DIFFERENT ADMINISTRATION METHODS FOR THE 3-/O15 D*eae* EPEC VACCINE STRAIN PROTECTING MEAT RABBITS AGAINST A 3 -/O15 CHALLENGE: PRELIMINARY RESULTS

BOHEZ L.[1], STAKENBORG T.[1], LAEVENS H.[1], PEETERS J.[1], VANDEKERCHOVE D.[1]

Veterinary and Agrochemical Research Centre (VAR),
Groeselenberg 99, 1180 Brussel, Belgium.
dovan@var.fgov.be

ABSTRACT

Six groups of six individually housed SPF rabbits, five weeks of age, were analysed for presence of enteropathogenic *Escherichia coli* (EPEC), *Clostridium spiroforme*, rotavirus and coccidiosis. All animals were negative for all pathogens tested, except one, which was positive for *C. spiroforme*. Four groups were vaccinated with an attenuated EPEC strain of the 3-/O15 pathotype (Δ*eae*) at 1.5 x 10^9 CFU/ml. The first group received the vaccine directly in the oral cavity by use of a syringe. The second group received the vaccine via the drinking water in individual water bottles. In the third group, the vaccine was sprayed on the rabbits' fur, and in the fourth group it was sprayed on the feed. The fifth (unvaccinated challenged) and sixth group (negative control) were not vaccinated. Three weeks later, groups one to five were challenged *per os* with 10^7 CFU of a 3-/O15 wildtype (WT) strain. The negative control group did not receive a challenge. All vaccinated rabbits excreted the vaccine strain on a detectable level, except three animals from group 2. After challenge, all rabbits from the unvaccinated challenged group excreted the WT strain in high numbers. Excretion in the vaccinated groups was much more limited. No significant differences were observed for body weight after challenge, due to some loss of virulence of the challenge strain, in combination with the rather small groups and the individual differences, causing large standard deviations. However, for feed intake a significant difference was observed after challenge between the unvaccinated challenged group and the negative control group (P = 0.0053), whereas no significant difference was found between the negative control group and the vaccinated groups. Symptoms of enteritis were seen in only one rabbit, of the unvaccinated challenged group. We conclude that all administration methods tested resulted in colonisation of the rabbits' gut by the vaccine strain. There are indications that all methods might yield an effective protection against a challenge infection with a 3-/O15 WT strain. However, the experiment must be repeated with more rabbits per group and a more virulent challenge strain.

Key words: EPEC, vaccine, administration methods.

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 Belgium and the Netherlands the pathotype 3-/O15 is the most prevalent. 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. Previous results showed that under field conditions the vaccine strain does not spread on a detectable level between cages. Consequently, individual vaccination of all rabbits is necessary. However, it should be possible to do this with a minimum of manual labour. For this reason, we have tested different administration methods of the vaccine.

MATERIAL AND METHODS

Vaccine and challenge strain

The attenuated 97/241.6 Δ*eae* strain (3-/O15) (STAKENBORG *et al.*, 2001) and wildtype (WT) strain 97/223.10 (3-/O15) were kept at -80°C in Luria Bertani broth (LB) containing 50% glycerol, and cultured in Penassay Broth (PAB) at 37°C. The attenuated vaccine strain was used at a concentration of 1.5×10^9 colony forming units (CFU) per ml, and the WT strain at 10^7 CFU/ml. A PCR test covering the deleted *eae* fragment was available to differentiate the WT EPEC strain from the attenuated mutant.

Animals and vaccination-challenge protocol

A total of 36 New Zealand White rabbits (Harlan, The Netherlands), aged 5 weeks, were used in this experiment. They were homogenously distributed over six groups. When they arrived, they were examined for possible presence of EPEC, *Clostridium spiroforme*, *Eimeria* and rotavirus, as described by PEETERS *et al.*(1986) and VAN OPDENBOSCH *et al.* (1981). The rabbits were individually housed. Water was available in individual bottles and they received a non-supplemented, pelleted feed (Konix, Belgium). Water and feed were given *ad libitum*.

Groups 1 to 4 were vaccinated at their arrival (day 0). Group 1 was vaccinated *per os* (directly in the oral cavity by use of a syringe) with 1 ml of the vaccine suspension. Because some leaking of the water bottles was anticipated, 2 ml of the vaccine suspension was added to 30 ml of drinking water in group 2. Three ml was sprayed on the rabbits' fur in group 3, to ascertain a minimum intake of the equivalent of 1 ml, and 1 ml of the vaccine suspension was sprayed on the feed in group 4. Three weeks after

vaccination (day 21), the rabbits of groups 1-5 were challenged *per os* with 1 ml of the WT strain suspension. Group 6 (negative control group) was neither vaccinated nor challenged.

