Immunosuppressive viral diseases in poultry

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Immunosuppressive viral diseases in poultry

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**Objective**

A. BACKGROUND

Poultry is the fastest growing livestock industry overall, benefiting from efficiency gains in production and associated price advantages, and from health considerations. Per capita consumption as well as the share of poultry in the meat market have shown a consistent increase in most countries; and the projections are for these trends to continue as consumer preference as well as price advantage continue to favour poultry. Moreover, production expansion is amplified at present due to the impact of BSE in bovine meat demand.

Poultry meat and other products derived from poultry provide an attractive source of protein for many and various reasons. Perhaps the principal reason is the fact that the "turn-around" time is relatively short. In the case of meat chickens (or broilers), this period averages between 5 and 8 weeks in general. A second reason for the attractiveness of poultry proteins lies in the fact that chickens are small animals and, therefore, require little space to satisfactorily perform their production functions.

Thirdly, chickens require little or no supplementary feeding, and, therefore again, require minimal use of available land. In conjunction with their dietary requirements it must be noted that chickens can convert food intake into edible product in a highly efficient manner. In addition, the by-products of chicken production such as manure can be successfully recycled in other areas of agriculture. From these reasons, we may conclude that poultry production will remain an important agricultural activity which probably will continue to grow.

As a result, production will be subjected to intensive housing and management systems in order to maximise the main advantages of poultry as a food source. Given this background we may also safely deduce that this intensification will be accompanied by an increase in the incidence of disease in these enterprises. Because the poultry industry is a worldwide activity in comparable housing circumstances one can expect similar disease problems all over the world. One can conclude that very few important diseases are unique to particular parts of the world. Moreover, a similar genetic stock is disseminated to all the major poultry producing nations.

Important developments in relation to poultry production in the EU are:

- an increasing consumption:
  - (1) In 1985 the EU produced 5 329 000 tons of poultry meat and in 1995 7 749 000 tons (broiler production represented an amount of ECU 8.5 billion of business for European poultry slaughterhouses);
  - (2) In 1985 the EU produced 77.2 billion eggs for 81.4 billion in 1995 (egg production represented about ECU 4.5 billion of business for wholesale egg-traders),
- an increasing demand for microbiological safety, concerning human health,
- an increasing disease incidence (new as well as well-known ones), concerning poultry health.

Immunosuppressive viral diseases have been a great concern for the poultry industry for several years. Indeed, the emergence of Chicken Anaemia Virus (CAV) and the “reemergence” of Infectious Bursal Disease Virus (IBDV) in variant or highly virulent forms have been the cause of significant economic losses. The destruction of the target lymphoid cells of chickens, as observed in IBDV-infected B-cells and in CAV-infected T-cells, leads to immunosuppression, disease or death. These immunosuppressive viral diseases have an important economic impact due to the direct losses they provoke but also to
indirect losses as a consequence of immunosuppression or due to the interaction they might have together or with other factors. The direct losses are due to specific mortality, depending on the virulence and the dose of the inoculum, the age and breed of the birds and the presence or absence of a passive immunity. Moreover, these infections are also responsible for indirect losses due to acquired immunodeficiency, impaired growth and condemnation of carcasses. Furthermore, the increased use of antibiotics and chemicals to fight against opportunistic (secondary) infections is a major concern for human health, if we consider the risks linked to the presence of residues in meat product, the release of residues into the environment and the increased antibiotic resistance.

Estimation of the economic impact of the immunosuppressive viral diseases of poultry is complicated by the multifaceted nature of the infection. Depending on the strain of virus, susceptibility and breed of flock, intercurrent primary and secondary pathogens, and environmental and managerial factors, the mortality rate may range from 1% to more than 50%. In addition to mortality, Infectious Bursal Disease Virus (IBDV) is immunosuppressive. Studies conducted in Ireland by Mc Ilroy et al. documented a 14% decrease in financial return from broiler flocks with subclinical IBD compared to unaffected flocks. An 11% depression in net income was recorded in flocks which showed serological evidence of IBD during a mean 42-day growing period, compared to non-exposed broilers. A 10% reduction in profit for the 991 flocks in the study infected by IBDV was attributed to relative depression in body mass and feed conversion efficiency, but not in survivability, compared to non-exposed flocks. The emergence of hypervirulent strains in Europe since 1987 has increased the financial impact on producers. A similar evaluation was performed by the same laboratory (Mc Nulty et al.) in order to evaluate the economic effect of subclinical CAV infection on broiler performance. Therefore, clinically normal broiler flocks were grouped in two categories: flocks in which none of the 10 birds sampled at slaughter had antibody to CAV and flocks in which 6 or more of 10 similar birds had CAV antibodies. Production and performance parameters of 25 flocks in each category were compared. The results showed that subclinical CAV infection has a significant effect on commercial broiler performance and profitability with a 13% higher net income, a 2% improvement of feed conversion ratio and 2.5% increase in the average weight per bird in the first category.

