

## EFFICIENCY OF A LIVE 316 F STRAIN VACCINE AGAINST PARATUBERCULOSIS IN RUMINANTS

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### Introduction

It is nearly a century ago that JOHNE and FROTHINGAM identified Mycobacterium paratuberculosis as the causative agent of PARATUBERCULOSIS, commonly named JOHNE'S DISEASE, in RUMINANTS. This disease is still, (and even more and more in some parts of the world), responsible for considerable economic losses to the dairy and beef cattle industry, related to reduced milk production, diarrhoea, emaciation, decreased fertility, and mortality (1,2,3,4).

The specificities of the epidemiology, the pathogeny and the diagnosis of this infection have made its control something difficult : e.g. the early contamination of the newborn calves, the late clinical expression of the disease in adult animals, the evolution of humoral and cellular indicators of the immune response in individually infected animals (5,6,7,8,9,10,11).

Propositions of control programs have been presented some years ago and recently updated (5). According to their authors, they rely on one or both of the following strategies : vaccination and/or sanitary measures.

The concept of vaccination has been developed by VALLEE and RINJARD (12,13) some 60 years ago. The following presentation wishes to expose the characteristics of a live 316F strain Mycobacterium paratuberculosis vaccine (\*\*\*), and the results obtained in France (dept. des Côtes d'Armor) when the use of this vaccine in newborn calves is associated with fecal culture in a large scale control program against PARATUBERCULOSIS in cattle.

(\*\*\*) NEOPARASEC<sup>R</sup> - RHONE MERIEUX - LYON - FRANCE

### I) - CHARACTERISTIC OF NEOPARASEC : live 316 F strain vaccine against PARATUBERCULOSIS in RUMINANTS

#### 1. The vaccine

NEOPARASEC<sup>R</sup> is a freeze dried live vaccine, containing Mycobacterium paratuberculosis, 316F strain, and reconstituted with an oil-in-water adjuvant at the time of use.

The vaccine is indicated for use in calves, lambs, and kids within the first month of life since it is at that time that the animals mostly contract the disease.

The dose for calves contains  $0.5 \cdot 10^3$  CFU in 2 ml; the dose for lambs and kids contains  $0.25 \cdot 10^3$  CFU in 1 ml for administration via the subcutaneous route.

Trials are currently being performed in order to check the interest of another route of administration for this vaccine : the intradermal route. In the laboratory, the humoral immune response is evaluated by using the complement fixation test ; the cell mediated immunity is evaluated by performing intradermal tests with avian and bovine tuberculins and Johnin (14).

#### 2. Efficiency in cattle

The efficiency of the vaccine has been demonstrated in controlled trials as well as under field conditions in control programs (15,16,17).

Vaccination of young replacement animals with a single injection during their first month of life (ie. when they are most susceptible to contamination) greatly reduces the number of shedders in the herd in subsequent years. The reduction of the number of *M. paratuberculosis* excretors, in parallel with the reduction of morbidity and mortality, illustrates the primary economic importance of vaccination practice as an integral part of any control program against the disease.

### 3. Efficiency in sheep and goats

Although the use of the vaccine in sheep and goats is not as old as in cattle, some recent data illustrate the interest of vaccination in these species and show similar results to those described in cattle. Significant data is being generated from extensive trials in New Zealand sheep flocks. In goats the success of a Norwegian program using a live paratuberculosis vaccine indicated that vaccination is as effective as in cattle, since the number of infected animals was greatly reduced (17,18).

### 4. Safety aspects

No general reaction to injection of the vaccine, by the subcutaneous route has been described. However local reactions have been observed in some vaccinated animals at the site of injection. The user must be made aware of such vaccination features and advised to take every precaution to ensure that the vaccine is administered aseptically and strictly subcutaneously to minimize these local reactions.

II) - **EFFICIENCY OF VACCINATION** associated with fecal culture, in a large scale control program in cattle, in the department of COTES D'ARMOR in FRANCE.