Bacteriological screening and clinical follow-up of rabbits

The rabbits were bacteriologically examined three times per week using rectal swabbings, as described by PEETERS *et al.* (1988a), to detect excretion of the vaccine and challenge strain. The excretion of biotype 3 *E. coli* was detected by the yellow color of these colonies on Simmons Citrate agar supplemented with sorbose (SCS) (PEETERS *et al.*, 1988b). The attenuated mutant was differentiated from the WT challenge strain by the above-mentioned PCR. The number of *E. coli* of pathotype 3-/O15 excreted was assessed by a semiquantitative method, with 0 designating no excretion, 0.25 excretion of up to 10 colonies, 0.5 excretion of up to 20 colonies, and 1 excretion of up to 50 colonies. If colonies were covering the entire agar plate, 2 was used for individual colonies versus 3 for confluent colonies. Three times per week, the rabbits were weighed and presence of diarrhoea was assessed.

Statistical analysis

The groups were compared for feed intake and body weight using a repeated measures analysis (SAS 8.02).

RESULTS AND DISCUSSION

During the initial screening, no EPEC, rotavirus or *Eimeria spp.* were detected. *Clostridium spiroforme* was detected in one rabbit.

All vaccinated rabbits excreted the vaccine strain on a detectable level, except for three animals in the group vaccinated via the drinking water. The water bottles of the same three rabbits had fallen from the cages and were found empty. It was not possible to assess with certainty whether these three rabbits had taken in a sufficient volume of the water or not, but we chose not to repeat the vaccination. In view of the bacteriological results obtained after challenge (see Figure 1 and below), we may conclude that all six rabbits in this group had ingested a sufficient dose of the vaccine strain. Excretion of the vaccine strain was detected from day 4 to day 20. From day 22 onward, the WT challenge strain was excreted by 6/6 rabbits in the unvaccinated challenged group with an average of 8/9 sampling days positive, whereas in the vaccinated groups excretion was more limited. In the group vaccinated *per os*, the WT strain was excreted by 4/6 rabbits, with an average of 2.5/9 sampling days positive. In the group vaccinated via the water, 3/6 rabbits were WT strain excretors, with an average of 1.8/9 sampling days positive. Two of the three rabbits that didn't excrete the vaccine strain, did not excrete the challenge strain either. This indicates that in spite of the accident with the water bottles, the vaccine strain had sufficiently colonised the rabbits' gut. In the groups vaccinated by spray on the fur and on the feed, 4/6 and 6/6 rabbits

