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VACCINATION WITH A LISTERIA STRAIN OF REDUCED VIRULENCE AGAINST EXPERIMENTAL LISTERIA ABORTION IN GOATS

R FENSTERBANK

INRA, Centre de Tours Nouzilly, Station de Pathologie de la Reproduction 37380, Monnaie, France

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Résumé

VACCINATION AVEC UNE SOUCHE DE LISTERIA DE VIRULENCE RÉDUITE CONTRE L’AVORTEMENT EXPÉRIMENTAL CHEZ LES CHÈVRES. — Une souche de Listeria monocytogenes de virulence réduite, la souche Aer, obtenue par trois mutations successives vis-à-vis de la streptomycine et de l’érythromycine, a été utilisée sur chèvres comme vaccin au cours de deux expériences successives. Les animaux ont été vaccinés une ou deux fois avec des doses comprises entre 6 x 10⁸ et 1,5 x 10¹⁰ germes par voie sous-cutanée. Aucun effet nocif n’a été observé, sauf l’avortement d’une chèvre vaccinée au cours de la gestation, avec réisolement de la souche vaccinale. Les chèvres ont été inoculées par voie sous-cutanée avec 5 x 10⁸ germes de la souche virulente L. monocytogenes ATCC 19115 à 95-100 jours de gestation. Treize sur 23 chèvres témoins (56,5 %) ont avorté ainsi que 1 sur 24 (45,8 %) vaccinées une fois et 5 sur 22 (22,7 %) vaccinées deux fois. Une protection significative peut donc être induite contre une infection sévère par vaccination et rappel avec la souche vivante L monocytogenes Aer.

Listeriosis mainly affects small ruminants in outbreaks of neurologic disorders and death or of abortions, which do not occur in an explosive manner, but sporadically with sometimes high death rates (Seeliger 1961). Epidemiology of listeriosis being not sufficiently known, control programs are difficult to devise. Thus, vaccination should be a very useful means of protecting animals. Actually, killed vaccines fail to afford a good protection (Osebold et al 1959, Asahi 1962, Von Koenig and Finger 1982) but live vaccines can induce a valuable resistance (Osebold et al 1959, Ivanov et al 1977, Von Koenig et al 1983, Gudding et al 1985).

The reduction of virulence, necessary for application in the field of a live vaccine, was achieved by the use of rough Listeria monocytogenes (Lm) (Ivanov et al 1977, Potel et al 1985) or by mutations in regard to streptomycin and erythromycin (Fensterbank 1986a). Protective immunity of rough forms was estimated on sheep (Gudding et al 1985) and that of mutants was measured on mice (Fensterbank 1986b). It was shown that strain Lm Aer, resulting from two successive mutations in regard to streptomycin (dependance then reversion to independance) followed by a third one in regard to erythromycin (resistance to 1000 μg/ml) was effective in protecting mice from lethal infection and in reducing the level of infection and the rate of abortions. On mice also, the protective immunity was shown to be early established, strongly enhanced by a booster, remaining stable for at least four months and being restored by a recall when vanishing.

These results on mice prompted us to test this vaccinal strain on small ruminants. In these animals, experimental listeriosis is difficult to standardize. However, subcutaneous injection of a virulent strain during pregnancy may cause abortion of a high number of control goats (Fensterbank 1986c). We used this model to estimate immunity induced in goats by one or two vaccinations with strain Aer. It was shown that actual protection could be obtained by vaccination.

Materials and Methods

1. Listeria strains and inoculations

Two Listeria monocytogenes strains were used: strain Aer, obtained by mutations that led to decrease in virulence without drop in immunogenicity (Fensterbank 1986a), as vaccinal strain and strain ATCC 19115 as challenge strain. After having been kept lyophilized, both strains were passaged twice on mice before use. They were grown on Brain Heat Infusion agar slants (Difco, USA) for 18 hours at 37 °C, harvested and adjusted to the required concentration in phosphate buffer saline after turbidimetric measure.

Challenge strain 19115 can be differentiated from vaccinal strain Aer by its phage type, different from that of strain Aer (strain 19115 is susceptible to phages 2671, 2425, 3552, 2389 and 4286, when strain Aer is susceptible to phages 2671, 3551, 3552, 1317 and 184) and by its susceptibility to erythromycin when strain Aer is resistant to 1000 μg/ml.
2. Animals and experimental design

In a first experiment, 60 adult alpine goats having already kidded once or twice were divided in three equal groups, one control and two vaccinated once or twice with 1.5 x 10^{10} CFU strain Aer. In a second experiment, one year later, 70 young alpine goats which had never kidded were divided in four groups: one control group and three vaccinated once or twice, before or after mating, with 10^9 CFU strain Aer in average (table 11. ).