#### 1. Introduction

The control program is organized by the FDGDS22 in the COTES D'ARMOR. This program is based on three major components : detection of excretors by fecal culture, hygienic measures, and vaccination.

It involves more than 500 herds (19,20).

All data are computerized on a herd as well as an individual cattle basis.

#### 2. Material and methods

a) Detection of excretors by fecal culture is performed on Herolds's medium, in herds with high clinical incidence in the previous year (more than 5% of adult cattle). Only cattle more than 2 years old are sampled. Excretors are usually slaughtered within the 3 months following the culture result. A second culture is performed on cattle negative to the first, and so on for the third. Interval between cultures is usually one year. Very few herds had more than 3 cultures.

b) Hygienic measures in the control program have been previously described elsewhere (19).

c) Vaccination is performed on the calves by a veterinarian during the first month of life with NEOPARASEC, administered at the recommended dose, subcutaneously. Vaccination is compulsory in the control program. However some calves may have been forgotten or neglected, mainly at the beginning of the program. This allows us to compare three groups of Animals (Group 1, Group 2, Group 3).

According to the year of birth, tested cattle have been divided in 3 groups. The first group (Group 1) is composed of cattle born before the beginning of the control program and specially before implementation of hygienic measures. The second category is composed of cattle born during the control program, and comprises vaccinated (Group 3) or non-vaccinated (Group 2) animals; Group 2 is constituted of cattle whose vaccination has been forgotten or neglected by the farmer.

### 3. Results

They are presented in the table 1.

For the first fecal cultures performed in 15219 cattle there is a significant  $p < 0.02$  Tst  $X^2$  difference (8.4%-6.5%) between infection rates of non- vaccinated cattle born before (Group 1) or during (Group 2) the control program. Within cattle born during the control program, there is a larger and significant  $p < 10^{-3}$  Tst  $X^2$  difference between vaccinated cattle (Group 3 : 1.2% positive) and non-vaccinated cattle (Group 2 : 6.5%).

The second culture has been performed on cattle negative to the first one, since positive cattle had been slaughtered as required in the control program ; and the third culture on cattle negative to the first and the second. For the second and third culture there is no significant difference between Group 1 and Group 2.

In Group 1, results show a significant  $p < 10^{-3}$  Tst  $X^2$  decrease of infection rate from the first to the third culture (8.5%-5.6%-2.9%). This decrease is also noted in Group 2 (6.5%-5.7%-2.7%).

From the first to the third culture, Group 3 results always present significantly  $p < 10^{-3}$  Tst  $X^2$  different results from the one observed in the two groups of non-vaccinated cattle (Group 1 and Group 3).

	1st culture		2nd culture		3rd culture	
	15219		5663		2106	
	Nb test.	%+	Nb test.	%+	Nb test.	%+
	↓	↓	↓	↓	↓	↓
<b>Group 1 : cattle born before the beginning of the control programme</b>	11865	8.4%	4580	5.6%	1683	2.9%
<b>Group 2 : cattle born during the control programme and not vaccinated</b>	1281	6.5%	349	5.7%	147	2.7%
<b>Group 3 : cattle born during the control programme and vaccinated</b>	2073	1.2%	734	1.7%	276	0.7%

**Table 1 : Effect of vaccination and/or hygienic measures on the percentage of positive fecal culture rate in paratuberculosis infected herds.**

#### 4. Discussion

Significant differences between the infection rates in group 2 and 3 show that vaccination is the most efficient measure. Differences between group 1 and 2 are significant only for the first culture. Hygienic measures are efficient but not sufficient to induce a definite decrease of infection rate in the not vaccinated group. We can notice a significant decrease of infection rate from 1st to 3rd culture in group 1. In these herds, with a high clinical rate (> 5% of adult cattle/year), the first two cultures and culling of excretors induced a definite decrease in clinical rate.

However, the survey clearly shows that vaccination, in association with hygienic measures and fecal culture detection of excretors, is able to decrease the infection rate in contaminated herd at a level that was not reached in three years by using only hygienic measures.

