

### THE EFFICACY OF A KILLED VACCINE AGAINST PARATUBERCULOSIS (SILIRUM®) IN CATTLE. A FIELD STUDY

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#### ABSTRACT

The efficacy of a killed vaccine against paratuberculosis in cattle has been assessed in a field study, using a histopathological method for the evaluation of paratuberculosis associated lesions in culled animals. Based on previous natural and experimental studies, lesions present in the intestine and associated lymph nodes, were classified as focal, multifocal -related to initial or latent forms, low bacterial load and subclinical-, and diffuse or severe, usually with high levels of mycobacteria in the tissues. Vaccination induced strong cellular and humoral responses that persisted in more than 90% of them until the end of the study. No new clinical cases appeared later than 6 mpv. The total number of culled animals (n=192), in the evaluated period, was higher in the control (48.1%) than in the vaccinated group (39%). A significant reduction in the percentage of culled animals that showed lesions associated with paratuberculosis infection has been also observed in vaccinated (56.6%) with respect to control (92.3%) cows. A clear decrease has been also observed in the number of cows with diffuse or severe lesions (15.1% of vaccinated vs 34.6% of controls) although some of them have appeared, at 30 mpv, in vaccinated cows. Vaccination has shown to be a valuable method for achieving a significant reduction of paratuberculosis in a herd with high percentage of losses.

#### INTRODUCTION

Vaccination has been considered to be one of the most efficient methods for the control of paratuberculosis. The use of either killed or live vaccines has been shown to reduce, in all susceptible species, the number of clinical cases and shedding of *Mycobacterium avium* subsp. *paratuberculosis* (Map) in faeces more rapidly and with lower economic costs than other procedures such as culling of faecal culture positive individuals (Jorgensen, 1983; Argente, 1992; Van Schaik *et al.*, 1996; Groenendaal, H. and Galligan, D.T., 2003). Pathological methods have been used for the assessment of the efficacy of vaccines (Nisbet *et al.*, 1962; Juste *et al.*, 1988). A classification of lesions that associates pathological features with the different stages of Map infection has been proposed (Pérez *et al.*, 1996; González *et al.*, 2005). In sheep, it has been shown that vaccination does not prevent the infection, but modifies the inflammatory response towards focal lesions restricted exclusively to the intestinal lymphoid tissue, whereas in unvaccinated animals, lesions spread to different mucosal areas (Juste *et al.*, 1994; García Marín *et al.*, 1995). In this field study, the efficacy of a killed vaccine against paratuberculosis in adult cattle has been assessed using a histopathological method for the evaluation of paratuberculosis associated lesions in culled animals.

#### MATERIALS AND METHODS

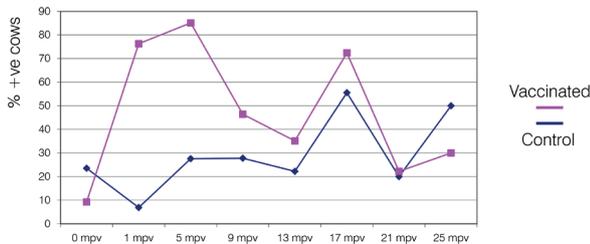
The study has been carried out in a Friesian dairy herd, showing approximately a 12% of annual clinical cases of the disease, and formed by 468 adult cows. A 75% of the total, regardless the age or clinical status, was vaccinated with a single dose of SILIRUM® (CZ Veterinaria) and the remaining 25% kept as unvaccinated controls. For a period of 30 months post-vaccination (mpv), all the culled animals, for any reason, have been recorded and pathological studies carried out in 79 of them. Cellular and humoral peripheral immune responses have been also assessed by IFN- $\gamma$  production and ELISA tests respectively.