Infectious Bursal Disease (IBD) - or Gumboro Disease - and Chicken Anaemia (CA) - or Blue Wing Disease - are highly contagious and distributed worldwide, with a high prevalence in countries with intensive poultry production. The selected host is the young chick in which a clinical disease occurs; while in older birds, the infection is essentially subclinical. Different breed susceptibility is described for IBDV with higher mortality rates in light breeds than in heavier. Serological surveys show a high prevalence of CAV and IBDV-antibody in breeder flocks, which has been acquired either following vaccination or subclinical infection. This immunity is passively transmitted to the offspring, protecting them at a young age against the clinical disease. Indeed, before 1987, IBD was satisfactorily controlled by vaccination and the incriminated strains, while highly contagious, caused less than 5% mortality. Therefore, the disease was essentially subclinical, with indirect economical losses due to immunosuppression. Since 1987, however, vaccination failures have been described in different parts of the world. In the USA, the new strains caused a slight increase in the mortality: virulent CAV is more important in Europe and in Asia, the new virulent strains were characterised by a specific mortality of up to 60% in layers and 25% in broilers. Thus, clinical IBD has become a predominant disease with additional losses due to specific mortality. Antigenic and molecular characterisation of these new strains has defined the new epidemiological situation. In the USA, the new strains are characterised by an antigenic variation that shows only a slight increase in virulence and are therefore called “variant” strains whereas, in Europe, the new strains still belong to classical serotype 1 strains but are characterised by a marked increase in virulence and are therefore called “hypervirulent” or “very virulent” IBDV (vvIBDV) strains.

During the 63rd General Session of the OIE, which was held in Paris, on 15-19 May 1995, it was estimated that Infectious Bursal Disease in poultry has considerable socio-economic importance. Not only to the national level as the disease is present in more than 95% of the Member Countries. At this session, 80% of the countries present reported the occurrence of acute clinical cases. Although isolates from different countries have been examined, the current typing confuses antigenic and pathogenic criteria, and the situation is sufficiently unclear to require more extensive comparative studies. Moreover, there is a wide variation in the general disease control procedures adopted which seldom conform to a specific or standard plan. These features justified the elaboration of a specific resolution.

Due to the high mutation rate of their RNA-polymerase, the emergence of viruses with new properties is a common mechanism of diversification of RNA viruses, allowing their persistence in immune populations. In the case of IBDV, these mutations might lead to antigenic variation or modification in virulence. In addition to antigenic differences in serotypes and subtypes, the viral strains can also be classified according to their virulence. Thus, IBDV strains can be defined as apathogenic (serotype 2); mild, intermediate or “hot” (serotype 1 vaccines); classical virulent (IBDV), variant (vIBDV), or very virulent (vvIBDV) (serotype 1). Serotype 2 strains cause neither mortality nor bursal lesions in SPF birds; serotype 1 vaccines cause no mortality but possess residual pathogenicity (with bursal lesions varying from mild to moderate or even severe) and virulent serotype 1 strains induce both mortality and bursal lesions. Classical virulent strains (e.g. strains FS2/70 and STC) cause up to 60 % mortality in SPF birds but only 1-2% in conventional chicks. Hypervirulent strains, on the other hand, cause up to 100% mortality in SPF birds, 20-25% in broilers and 50-60% in layers. However, there is a great deal of confusion in these descriptions. In particular, the term “hypervirulent strain” has been used to describe both European very virulent strains and variant American strains causing less than 5% mortality. In the absence of identification of specific virulence determinants, the only valuable criteria for the classification of IBDV strains as “pathotypes” should be their virulence (mortality, bursal lesions) in SPF birds. Moreover, increased virulence seems independent of antigenic variation and the search for virulence markers is still in progress.