Goats were mated after oestrus synchronization. After the accidental death of 5 animals and the rejection 30 which obviously were barren, 95 goats were challenged with Lm strain ATCC 19115 when 95-100 days pregnant. The challenge strain was injected by subcutaneous route at the dose of 5 x 10^8 CFU for the first experiment and at the dose of 4.3 x 10^8 CFU for the second one.

Later on, 22 other goats were found non pregnant.

3. Examination procedures

Duration of pregnancy, abortions and deliveries at term were recorded.

Cultures were made from uterine discharge collected with vaginal swabs sampled on the day of delivery and the day after. In addition, duration of vaginal excretion was tested by sampling swabs twice a week for four weeks on 5 goats which aborted. Colostrum was sampled two successive days also: it was centrifuged, and cream and pellet were cultured separately. All aborted foetuses and dead kids were necropsied and cultures were made from their spleen, liver and gastric content.

From both experiments, 26 goats were necropsied. Samples of organs were ground in a mixer (Kenwood, Woking, UK); lymph nodes from goats, livers and spleens from foetuses were homogenized in a stomacher (Prolabo, Paris).

Tissue homogenates were seeded onto Listeria Selektivagar (Merck, Darmstadt, RFA) without enrichment procedure. After a 24 and 48 hours incubation at 37 °C, plates were examined by transillumination with a magnifier. Colonies resembling Lm were identified (Gram, catalase, esculin agar, litmus milk) and one strain per animal was subjected to phage typing and examined for its resistance to erythromycin for differentiation between vaccinal and challenge strains.

Results

1. Innocuousness

After vaccination with Aer at the highest dose, ie 1.6 x 10^{10} CFU, temperatures rised to a peak of 40.1 °C in average (40.8 °C on one animal) on the day after and returned to the normal on the third day. There was no loss of appetite and animals did not seem limp. Local swelling was very moderate and disappeared quickly.

One goat out of 18 aborted 14 days after a recall done at 67 days of pregnancy. The strain recovered from a vaginal swab showed same phage-type and same resistance to erythromycin than vaccinal strain Aer.

2. Clinical results (table 1)

On the pool of both experiments, 29 goats aborted: 28 from 6 to 19 days after challenge and a 29th one (goat 49) 28 days after challenge. None showed any illness before abortion. Forty goats kidded at term from 49 to 61 days after challenge. Four goats died from 4 to 10 days after abor-
tion: all showed important lesions of metritis and numerous microabcesses were observed on the liver of one. One goat was slaughtered because of paralysis two days after normal kidding and no gross lesion was seen.

Four pregnant animals died from accident or were emergency slaughtered from 21 to 38 days after challenge.

3. Bacteriological results

Lm were reisolated in very large number from all swabs collected on the 28 goats that aborted. On the 5 animals specially examined for duration of uterine excretion, the number of reisolated Lm decreased with the time and excretion gave up on the third week after abortion. Organisms were recovered in fewer number in the colostrum of 19/24 aborted goats (4 colostrums were not available). All aborted foetuses were heavily infected.

No Lm was recovered from swabs, colostrum and foetus of the goat 49 that aborted 28 days after challenge. Likewise no Lm was reisolated from swabs and colostrum from 40 goats that kid ded at term, neither from 3 kids that died soon after birth.

At necropsy, Lm were reisolated in large number from the four animals that died from 4 to 10 days after abortion, and in very few number from the lymph nodes of the head of two and from the liver of one out of 16 goats slaughtered from 40 to 54 days after abortion. In contrast, no Lm was recovered from four goats which died or were emergency slaughtered from 21 to 38 after challenge, neither from their 11 foetuses, nor from one goat which died two days after delivery at term.

On the whole, Lm were regularly reisolated from day 6 to day 19 after challenge and never from day 21 after challenge until the end of the experiments, except on three goats necropsied from 40 to 54 days after abortion.

All reisolated strains of Lm showed same phage-type and same susceptibility to erythromycin than challenge strain ATCC 19115.

4. Protection

Rates of abortions in controls from both experiments being not statistically different in spite of slight differences in the design of each experiment, data were pooled in order to make the analysis easier.

On the whole (table 2), rate of abortions in goats vaccinated once (45.8 %) is not significantly different from that of controls (56.5 %), but rate of abortions in goats vaccinated twice (22.7 %) is at the same time significantly lower than that in controls (P < 0.01) and than that in animals vaccinated once (P < 0.05).