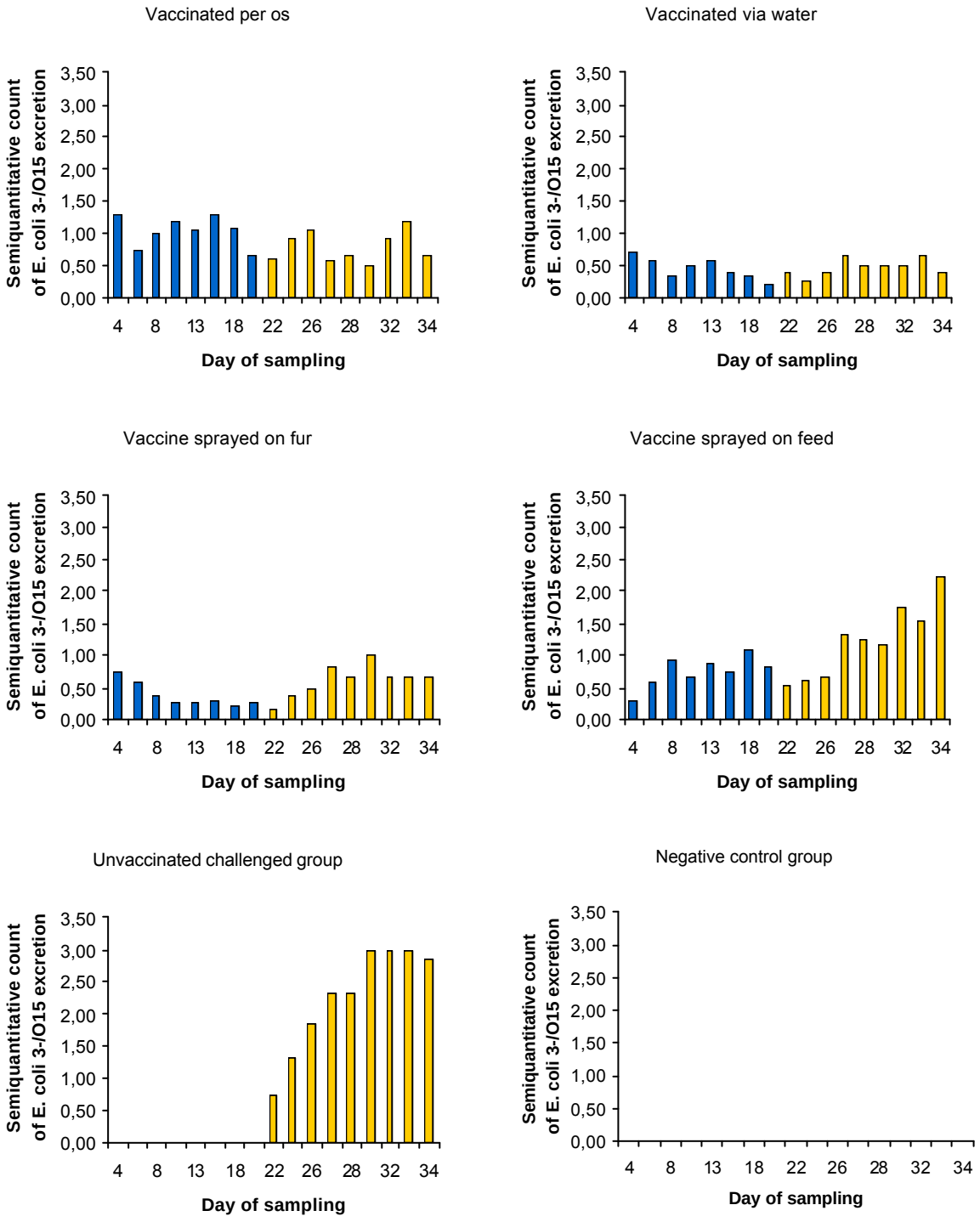

Figure 1. Semiquantitative excretion of the vaccine and the challenge strains in the respective groups. *E. coli* strains isolated from day 4- 20 belong to the vaccine strain (blue). Those isolated after day 20 belong to the challenge strain (yellow).

respectively became WT strain excretors, with respective averages of 3.2 and 4.3/9 sampling days positive. These results confirm that colonisation by the challenge strain and subsequent excretion is effectively kept low by the vaccine strain (Figure 1).

Statistical analysis revealed a significant difference (P = 0.0053) for feed intake after challenge between the unvaccinated challenged group and the negative control group, whereas no significant difference existed between the vaccinated groups and the negative control group (see Figure 2). For body weight, no significant differences were detected between any of the groups. This can be explained by the lower virulence than expected of the challenge strain. Moreover, the low number of rabbits per group and the rather large differences in body weight between the animals resulted in a large standard deviation. In only one rabbit, of the unvaccinated challenged group, severe diarrhoea was observed, accompanied by a decrease in body weight.