CAV and IBDV have numerous similarities. Indeed, they are small, non-enveloped viruses which are very resistant to heat and to many disinfectants. Both of them cause disease in young chicks, by destroying immature lymphoid cells. Both induce immunosuppression by provoking apoptosis in their target cells. The younger the chicks are at infection, the more severe will be the immunosuppression. These viruses might have the same target organs (thymus, bursa of Fabricius, spleen), which makes the diagnosis difficult sometimes. But, in each disease, good protection is achieved by the induction of neutralising antibodies.

Cav and IBDV have had crucial differences which have important consequences for diagnosis and control. First, Cav is a DNA virus, undergoing few mutations and for which only one serotype is described. Worldwide, the Cav genome is highly uniform, particularly in the coding regions and the regulatory sequences. Therefore, diagnostic assays and vaccines are universally applicable. IBDV, on the other hand, is a RNA virus possessing a high mutation rate, which might lead to viruses with new properties such as antigenic variation or increased virulence. This situation needs more attention and efficient tools for a constant monitoring of the field. The second important difference between the two viruses lies in their mode of transmission. Indeed, although transmission of IBDV occurs exclusively by the horizontal mode, Cav can also spread vertically.
through the egg. This special mode of transmission needs an additional control of the disease, with adequate tools, to insure that parental flocks are seropositive before lay.

Clinical and subclinical CA and IBD cause economic losses but they might also interact together or with Marek’s Disease Virus (MDV). It is therefore essential to control both clinical and subclinical diseases. Due to the high resistance of IBDV and CAV to environmental exposure, hygienic measures alone are often ineffective and vaccination is thus essential. The economic impact of immunodepressive viral diseases, both clinical and subclinical, warrants the search for and the use of efficient vaccines. First of all, the development of more sensitive and rapid diagnosis methods warrants a better definition of the epidemiological situation of the diseases and particularly, a control of the presence of CAV-antibodies in breeders. Finally, for vaccination, recombinant vaccines might have several advantages over conventional vaccines. These improvements could only be obtained through a better understanding of the pathogenesis of these diseases and of the underlying molecular mechanisms.

B. OBJECTIVES AND BENEFITS

1. The main objective of the Action is to contribute to reduce the economic impact of both clinical and subclinical forms of the Immunosuppressive Viral Diseases of Poultry by promoting the exchange of knowledge and technology in the fields of epidemiology, diagnosis, vaccination, immunology, molecular biology, pathogenesis of these viruses and interactions between IBDV and CAV. This will help to reduce the economic impact of the disease and to improve animal welfare as well as reinforce the place of Europe in international trade. Furthermore, the increased use of antibiotics and chemicals to fight against opportunistic (secondary) infections is an important concern for human health, if we consider the risks linked to the presence of residues in meat product and the release of residues in the environment.

2. The benefits of a European network on Immunosuppressive Viral Disease of Poultry will be:
   - a better definition of the epidemiology (including molecular) of Infectious Bursal Disease and Chicken Anaemia in Europe and the subsequent monitoring of the field,
   - an improvement and standardisation of the diagnosis procedures for the detection of IBDV (especially vvIBDV) and CAV,
   - a standardisation of the nomenclature of IBDV strains: several criteria (including serotypes and pathotypes) will be selected and a general classification will be proposed,
   - a better understanding of the basis for virulence and attenuation,
   - a better comprehension of the pathogenesis of the diseases, including mechanisms of persistence,
   - an improvement of the vaccination procedures for a better control of the diseases,
   - a wider evaluation of the interactions between CAV and IBDV,
   - an overall increase in the quality of the final product,
   - a positive impact on public health with regard to zoonosis, drug resistance and residues,
   - an improvement of animal welfare.

3. A new COST Action is required:
   - to extend the knowledge and the technology in the fields of the pathology, the biology, the immunology and the genetics of the Immunosuppressive Viral Disease of Poultry. This should place Europe at the head of several fields,
   - to transfer this knowledge and technology to the field situation and into the commercial practices (in both poultry and pharmaceutical industries),
   - to provide a European framework to develop regulatory guidelines for the control and vaccination. This should help to follow the biosafety rules and warrant a lower impact on public health and environment.

