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Baselga M. (ed.), Marai I.F.M. (ed.).
Rabbit production in hot climates

Zaragoza : CIHEAM
Cahiers Options Méditerranéennes; n. 8

1994
pages 543-550

Article available on line / Article disponible en ligne à l'adresse :

<http://om.ciheam.org/article.php?IDPDF=95605336>

To cite this article / Pour citer cet article

Soliman S.A.A., Gorgi S.F., Gergis S.M. **Preliminary trials for protection against snuffles in rabbits with live avirulent M 9 Pasteurella multocida vaccine.** In : Baselga M. (ed.), Marai I.F.M. (ed.). *Rabbit production in hot climates*. Zaragoza : CIHEAM, 1994. p. 543-550 (Cahiers Options Méditerranéennes; n. 8)



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Preliminary trials for protection against
snuffles in rabbits with live avirulent

M 9 *Pasteurella multocida*

Vaccine

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INTRODUCTION

Pasteurellae colonize the mucosal surfaces of the pharynges of rabbits in the carrier state or with recurrent rhinitis (Flatt 1974, Luetal 1978). In the early stages of experimentally induced disease produced by intranasal inoculation of *pasteurellae* can be isolated only from the tissue surfaces of the naso - and oropharynx - Subsequently, ascending infections of the sinuses and middle ears are observed (Watson et al; 1975). These findings suggest that *P.multocida* proliferates initially on the nasopharyngeal mucosae and this may be an important aspect of the pathogenesis of rabbit pasteurellosis.

Various techniques have been attempted to control and eliminate pasteurellosis. Vaccination studies of rabbits with killed *P.multocida* have shown variable results (Alexander et al; 1952 and Bapat and Sawhney 1972). It was noticed that although killed vaccines may protect against mortality, they do not protect against clinical disease or colonization to overcome this,

streptomycin dependant vaccines have been tried (Chengappa et al 1980, Digiacomo et al.; 1987 and Barbara et al; 1989). These vaccines were effective in preventing clinical disease caused by homologous strains and partially effective in preventing chronic infection.

In this study we evaluate the protective efficacy of the live avirulent M 9 P.multocida Vaccine against experimental rabbit pasteurellosis.

MATERIALS AND METHODS

Experimental Animals:

A total of 60 rabbits were used in this study. They were 4 week old female New Zealand white rabbits obtained from a commercial supplier. Rabbits were determined to be free of P.multocida infection by three nasal cultures obtained over 2 weeks and anti - pasteurella antibody assays of their sera. Rabbits were housed individually in stainless steel cages with wire floors. Commercial feed and water were available ad libitum.

Experimental Design:

Rabbits were divided into six groups and treated as follows, non vaccinated controls (3 groups) S/C vaccinated group, spray vaccinated group, intranasal vaccinated group.

Vaccinal strain :

M - ninevax produced by schering corporation, omaha,

Nebraska Lot. No 65155 was used. This vaccine is a mutant Cu vaccinal *P. multocida* strain which belong to serovar A: 3x4. The vaccine titer was determined before rabbit inoculation by plating serial dilutions of the culture reconstituted in 0.01 M phosphate - buffered saline, ph 7.2. Rabbits were sedated with ketamine hydrochloride and held in dorsal recumbency while 0.25 Ml of vaccine was administered intranasally.

Challenge Exposure :

Vaccinated rabbits were challenged by virulent *P. multocida* strain A: 3 which was isolated from diseased rabbits. The same route used for vaccination was used for challenge. Serial 0.5 log dilutions were made to achieve challenge exposure doses and plate counts were done to determine the exact dose.

Antibody Assay :

Anti - *pasteurella multocida* antibodies were assayed in sera of rabbits collected at different intervaked after vaccination by using the indirect hemagglutination test as described by carter and Rappay (1962).

RESULTS AND DISCUSSION

The development of a rabbit model of pasteurellosis is basic for vaccination studies. Conjunctival inoculation of rabbit with virulent strains of *P. multocida* induced pasteurellosis (Al-Lebban et al., 1988). Inoculation over a mucous membrane induced

disease similar to septicaemic pasteurellosis in rabbits and allowed us to study host - parasite relationships in a model that not only mimicked a syndrome seen in the field, but also was induced by a natural route of infection - Furthermore, because the first line of defence is at mucosal surfaces, this model allowed us to compare the efficiency of subcutaneous vaccination with that of mucosal vaccination when organisms gained entrance over a mucosal surface. The reproducibility of the LD₅₀ of *P. multocida* strain A:3 made it possible to challenge expose rabbits with a standard dose and to enable us to compare protection among experiments.

In this preliminary study we have shown that rabbits could be successfully immunized with the M 9 live avirulent mutant *P. multocida* vaccine administered either by the spray or intranasal inoculation methods. Subcutaneous vaccination only resulted in partial protection (Table -1). There were no statistical differences in the levels of antipasteurella multocida antibodies between the differently vaccinated and non vaccinated controls (Table -2). The reason for using this mutant vaccine was that we found that the parent CU strain was extremely virulent for rabbits by the different routes of inoculation. All rabbits that received the cu *P. multocida* strain died shortly after inoculation, although the fact that this vaccine was found to be avirulent for fowls.

Challenge test results in this study point out to the possibility of using the M 9 vaccine in the field for protection of rabbits against snuffles rather than septicaemic pasteurellosis. In our trial one application of this vaccine on mucosal surfaces

enhance protection, but further investigations are needed to determine the number of immunizations that could be sufficient to elicit better protection. Also the protective role of the local immune responses induced by this vaccine is now under investigation in our laboratory.

Chengappa et al (1980) used mutant serotype A:12 in their live vaccine studies and their results were similar to our observations with the M 9 live avirulent mutant vaccine. They reported complete protection in rabbits challenged with the homologous serotype, citing no evidence of disease or lesion.

Generally speaking live vaccines usually provide qualitatively and quantitatively better protective immunogens because chemical and physical procedures used to prepare killed vaccines may adversely alter immunogens. Also live vaccines may stimulate longer lasting immunity because the organism multiplies in the host and this continues stimulating the immune system both locally and systemically.

SUMMARY

The live avirulent mutant M 9 vaccine was administered to three different groups of rabbits by the subcutaneous, intranasal and spray methods. Vaccinated and non vaccinated control rabbits were challenged one month after vaccine administration by the same routes. The results obtained indicated that this vaccine can afford protection against clinical disease (snuffles) when it was applied by the spray method or intranasal method rather than by the subcutaneous method.

Table (1) Challenge test results of variously vaccinated and non vaccinated control rabbits.

Vaccine administered	Total No.	How Challenged	No. dead or discased	No. alive or Healthy	Protection %
Subcutaneously	15	Subcutaneously	6	9	60.0
Intranasaly	15	Intranasaly	4	11	73.3
Spray	15	Spray	3	12	80.0
Non Vaccinated	5	Subcutaneously	5	0	0
Controls	5	Intranasaly	5	0	0
	5	Spray	5	0	0

Table (2) : Geometric mean titers of anti- pasteurella multocida antibodies one month after vaccination with M 9 vaccine administered by different routes.

Vaccine administered	Pre- vaccination	one month after vaccination
Subcutaneously	13 ± 6.3	17 ± 2.5
Intranasaly	11 ± 5.5	15 ± 3.3
Spray	12 ± 4.8	15 ± 4.1
Control non Vaccinated rabbits	12 ± 3.6	13 ± 5.8
	12 ± 3.6	13 ± 5.8
	12 ± 3.6	13 ± 5.8

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