#### Conclusion

Laboratory data and field use results demonstrate the interest of using a live 316 F strain vaccine, for the vaccination of calves, lambs and kids during their first month of life, in a control program against paratuberculosis in ruminants relying on the 3 major aspects : vaccination, hygiene, detection of excretors. The last two aspects are not sufficient when they are used alone.

#### Summary

Paratuberculosis, commonly named Johne's disease is identified in many parts of the world as a major sanitary problem due to its epidemiological, pathological and economic importance.

Many countries consider that vaccination is a necessary tool to control and eradicate the disease.

The authors present the results of a Mycobacterium paratuberculosis, 316F strain, freeze-dried live vaccine, reconstituted in an oil-in-water adjuvant, NEOPARASEC<sup>®</sup>, for the vaccination of calves, lambs and kids within the first month of age.

Laboratory trials demonstrated the activity of the vaccine as evidenced by the humoral immune response (fixation complement test) as well as the cell-mediated immune response (delayed skin hypersensitivity), when the vaccine is administered via the subcutaneous route or the intradermal route.

Large scale field survey (15000 animals, 500 herds) performed by the GDS22 in France have recently demonstrated that the use of NEOPARASEC<sup>®</sup> is the key factor in control programs against PARATUBERCULOSIS in cattle ; and have shown a significant difference of positive fecal culture rate (8,5% to 0.7%) between non vaccinated and vaccinated animals.

Hygienic measures of control alone can not give such results in not vaccinated cattle.

#### Résumé

La Paratuberculose, communément appelée Maladie de Johne, est considérée dans plusieurs parties du monde comme un problème sanitaire majeur en raison de son importance épidémiologique, pathogénique et économique.

De nombreux pays considèrent que la vaccination constitue un outil nécessaire pour contrôler et éradiquer la maladie.

Les auteurs présentent les résultats d'un vaccin vivant lyophilisé contenant la souche Mycobacterium paratuberculosis 316 F, reconstitué en adjuvant huileux O/W, NEOPARASEC<sup>MD</sup> pour la vaccination des veaux, agneaux et chevreaux durant leur premier mois de vie.

Au laboratoire, des essais ont démontré l'activité de ce vaccin, mise en évidence par la réponse immunitaire humorale (réaction de fixation du complément) connue par la

réponse immunitaire cellulaire (hypersensibilité cutanée retardée), quand le vaccin est administré par la voie sous cutanée ou la voie intradermique.

Sur le terrain, le suivi sur une grande échelle (15000 animaux, 500 troupeaux) réalisé par le GDS22 a démontré que l'utilisation du vaccin NEOPARASEC est un facteur clé du programme de contrôle de la paratuberculose chez les bovins ; et a démontré une différence significative de taux de culture fécale positive (évoluant de 8,5% à 0,7%) entre les animaux non vaccinés et les animaux vaccinés.

Des mesures hygiéniques de contrôle, seules, ne peuvent pas donner de tels résultats chez des animaux non vaccinés.

### Resumen

Paratuberculosis, comunmente Llamada Enfermedad de Johne's, es identificada en muchas partes del mundo como un problema sanitario mayor, debido a su importancia epidemiologica, patologica y economica.

Muchos paises consideran la vacunacion como una herramienta necesaria para controlar e irradiar la enfermedad.

Los autores presentan los resultados de la cepa vacunal viva Mycobacterium paratuberculosis, 316 F, congelada-desechada, reconstituida en aceite en aguacomo adjuvante, NEOPARASEC, para la vacunacion de terneros, corderos y cabritos durante su primer mes de vida.

Pruebas laboratoriales han demostrado la actividad de la vacuna y su evidencia a traves de la respuesta inmune humoral (fijacion del complemento) asi como la respuesta inmune mediada por celulas (Hipersensibilidad retardada de la piel) cuando la vacuna es administrada via subcutanea o intradermica.