#### RESULTS AND DISCUSSION

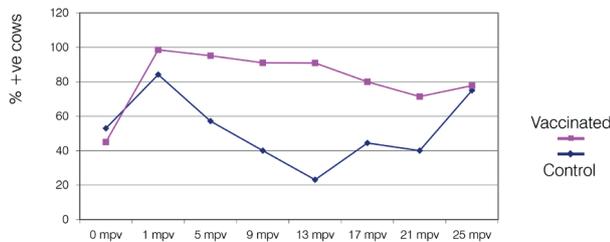
Vaccination against paratuberculosis induced a strong humoral immune response in the majority of vaccinated animals. The number of cows positive to ELISA test reached its highest point at 5 mpv (85% approx.) and began to decrease until 21 and 25 mpv showing no differences with controls (Figure 1). As it can be seen in Figure 2, a strong cellular immune response was also induced by vaccination. The percentage of vaccinated

animals positive to IFN- $\gamma$  test was 100% at 1 mpv, decreasing slowly to approximately 80% at the end of the study.

**Figure 1.- Percentages of cows positive to ELISA test according to their vaccination status and date post-vaccination.**

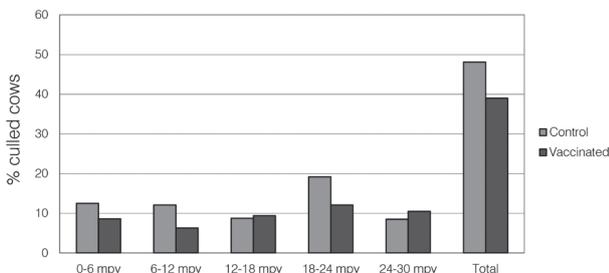


**Figure 2.- Percentages of cows positive to IFN- $\gamma$  assay according to their vaccination status and date post-vaccination.**



Thirty months after vaccination, 191 animals have been culled from the herd for any reason (48.1% of the unvaccinated group and 39% of the vaccinated). Percentage of culled animals, in all periods in which the study was divided, was higher in unvaccinated animals than in vaccinated cows, except in 12-18 mpv period (9.4% and 8.75%) and 24-30 mpv (10.5% and 8.5% in vaccinated and control respectively), where no significant differences were found.

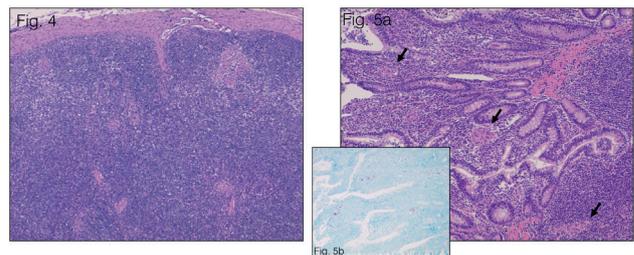
**Figure 3.- Percentages of culled animals according to their vaccination status and period of culling**



Pathological studies were carried out in samples of intestine and associated lymph nodes from 79 of the culled animals. Lesions were classified (González *et al.*, 2005) as focal/multifocal, formed by well-defined small granulomas located in the Peyer's patches or lymph

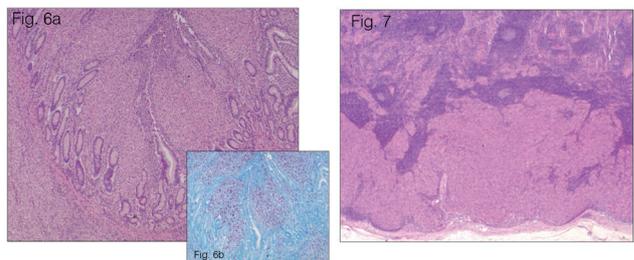
nodes, thought to be subclinical forms related to initial or latent phases of the disease respectively, with low mycobacterial load (Figures 4 and 5), and diffuse or severe, showing a widespread granulomatous enteritis associated with clinical signs and high levels of Map in the tissues (Figures 6 and 7).