4. COST is the most appropriate vehicle because:
   - much of the information that would result from projected research (outlined under WGs) may already be available but needs to be coordinated/assimilated and brought to the attention of the industry/research, - due to their complexity, these diseases require a multidisciplinary approach which can only be achieved by combining scientific expertise and resources (strains, reagents) from different Member States,
   - as stated by the OIE resolution (No XVIII, 1995), the first requirement for the progress in the diagnosis and the control of immunosuppressive viral diseases of poultry is a coordination between States. This Action follows the principle of concerted action and could serve as basis for grant applications and joint programs with other COST Actions in the field of Poultry,
   - there is a great demand to transfer knowledge and technology to the poultry industry and to establish a framework between research units and industry.

C. SCIENTIFIC PROGRAMME

Different working groups will be constituted and will focus on selected topics. Regular meetings and exchanges will be held in each working group and one or two general extraordinary meetings will be considered. The activities of each working group will be managed by a coordinator assisted by a substitute. The collaboration of external experts could also be envisaged.

Working Group 1: Epidemiosurveillance

The task of this group will be to collect data and to coordinate research among different labs in order to propose a universal nomenclature and a concerted strategy against IBD and CA.

The main objectives of the working group will be:
- selection of representative vvIBDV isolates according to their pathogenicity (SPF chicks), their antigenicity (MAbs) and their genotype sequences (RT-PCR and RE analysis),
- selection of representative CAV isolates following similar criteria,
- constitution of a panel of MAb by competition studies and epitope mapping
- characterisation of different isolates on SPF chicks and comparison between European and with some non-European strains (USA, Asia, Africa),
- analysis of the sequences and comparison with serotype I (classical, attenuated, variant) and serotype II IBDV strains,
- search for specific markers for vvIBDV (antigenic (VP2-VP3) or pathotypic (rest of the genome)),
- evaluation of the host range and possible reservoirs.

The expected benefits from WG1 in the Action are:
- Epidemiology:
  - standardisation of a capture Elisa test,
  - establishment of a European reference panel of Mabs,
  - evaluation of the incidence and prevalence of IBD and CA in Europe,
- Nomenclature:
  - determination of criteria for the classification of strains as pathotypes,
  - proposition for a standardised denomination of strains,
- Centralised data processing:
- creation of a general data bank concerning IBD and CA,
- constitution of a collection and a genomic bank of vvIBDV strains,
- establishment of phylogenetic trees by analysis of sequence,
- availability of selected material for the other working groups.

Working Group 2: Diagnosis and economic impact
This group will evaluate and coordinate the use of diagnostic procedures for field applications. This activity will be linked with these of the working group 1.

The main objectives of this group will be:
- the evaluation and validation of current diagnostic procedures at two levels:
  1. Serology: ELISA, SN, AGID
  2. Viral isolation
     - Immunofluorescence, Immunoperoxidase
     - Antigen-Capture ELISA
     - RT-PCR and RE analysis on field isolates
     - the establishment of standardised procedures (references)
     - the exchange of material (sera and antigens)
     - the evaluation of the needs for and of the importance of new diagnostic procedures (recombinant technology, cell mediated immunity).

Some emphasis will be put on the improvement of diagnostic tools and the development of new diagnostic techniques.
Specific attention will be paid to broiler production regarding the necessity of carrying out CAV and IBDV antibody surveys in different commercial operations (at slaughter) in different countries. This study will indicate if subclinical disease is likely in the case of CAV and allow the evaluation of the immune status at the end of the growing period in relation to vaccination status in the case of IBDV.
Particular care will also be taken to estimate the economic impact of the diseases on the poultry industry. Such evaluation is complicated by the multifaceted nature of the infection (presence of secondary or opportunistic infections) and by the serious lack of tools to perform a satisfying "economic study in parallel with epidemiology". For example, in IBDV, the prevalence of the disease could not be determined serologically because of widespread vaccination and circulation of apathogenic strains: serology cannot differentiate between infected and healthy (vaccinated) flocks. Viral isolation needs supplemental tools to characterise the viruses because strains with different virulence (and with different economic impact) can co-circulate. The integration of participants from the poultry industry will be planned in a second phase when a well-defined framework will have been determined.

The expected benefits from WG2 in the Action are:
- standardisation and guidelines for diagnostic procedures,
- evaluation of the incidence and prevalence of the different forms of the diseases (with WG1),
- development of practical tools to measure immunodepression (with WG4),
- recommendations concerning the integration of diagnostic tools in economic studies,
- integrated approach to the economic impact of the diseases in parallel with epidemiology.