Supervivencia en el Terreno a gran escala (15.000 animales, 500 rebanos) llevados a cabo por el GDS22 en Francia han demostrado recientemente que el uso de NEOPARASEC es un factor basico en programas de control contra la PARATUBERCULOSIS en el ganado, y han mostrado la diferencia significativa en los niveles positivos de los cultivos fecales (8,5% hasta 0,7%) entre animales no vacunados y vacunados.

Medidas higienicas de control solamente no pueden proporcionar estos resultados en ganado no vacunado.

### References

1. Thoen, C.O., Baum, K.H., Current knowledge on paratuberculosis, JAVMA 192(11):1609-1611.1988.
2. Proceedings of the Second International Colloquium on paratuberculosis. Maisons-Alfort. FRANCE. 1988.
3. Cottureau, Ph. La paratuberculose des ruminants. Cah. Méd. Vét., 39:275-289. 1970.
4. Chioldini, R.J., Van Kruiningen, H.J., Merkal, R.S., Ruminant paratuberculosis (Johne's disease). The current status and future prospects. Cornell vet.74:218-262.1984.
5. Proceedings of the Third International Colloquium on paratuberculosis. ORLANDO. USA. 1991.(in press).
6. Buergelt,C.D., Hall, C., Mc Entee, K., Ducan, J.R., Pathological evaluation of paratuberculosis in naturally infected cattle. Vet.Path.15:196-207.1978.
7. Merkal R.S., Paratuberculosis : Advances in cultural, serologics and vaccination methods. JAVMA, 84:939-943, 1984.
8. Desmettre, Ph.,Valette,L., Experimental diagnosis of Johne's disease caused by infection with Mycobacterium paratuberculosis,Col.Int. sur les Mycobactéries atypiques. Lyon-France.1979.
9. Whitlock, R.H., Simpson,M.M.,Chronic Diarrhea in cattle : differential diagnosis in 14th World Congress on diseases of cattle. Dublin. 307-317.1986.10. Anonymous, Quest continues for fast, reliable test for bovine paratuberculosis, JAVMA, 197(3): 299-304. 1990.
11. Brugère-Picoux, J., Le diagnostic de la paratuberculose chez les ruminants, Rec. Méd.Vét.163,(5):539-546,1987.
12. Vallée, H., Rinjard, P., Etude sur l'entérite paratuberculeuse des bovidés. Rev. Gén. Méd. Vét. 409:1-9.1926.
13. Vallée,H.,Rinjard, P., Vallée, M., Sur la prémunition de l'entérite paratuberculeuse des bovidés, Rev.Gén.Méd.Vét. 516:777-779.1934.
14. Saint-Marc,B., Guillemain,F.,Milward, F., Reynaud,G., Lacoste,F., Brun,A., Vaccination against paratuberculosis:New perspectives.in Proc. of the Third International Colloquium on paratuberculosis,

Orlando, USA, 1991, (in press). 15. Jorgensen, J.B., The effect of vaccination on the excretion of *Mycobacterium paratuberculosis*. Paratuberculosis diagnostic methods, their practical application and experience with vaccination. CEE. Bruxelles, 131, 1984. 16. Stuart, P., Vaccination against Johne's disease in cattle exposed to experimental infection. *Brit. Vet. J.* 121:289-312.1965. 17. Mac Diarmid, S.C., The control of Johne's disease by vaccination, *Surveillance* 16,2:7-10,1989. 18. Saxegaard, F., contrôle de la paratuberculose (Maladie de Johne) des chèvres par vaccination en Norvège. *Les maladies de la chèvre*, Niort, 541, 1984. 19. Hillion, E., Argenté, G., Plan de lutte contre la paratuberculose dans les Côtes du Nord : Aspects sanitaires et économiques. *Le Point Vét.* 19:123-130.1987. 20. Argenté, G., Efficiency of vaccination and other control measures estimated by fecal culturing in a regional program. in Proc. of the Third International colloquium on paratuberculosis, Orlando, USA, 1991, (in press).