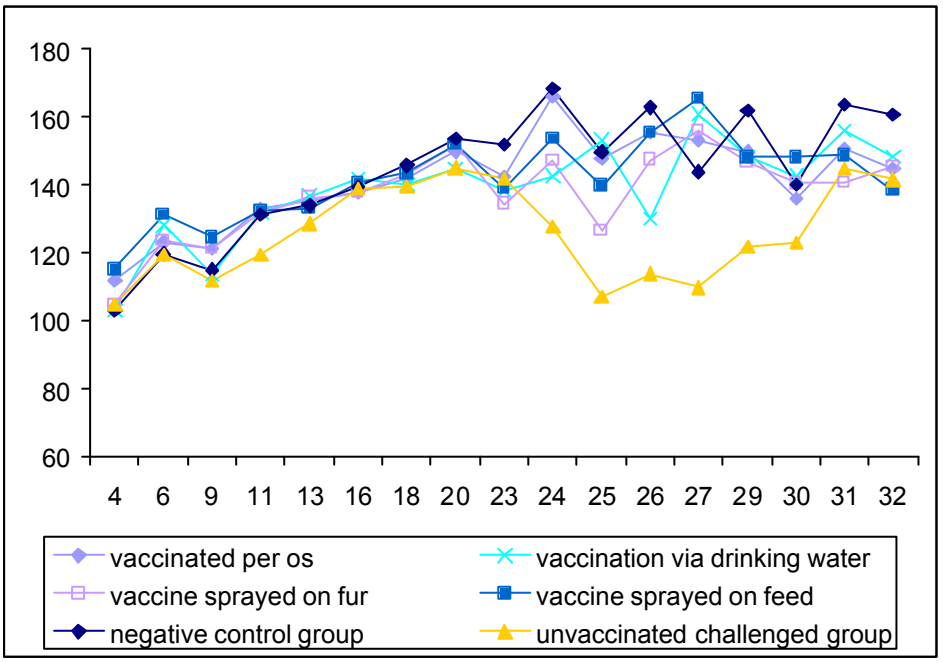

Figure 2. Average feed intake per group. The difference between the negative control group and the unvaccinated challenged group is significant (P = 0.0053), whereas no significant difference exists between the negative control group and the vaccinated groups.

CONCLUSION

All vaccine administration methods were effective in view of colonisation of the gut by the vaccine strain. Except for three rabbits vaccinated by the drinking water, all vaccinated rabbits excreted the vaccine strain on a detectable level. Moreover, all vaccinated rabbits that became challenge strain excretors excreted only low numbers of this strain. No clinical symptoms were seen in the vaccinated groups after challenge,

and no significant difference was seen for feed intake between the negative control group and the vaccinated groups, in contrast with the significant difference between the negative control and the unvaccinated challenged group. However, the challenge strain did not have a clear effect on the rabbits' body weight, probably for loss of some virulence. The experiment should therefore be repeated with a more virulent challenge strain. The use of more rabbits per group would offer supplementary proof of the vaccine's protective effect. As far as limitation of challenge strain excretion was concerned, best results were obtained in the groups vaccinated *per os*, via the water and via spray on the fur. However, in view of the possible use of disinfectants in the drinking water, and the fact that vaccination *per os* is more labour intensive, administration by spray on the rabbits' fur might be the vaccination method of choice under field conditions.

ACKNOWLEDGEMENTS

This study was funded by the Belgian Ministry of Health, Food Chain Safety and Environment, Department of Contractual Research (DG4).

REFERENCES

PEETERS J. E. 1989. Ademhalings- en spijsverteringsstoornissen in de industriële slachtkonijnenhouderij. *Diergeneesk. Memorandum,* 124-126.

PEETERS J. E., GEEROMS R., CARMAN R. J., WILKINS T. D. 1986. Significance of *Clostridium spiroforme* in the enteritis-complex of commercial rabbits. *Vet. Microbiol.,* 12, 25-31.

PEETERS J. E., GEEROMS R., ORSKOV F. 1988a. Biotype, serotype, and pathogenicity of attaching and effacing enteropathogenic *Escherichia coli* strains isolated from diarrheic commercial rabbits. *Infect. Immun.,* 56, 1442-8.

PEETERS J. E., GEEROMS R., VROONEN C., POHL P. 1988b. A selective citrate-sorbose medium for screening certain enteropathogenic attaching and effacing *Escherichia coli* in weaned rabbits. *Vlaams Diergeneesk. Tijdschr.,* 57, 264-270.

PEETERS J., GEEROMS R., GLORIEUX B. 1984. Experimental *Escherichia coli* enteropathy in weanling rabbits: clinical manifestations and pathological findings. *J. Comp. Pathol.,* 94, 521-528.

STAKENBORG T., VANDEKERCHOVE D., RÜLLE K., PEETERS J. 2001. Protection contre une inoculation d'épreuve des lapins vaccinés avec une souche EPEC 3-/O15 délétée dans le gène *eae*. 9èmes J. Rech. Cunicole, 28-29 nov. 2001, Paris, 123-126, ITAVI, Paris.

VAN OPDENBOSCH E., WELLEMANS G., STROBBE R., DE BRABANDER D. L. A, BOUCQUE C. V. 1981. [Evolution of anti-rota virus antibodies in the milk of cows treated in the last month of pregnancy either by adjuvated rotavirus vaccine or by the adjuvant fraction of the vaccine (author's transl)]. *Comp. Immunol. Microbiol. Infect. Dis.,* 4, 293-300.