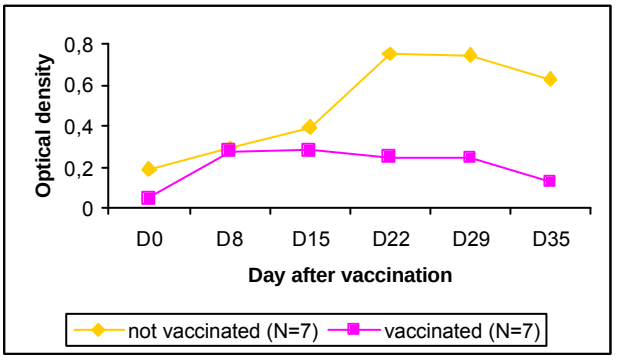

Figure 2. Serological results of the EspB-based ELISA for the seven vaccinated animals from which blood samples were taken, and their seven litter mates in the control group. The difference between the two groups is significant from day 22 onward (*P* = 0.006).

In view of the bacteriological results, only the sera of the seven blood sampled vaccinated rabbits and their non-vaccinated littermates were analysed. The results are shown in Figure 2.

There is no information available in literature on the potential immunoprotective effect of anti-EspB antibodies. However, anti-EspB antibodies are useful as a marker for the immunostimulating effect of both the vaccine and wildtype strains. The rabbits were kept in a contamina ted environment and were thus continually exposed to EPEC. This explains why in the vaccinated and in the control rabbits both an immediate rise in anti-EspB antibodies is detected.

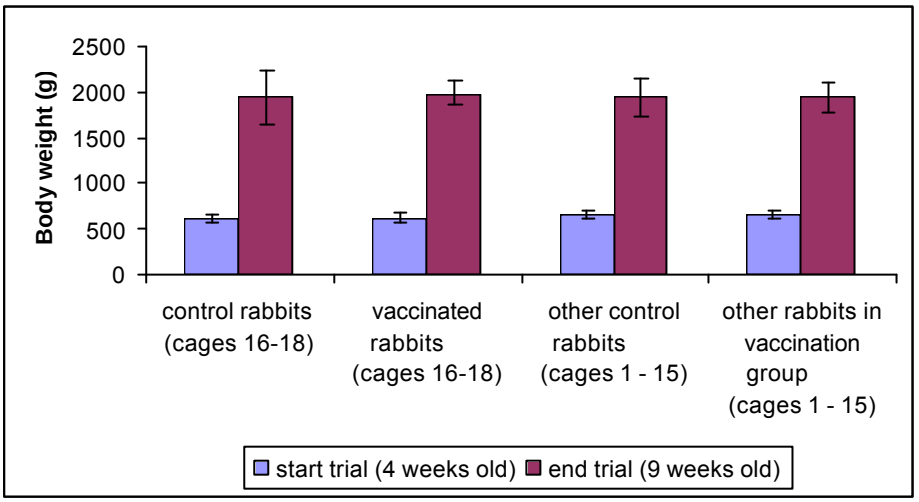

Figure 3. Average body weight with standard deviations for the control and vaccination group, subdivided by cages. The difference between the groups is not significant.

One week after vaccination the level of antibodies in the vaccination group reaches a platform, whereas in the control group it continues to rise until day 22. This might indicate that the vaccine strain effectively limits contact possibilities for the EPEC present in the rabbitry, even though these EPEC belong to another bio/serotype. In the field, colibacillosis is mainly a problem during the first two weeks after weaning. After that, the rabbit's intestinal flora has stabilised and the rabbit gut becomes less susceptible to all but the most virulent EPEC. Less EPEC colonisation of the gut results in less immunostimulation, which could account for the lowering level of antibodies from day 29 in the control group. In the vaccinated rabbits the level of antibodies also starts to decline after day 29, probably caused by a combination of the elimination of the vaccine strain from the gut, and the lowered susceptibility of the rabbits to EPEC. Moreover, the infection pressure of EPEC in the cages might be lowered by the vaccination.

For body weight (Figure 3), feed intake and feed conversion (results not shown), similar results were obtained in the control and vaccination group, in the respective subclasses, and in the vaccinated and non-vaccinated cages. The vaccine does not seem to negatively influence production parameters.

CONCLUSION

The vaccine strain 3-/O15 Δ*eae* does not spread on a detectable level, and should therefore be administered to each animal individually. The serological results of an EspB-based ELISA yielded a significant difference between the vaccinated and control rabbits. This might indicate that the vaccine strain effectively limits wildtype strains in their possibilities to colonise and thus immunostimulate the rabbits. Results for zootechnical parameters of the vaccinated animals, though obtained from only 9 rabbits, indicated nonethe-less that the vaccine strain did not have any adverse effects in this respect.

ACKNOWLEDGEMENTS

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