**Figure 4.- FOCAL LESION. Small well-defined granulomas in ileal lymph node cortex and paracortex (H-E 40x). Figures 5a and 5b.- MULTIFOCAL LESION. Small well-defined granulomas located in ileocecal valve Peyer's patch and related mucosa with low Map load (H-E 40x; Z-N 100x).**



**Figures 6a and 6b.- DIFFUSE LESION. Severe and diffuse granulomatous enteritis affecting mucosal and submucosal areas in distal ileum with high Map load in tissues (H-E 4x001; Z-N 40x).**

**Figure 7.- DIFFUSE LESION. Diffuse granulomatous lymphadenitis with destruction of normal histology in ileal lymph node (H-E 40x).**



As it can be seen in Table 1, a significant reduction in the number of cows with paratuberculosis associated lesions have been observed in vaccinated (56.6%) with respect to control animals (92.3%). A clear reduction in percentage of diffuse or severe lesions present has been also seen (15.1% of vaccinated vs 34.6% of controls).

**Table 1.- Distribution of cows according the lesional type and vaccination status of culled animals .**

Type of lesion	Control	Vaccinated
	Nº of cows (%)	Nº of cows (%)
Without lesion	2 (7,7)	23 (43,4)
Focal/Multifocal	15 (57,7)	22 (41,5)
Diffuse	9 (34,6)	8 (15,1)
Total lesion	24 (92,3)	30 (56,6)

When considering the different dates post-vaccination, similar results were observed (Table 2) with a progressive reduction in the number of animals showing lesions from 24 mpv in vaccinated cows and an increase in animals showing diffuse or severe lesions in control cows.

animals, presumably already infected, would be the modification of the inflammatory response towards focal or latent lesions, subclinical or even the total disappearance of pathological changes.

**Table 2.- Percentages of lesional types of paratuberculosis according to vaccination status of the animals and period of culling.**

GROUP	Type of lesion	0-12 mpv (n=6)		12-24 mpv (n=12)		24-36 mpv (n=8)		Total	
		Nº animals	%	Nº animals	%	Nº animals	%	Nº animals	%
CONTROL (n=26)	Without lesion	0	0	1	8,3	1	12,5	2	7,7
	Focal/Multifocal	5	83,3	6	50	4	50	15	57,7
	Diffuse	1	16,7	5	41,6	3	37,5	9	34,6
VACCINATED (n=53)	Without lesion	3	30	10	47,6	10	45,5	23	43,4
	Focal/Multifocal	6	60	7	33,3	9	40,9	22	41,5
	Diffuse	1	10	4	19	3	13,6	8	15,1

Vaccination of adult cows has been shown to be an efficient method for reducing the number of clinical cases, as seen by the reduction of cows showing diffuse lesions, that are probably high shedders (Pérez *et al.*, 1996; González *et al.*, 2005). In experimental studies (Nisbet *et al.*, 1962; Juste *et al.*, 1994) it has been shown that vaccination does not prevent infection of animals, but modifies the inflammatory response towards a limitation of the progression of granulomatous lesions. The results obtained in this study support this hypothesis, since an increase of cows with no lesions and showing focal forms was observed in vaccinated animals in contrast to controls. It has to be taken into account that animals with severe lesions have been encountered at 36 mpv, suggesting that protection conferred by the vaccine is not absolute. This could be due to individual factors, that predispose to poor immune responses (Doyle, 1964), or the advanced stage of Map infection at the time of vaccination that has not enabled the vaccine to induce any modification in the inflammatory response.

This is a preliminary part of a field study in which the effect of vaccination on fecal shedding and milk production is being also assessed.

## CONCLUSIONS

Vaccination of adult cows against paratuberculosis with a single dose of SILIRUM® has shown to be a valuable method for reducing the number of culled animals and those showing severe lesions associated with clinical signs.

The effect of the administration of SILIRUM® to adult

Strong cellular and humoral immune responses mounted after vaccination persist in the animals for at least two years post-vaccination.

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