Working Group 3: Vaccination
The role of this group will be to evaluate the current vaccination procedures (live or inactivated vaccines) and to analyse the possible cause of failure during prophylaxis. Critical parameters like the age of vaccination and interference with Maternally Derived Antibodies (MDA) will be considered. But a special attention will be put on new vaccine technologies with an emphasis on:
- recombinant vaccines: Fowlpox, HVT,
- sub-unit vaccines: Baculovirus, prokaryotes,
- reverse genetics system for IBDV: generation in vitro of infectious clones of different antigenicity and pathogenicity (reassortants, recombinants or mutants) and evaluation of the genetically engineered virus for vaccination,
- DNA vaccination,
- in ovo vaccination,
- immune complex vaccines.

Some emphasis will be put on the interference of MDA in the vaccination schedules and the way how the immunity gap between passive and active immunity could be reduced. Particular attention will be put on developing recommendations which could be proposed as guidelines for the European Pharmacopoeia and for the European Agency for the Evaluation of Medicinal Products (EMEA) in London with regard to vaccines against CAV and IBDV. The standardisation of vaccines being agreed by manufacturers will therefore also be considered.

The expected benefits from WG3 in the Action are:
- the establishment of a vaccination schedule,
- the reduction or suppression of the immunity gap between maternal and active immunity,
- the standardisation of the use and the identification of vaccines,
- guidelines for future trends in vaccination.

Working Group 4: Pathogenesis
The objectives of this group will be to coordinate ongoing basic research in different labs in order to stimulate the exchange of knowledge, technology and reagents and to promote new research areas. As the target cells for IBDV and CAV are B and T lymphocytes respectively, special attention will be placed on immune cells and the mechanisms of immunodepression.
Particularly, avian immune system has been highly studied as a model for other immune systems and it may be useful to bring this sort of information to the attention of CAV and IBDV virologists. The main topics include:
- Immunology:
  - analysis and measurement of the immunodepression,
  - specific role of some immunological mediators (cytokines),
  - importance of different cell populations (lymphocytes, macrophages, follicular dendritic cells),
  - importance of humoral and cell-mediated immunity in protection,
  - tentative definition of protection criteria,
  - In conjunction with WG5:
- interaction of the virus with the host,
- identification of the cellular and viral receptors,
- molecular basis for the virulence and the attenuation,
- role of apoptosis in the virulence of strains,
- Genetic Resistance to IBDV and CAV.

The expected benefits from WG4 in the Action are:
- a better definition of the immunodepression and its evaluation,
- a better understanding of the underlying mechanisms of virulence,
- a definition of tools for the measurement of the immune response in the disease and protection,
- an increased contribution of basic avian immunology to CAV and IBDV virology.

Working Group 5: Molecular virology

The role of accessory, structural and non-structural genes in the biology of IBDV and CAV will be envisaged. The continuation, coordination and improvement of research already undertaken in this area will maintain the advances made by European working teams. This should lead to a better understanding of the replication and translation strategies, the regulation of expression and the mechanisms of assembly of the viruses. This will give new insights into the pathogenesis of the diseases and the role of the different genes in virulence.

In IBDV, work will focus on:
- VP1, the viral polymerase,
- VP2 and VP3, the structural genes,
- VP4, the viral protease,
- VP5, new protein with a regulatory function,
- non-coding regions and 5' and 3' extremities.

In CAV, particular attention will be put on:
- VP3 or apoptin and its role in disease,
- the VP1 and VP2 genes,
- non-coding regions,
- non-characterised ORFs.

The expected benefits from WG5 in the Action are:
- genomic mapping,
- relation between structure and function,
- generation of molecular tools which could be tested in the other WG.

In summary, the general expected outcomes of the Action will be:
- a handbook with state-of-the-art knowledge and recommendations for future research and current practices. This will provide guidelines for the OIE and EU,
- the stimulation for future concerted actions and grant applications,
- the standardisation of tools and nomenclature,
- the transfer of knowledge and technology, especially to the poultry industry,
- the integration of the generated knowledge into current practice,
- the training of young scientists,
- the production of scientific publications and monographs (for instance on hypervirulent IBDV, on the young chick, on the interactions IBDV-CAV) which will reinforce the international position of European scientific teams,
- a web site for the rapid and effective dissemination of information in the handbook as well as further up-to-date information will also be considered.

