ABSTRACT

"Comparative study of the immune response induced by two types of vaccines (inactivated VLP) against Bluetongue virus in sheep"

The Bluetongue (BT) is an infectious, not contagious disease transmitted by midges of genus *Culicoides* that concerns different species of ruminants. The animals that develop the disease are mainly the sheep, where BT is able to reach high rates of morbidity and mortality. The BT disease does not affect humans, but it receives great importance due to its high power of transmission and diffusion provoking serious socioeconomic and sanitary consequences, which have influence in the international trade of animals and products of animal origin. For all this, BT is included in the notifiable list diseases of the World Organization for Animal Health (OIE).

This disease is caused by a virus belonging to the *Orbivirus* genus (*Reoviridae* family*),* which is composed by 7 structural proteins (VP1-VP7) and five non-structural (NS 1, 2, 3, 3A and 4). A particularly important protein in the immune response is the VP2, since it is responsible for the formation of neutralizing antibodies and serotype-specific.

In the current context of Europe, where 6 serotypes are present (1,2,4,8,9,16), the vaccination has demonstrated to be a basic tool for the control and the eradication of this disease since it has been observed in Spain by the eradication of the serotypes 2 and 4.

The absence of cross immunity between serotypes, supposes a great problem since it implies immunizing to the whole livestock for each serotype. Vaccines currently available are mainly monovalent and only bivalent in some cases. To date it has not been able to develop a diagnostic method that allows differentiation between vaccinated animals with inactivated vaccine and infected animals.

To solve the problems previously described, VLP vaccines (virus like particles) have been developed. These vaccines are composed by a complex of structural proteins (VP2, VP3, VP5, VP7), that assemble into virus-like structures, lacking the genetic material, which involves replicative inability. In their production, expression vectors based in Baculovirus are used. Such vaccines show great advantages such as high level of safety, the ability to distinguish vaccinated animals from infected, thanks to the absence of NS1 nonstructural proteins, and the ability to generate multivalent vaccines by including in them different VP2 from different serotypes . These are the reasons why they have great potential to become the future vaccines,

but it is necessary to prove that these vaccines induce a good immune response and provide effective protection in animals, given current conditions in Europe.

The aim of this thesis is the assessment of humoral and cellular response produced by VLP vaccines against the BT virus and its comparison with the inactivated vaccines, assessing the advantages and disadvantages of both types of vaccines in the environment of control and eradication of disease. To that purpose, techniques have been adapted to characterize the immune system of sheep under physiological conditions, and in the immune response that occurs as a result of the vaccination or after the virus challenge.

To characterize the immune response produced by vaccines, we have studied different parameters such as production of antibodies, viremia levels, or cytokines kinetics. To carry out this work we have optimized the techniques for the detection of 6 cytokines encoding mRNA by real time RT-PCR (IL2, IL4, IL10, IL12, IFNγ and TNF) and the detection of different cell populations of the immune system in peripheral blood of sheep by flow cytometry ($CD4^+$, $CD8^+$, $CD25^+$, $CD14^+$, B lymphocytes, the subpopulation of T lymphocytes γδ TCR1-N6 and $WC1^+$).

Within the European project BTVAC (FP6-2005-SSP-5), a trial of two VLPs vaccines (monovalent BTV1VLP, and bivalent BTV1+BTV4VLPs) as well as a monovalent inactivated vaccine against serotype 1 has been realized in sheep. In this vaccine trial, it has been carried out a daily monitoring of the animals with the registration of clinical symptoms, rectal temperature and surface temperature by infrared thermography. Also periodic blood samples were taken for virus detection by real time RT-PCR, for analyzing the antibody levels by ELISA, for detection of PBMCs cell populations by flow cytometry and mRNA extraction to analyze the expression of genes encoding for cytokines by real time RT- PCR.

The results of this thesis indicate that the immune response generated prevents the onset of symptoms, lesions and viremia after exposure to the homologous virulent virus, with the use of either monovalent vaccines (inactivated and VLP) or with the bivalent VLP vaccine, indicating the safety of both types of vaccine to be used even in the presence of the vector. In the detection of clinical symptoms, the possible use of infrared thermogrphy as a screening tool for fever has been analyzed, yielding results with an enough sensitivity and specificity for its use as a tool for mass detection, this tool can be used in monitoring sentinel animals.

The evolution of cytokine expression has shown the importance of IL2 in response to monovalent VLP vaccine and IL12 in response to vaccination with bivalent VLP and after challenge of control animals with their respective homologous virulent virus. On the other hand, cytokines such as IFNγ, whose importance in the response against BTV has been previously described, have not been shown in some cases, due to the variability introduced by the technique as well the variability between animals. The analysis of PBMCs populations showed

for both VLP vaccines the increase in the $CD25^+$ population after vaccination, being more evident in the case of animals vaccinated with monovalent VLPs. Animals vaccinated with inactivated vaccine showed a significant increase in the percentage of B lymphocytes. Both animals, vaccinated with inactivated vaccine and non-vaccinated, showed an increase in $CD8^+$ T cells after challenge with the virus, while significant variation was not observed in the case of those vaccinated with VLPs.

The response generated by both types of vaccine is enough to generate protective antibodies in the case of monovalent vaccines for serotype 1 (VLP and inactivated) and in the case of the bivalent VLP vaccine for serotypes 1 and 4. Finally, the results obtained in the control groups indicated that serotype 1 has a greater pathogenic capacity, than the serotype 4.