Discussion

In both experiments, vaccination with strain Aer was very well tolerated, even with the highest dose of 10ⁱ⁰ CFU: the rise of temperature was moderate and transient. There was no loss of appetite and local swelling was slight, often escaping notice. The same tolerance was observed after several thousands vaccinations in the field (Guer rault et al, essai de vaccination des caprins contre la listériose, submitted for publication). We know that after SC vaccination at the neck, the homolateral prescapular lymph node is infected for a short time, generally inferior to a week, and that generalized infection may occur, reaching spleen and liver on only some animals (Fensterbank 1986). However, one goat recalled at the 67th day of pregnancy aborted in these experiments. This event seems to be marginal: we did not observe abortions on 98 sheep and on the 16 other goats of the same groups vaccinated at the station during gestation, and very rarely on pregnant goats vaccinated in the field. It must be kept in mind that, as a rule, live vaccine should not be injected to pregnant animals.

In the first experiment, protection was poor after a single vaccination, but evident after a recall. With the limited number of animals, differences in rates of abortion were not significant to the chi-square
A second experiment was planned to corroborate or invalidate these first results. Challenge strain was the same in both experiments, and dose of challenge and stage of pregnancy were very comparable in both experiments. In contrast, goats were younger in the second experiment than in the first one, but this did not seem to have any effect since clinical results in control groups were not significantly different. Doses of vaccine were also different, roughly ten fold less in the 2nd experiment than in the first one, but it was shown in mice that difference of one log₁₀ in the dose of vaccine was very few effective and, actually, there were even less abortions in animals having received the lower dose of vaccine. In addition, vaccinations had been performed later in regard to challenge in the 4th group of the second experiment, in order to study the duration of immunity. Unfortunately, no effect was observed because of too limited number of animals. In this 2d experiment, rates of abortions were about the same in controls and in animals vaccinated once, but all goats vaccinated twice kidded at term. Both experiments were performed one year apart and the fact that the results were roughly similar enhances the confidence in the effect of vaccination.

In experimental conditions, an actual protection was induced only when a recall was performed. We already observed on mice that a recall increases strongly the immunity induced by a primary vaccination. Nevertheless, single immunization afforded in mice an evident level of protection and seemed to induce in small ruminants an immunity sufficient to stop the course of encephalitis in epidemic conditions in most of the flocks of the field (Guerrault et al, essai de vaccination des caprins contre la listériose, submitted for publication). In contrast, single vaccination afforded on goats only a poor protection against experimental abortion. However, we must keep in mind that experimental challenge is very different from natural contamination. The effect of single administration of a large number of Lm injected by parenteral route leading likely to short-circuit of some immunological barriers is much more severe than natural repetition of lower doses of organisms having to pass through mucous membranes and afferent lymph node and a rate of 56.5 % of abortions seems having never been observed in field conditions. We tried to reproduce the natural passage of Lm through mucous membranes by inoculating them by conjunctival route, but we did not succeed in obtaining regular abortion or encephalitis. We can also wonder whether abortion, the only model easily accessible to experimentation, is valuable in representing the natural disease which includes other symptoms such as encephalitis.

Nevertheless, this model allowed to display, in severe conditions of experimental infection, a protection induced by vaccination and recall. These results corroborate previous findings on mice and the advantage of strain Aer used as vaccine. Thus, for field application, a single vaccination may be enough to protect from natural contamination, but in case of severe epizootic, a recall would increase immunity up to a much higher level.

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Abstract

A live Listeria monocytogenes strain of reduced virulence, strain Aer, obtained by three successive mutations in regard to streptomycin and erythromycin, was used as vaccine on goats in two successive experiments. Animals were vaccinated either once or twice by subcutaneous route with doses varying from 6 x 10⁸ to 1.5 x 10¹⁰ CFU. No side effect was observed, except in one goat, vaccinated as pregnant, that aborted and from which the vaccinal strain was reisolated. The goats were challenged by subcutaneous route with 5 x 10⁸ CFU L monocytogenes virulent strain ATCC 19115 at 95-100 days of pregnancy. Thirteen out of 23 controls (56.5 %) and 11 out of 24 once vaccinated goats (45.8 %) aborted whereas only 5 out of 22 (22.7 %) twice vaccinated goats aborted. Significant protection against a severe challenge can thus be afforded by vaccination and recall with the live strain L monocytogenes Aer.

Références


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VACCINATION AGAINST LISTERIOSIS IN GOATS


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