Interactions between work groups are summarized in Table 1.

D. ORGANISATION AND TIMETABLE

The organisation and the coordination of the COST Action will be assumed by a Management Committee (MC) constituted of a maximum of two members from each participating country. The MC will be set up during a meeting in Brussels organised by the COST Secretariat. The first task will be the designation of the chairpersons of the MC and of the different working groups (WG) and providing an overall description of the tasks of each WG. Thereafter, the MC will have the responsibility of organising the first seminar with all the participants in the COST Action.

The role of the MC, during the Action, will be:
- to supervise, coordinate and to give some expertise when necessary,
- to constitute technical committees if necessary,
- to elaborate the annual and final reports,
- to make publishing decisions regarding common work performed during the Action,
- to finalise the more important recommendations concerning:
  - the nomenclature of IBDV,
  - the standardisation of current diagnostic procedures and the validation of new ones,
  - the vaccination schedules
  and to transmit them to the relevant authorities like EU, OIE and local veterinary services,
- to formalise the constitution of a European IBD-Network,
- to distribute information during international meetings and congresses,
- to negotiate the possible transfer of technology to industry.

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In each WG, a coordinator and a substitute, will be designed with the mission:
- to follow the course of the programs,
- to write a report after each annual meeting,
- to transmit the information to the MC (as members),
- to propose exchange and training programs to the MC,
- to promote the mobility of researchers and technicians between laboratories involved in this working group. This last point will be one of the most important activities in order to make this COST Action successful.

In each WG, workshops (2-3 days) will be organised at least annually in one of the participating countries and the proceedings will be published. Exchanges of people will be considered during these meetings and transmitted to the MC by the coordinator.

The duration of the COST Action will be five years. Two extended international meetings will be organised, one at the

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beginning and one at the end of the Action. They could be combined with international meetings like the WVPA meeting (held every 4 years) or with the organisation of the next International Symposium on Infectious Bursal Disease and Chicken Infectious Anaemia.

External experts from the USA, Asia and Australia will be invited to participate in several meetings according to needs and topics.

Several laboratories will be involved in more than one WG as the scientific programmes are broad and encompass a range of interrelated disciplines.

The MC will meet about once a year during the interWG meetings, when possible.

A separate evaluation meeting, with possible inclusion of non-European Experts and members of the Technical Committee, will be held each year.

Timing, milestones and bottlenecks:
The timetable of the Action is summarized in Table 2. The time required to pursue the scientific projects will be five years. During this time, the following activities are planned:
- two Management Committee meetings/year,
- one Working Group meeting (workshops)/year,
- a starting seminar organised by the MC,
- a mid-term general COST meeting at the end of the 3rd year,
- a final general COST meeting at the end of the Action (5th year),
- visits to the collaborative institutes,
- exchange of scientists and technicians,
- inter-working group meetings will be organised to stimulate the interrelationship between WG,
- the last management meeting will finalise the publications and the handbook,
- some extraordinary meetings over particular topics (CAV, status of the young chick, economical impact of subclinical diseases) could also be considered.

Work in some WGs will be carried out in a single continuous effort; whereas, in other WGs, action will more likely consist of clearly defined phases. Nevertheless, in each case, milestones will have to be clearly defined in the beginning of the Action by the MC and managed by the chairman within each WG.

Special attention will be paid to the possible existence of bottlenecks throughout the Action. These might occur particularly each time that a new phase will be initiated:
integration of the poultry industry in the frame of WG2, transfer of basic avian immunology knowledge to CAV and IBDV virology and diagnosis, generation and dissemination of molecular tools, etc.

At the end of the fourth year, the Evaluation MC Meeting will pay special attention to the milestones, the occurrence of bottlenecks and the good achievement of the final outcomes of the Action.

E. ECONOMIC DIMENSION

For the moment, the following countries have participated in the constitution of the proposal or have indicated their interest and should sign the MoU: Belgium, Denmark, Finland, France, Germany, Hungary, Spain, Switzerland, The Netherlands and United Kingdom but we can consider the participation of more than 10 European Countries in the project.

For each participating country, 3-5

Coordinator

N/A
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