**SUMMARY**

West Nile Disease (WND) is a viral infection caused by an arbovirus of the Flaviviridae family (WNV). Viral transmission is mediated by arthropods, principally of the Culex genus, and involves avian species where the virus may replicates efficiently. Other animal species, such as human and horses, may occasionally become infected but are considered as dead-and host and, hence, not responsible for the spreading of the infection.

The management of serological surveillance against West Nile, especially in equines, may run into some critical issues related either to "false positivities" induced by viruses antigenically correlated to WNVirus, or by seropositivity induced by vaccination campaigns: regarding the latter, overcoming the problem would require the availability of a "DIVA" serological test, enabling the differentiation of "true" positivities (induced by a viral expotion) from those induced by vaccinations. A serological test with these characteristics must obviously be based on viral antigens not present in the vaccine formulation used: at present, the vaccines used for WNV are of recombinant type and are based on the surface structural proteins "E" and "M"; the selection of the non-structural protein "NS5" should therefore prove suitable for the purpose of this research, namely the development of a DIVA test. "NS5", is the largest and most conserved protein in Flaviviruses and is involved in the replication of viral RNA, with the N-terminal portion having methyltransferase activity (MTase) wherease the C-terminal portion, an RNA-polymerase-RNA-dependent (RdRp) activity.

The definition of the working methodology used to accomplish the project, has started from epidemiological considerations, that show the circulation, in Italy, of two different viral lineages, the "lineage 1" (such as the "Livenza" strain) and the "lineage 2" (such as the "Rovigo" strain). Other considerations have related to the comparison of the polypeptide sequences (905 amino acids) that in the two different lineages correspond to the "NS5": these data show a sequence similarity of about 94%, with amino acid substitutions homogeneously distributed over the entire region. Due both this evidence and the fact that there is no available data regarding the immunogenic potential for the two polypeptides, the work plan included the expression of NS5 from both lineages, in form of subportions of about 30 and 70kDa corresponding to the "MTasi" domain (at the N-terminal) and the "RNA polymerase RNA dependent" domain (RpRd; at the C-terminal) respectively. The synthesis of the four sequences (two for each lineage) was than commissioned to a "gene synthesis" service, giving the opportunity to modify the sequences according to the codon usage of the host chosen for their expression, a system based on the *Escherichia coli* protein synthesis machinery.

The four sequences (sent in a storage vector) were amplified and then cloned into a first expression vector, the "pET/SUMO (Invitrogen)": the SUMO "fusion partner" has a mass of about 11kDa and is

the fungal counterpart (*Saccaromices cerevisae*) of the mammals' SUMO-1; literature data indicate that this polypeptide is strongly effective in conferring solubility characteristics to co-expressed proteins (the sub-polypeptides of NS5), assuming hence a role of "intramolecular chaperone". The expression of polypetides has been conducted by means of an E.coli cell free expression system (Promega), that enables the synthesis of recombinant proteins in an acellular enviroment, thus avoiding the generation of a Genetically Modified Microorganism (MOGM), the handling of which requires prior authorisation from the Ministry of Health. Immunological characteristics of the proteins expressed in this way have been checked by immunoblotting and ELISA assays, using positive and negative WNV horse sera. Results from these tests, showed an evident reactivity of all sera (positive and negative) sera against the SUMO partner: due to this evidence, a second experimental strategy hase been undertaken using a different expression vector, namely the pET200/D-TOPO (Invitrogen), devoided of any fusion partner excepting a small polypeptide, the "express epitope", to be eventually exploited for the detection phase. The drawbacks encountered during the experimentaions as well as the limited time available to accomplish the research project, have limited the use of this new strategy to a single polypeptide, namely the MTasi of lineage 2: preliminary results so far obtained confirm the suitability of this choice, that enables the reduction of undesirable antigen/antibody reactions and a sharper result.