



**European Cooperation
in the field of Scientific
and Technical Research
- COST -**

Brussels, 15 May 2014

COST 033/14

MEMORANDUM OF UNDERSTANDING

Subject : Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action FA1401: European network on the factors affecting the gastro-intestinal microbial balance and the impact on the health status of pigs (PiGutNet)

Delegations will find attached the Memorandum of Understanding for COST Action FA1401 as approved by the COST Committee of Senior Officials (CSO) at its 190th meeting on 14 May 2014.

MEMORANDUM OF UNDERSTANDING
For the implementation of a European Concerted Research Action designated as
COST Action FA1401
EUROPEAN NETWORK ON THE FACTORS AFFECTING THE GASTRO-INTESTINAL
MICROBIAL BALANCE AND THE IMPACT ON THE HEALTH STATUS OF PIGS
(PIGUTNET)

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 4114/13 “COST Action Management” and document COST 4112/13 “Rules for Participation in and Implementation of COST Activities”, or in any new document amending or replacing them, the contents of which the Parties are fully aware of.
2. The main objective of the Action is to increase the knowledge about the effect/interaction of environmental and genetic factors on the composition of the microbiota in the gastro-intestinal tract of pigs and to improve the risk management associated with antibiotic resistance in pig production.
3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 84 million in 2014 prices.
4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.
5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of section 2. *Changes to a COST Action* in the document COST 4114/13.

A. ABSTRACT AND KEYWORDS

The “hoped for” reduction in the use of antibiotics in pig by EU producers has not materialized as they are still being widely used for the control of enteric infectious diseases. This practice can spread antibiotic resistance in the farm environment and poses a threat to consumer health. Whilst it is widely recognized that a diversified gastro-intestinal tract (GIT) microbiota is essential for optimal health and performance, the underlying factors favoring the development and maintenance of a balanced intestinal microbiota are not fully understood. PiGutNet will establish the first European network focused on this topic, joining specialists in all research areas. It will define both environmental and host genetic factors affecting the GIT microbiota and the complex interactions between microbiota and gut maturation, to maintain a healthy gut throughout life. The network will coordinate databases and unravel innovative tools to define the status of intestinal eubiosis in pigs. The most important outcomes will be genome/metabolome-wide association studies and the provision of a road map to increase pig resistance against GIT infections. This will have an important translational potential, being the foundation for European companies to develop strategies in the areas of feed additives and animal husbandry, resulting in improved animal health and welfare, consumer protection and competitive advantage for the European agriculture.

Keywords: swine, microbiome, gut health, antibiotic resistance, animal health and welfare

B. BACKGROUND

B.1 General background

The European Union (EU) is facing major challenges in the area of farm animal production. Pork is the most important meat source in many European countries but its manner of production is criticized by consumers and other groups for paying insufficient attention to animal welfare and the wide use of therapeutic antibiotics to control GIT diseases. The latter practice promotes the development of multi-resistant bacteria, challenging animal health and welfare as well as food safety. The EU has prioritized to combat the threat of antimicrobial resistance to human and animal health, promoting actions such as the new EU program HORIZON2020 and the ANIHWA (Animal Health and Welfare) ERA-Net initiative. Therefore, strategies are needed to improve animal health, especially in the juvenile pig. The stability of the GIT microbiota, therefore, is of utmost importance for achieving this goal.

The establishment of a “balanced” intestinal microbiota (eubiosis) is determined by host factors and

a broad spectrum of environmental factors with husbandry and feeding practices being the major sources of variation. The physiological development of the porcine GIT with its immune system forms an effective barrier against pathogen establishment and (or) invasion. Thus, optimizing GIT maturation is a major goal for preventive strategies against infectious diseases in pig husbandry. A reduction of undesirable intestinal microbiota, designed as “dysbiosis”, can be achieved only by a wide coordinated contribution of the knowledge of several disciplines and with the transdisciplinary skills of scientists and experts.

The COST framework represents the best tool to systematically identify the host and external factors that limit the preservation of a good health status of pigs throughout life. PiGutNet will establish the first network focused on this topic, bringing together a broad range of specialists in all relevant research areas including animal science, veterinary medicine, animal and human nutrition, genetics, food science, microbiology, systems biology, immunology, physiology, toxicology and different industries related to feed production. A COST Action would coordinate databases and tools to define the microbial conditions for eubiosis in the pig, open new opportunities for the genomic selection by genome-wide association studies and, finally, create a road map to develop strategies to increase pig resistance against intestinal infections and conditions of dysbiosis. This will have an important translational potential, being the foundation for European companies and farmers to develop most effective strategies in the areas of feed additives and feed production, pig breeding, animal husbandry and biosecurity monitoring with a clear potential for competitive advantages for livestock agriculture in COST countries.

B.2 Current state of knowledge

The gut-resident microbiota is unique and continually evolving for each domestic animal species. It is shaped by its environment and by the host genotype. Suitable farm management system leading to early exposure of piglets to a diverse microbiota is required to drive the development of gut-associated lymphoid tissue (Lewis et al., 2012. *Pediatr Allergy Immunol.* 23:265-9) and promote the maturation of the host gut. Conversely, dysbiosis of the intestinal microbiota may be associated with diarrhea and predispose the pig to other health problems (Lalles et al. 2007. *Proc Nutr Soc.* 66:260-8). Recent outcomes of the EU research project Interplay and of other European and national projects have described the long term effects of pre- and early- postnatal nutrition, rearing environments and antibiotic treatment of the sow (to modify gut microbiota), upon the intestinal barrier function and stress responsiveness. The pressure imposed by the EU ban on in-feed antibiotics as growth promoters has stimulated a more profound awareness of this problem and the

use of a more integrated approach to understand the relationships between gut maturation and the GIT microbiome. The application of new technologies has allowed a more holistic approach to the characterization of the gut microbiota as evidenced by several research networks related to the study of human microbiome (particularly in EU, USA and China). Similar approaches in rodents have recently shown that host genetics define murine gut microbiota (McKnite et al. 2012. PLoS ONE 7(6):e39191). In contrast, the opportunities provided by recent advances in our knowledge of the pig genome have not yet been fully utilized. For example, whilst, it is known that the multiplication of two strains of *Escherichia coli* (F4 and F18 fimbrial types) is related to two host genes that required for bacterial adhesion. Equivalent studies have not been completed for other important porcine pathogens or commensals. Consequently, a framework to promote multi-disciplinary studies of the pig genome alongside gut health-related traits with experts in different fields, including animal genetics and nutrition, is urgently required. In conclusion, PiGutNet takes all these aspects as a starting point and will comprehensively identify the causes that lead to the emergence of microbial dysbiosis in the GIT of pigs. The methodological aspects include the examination of host genetic factors, different feeding or husbandry strategies, and their interactions, to obtain new insights into this complex problem and to offer potentially new practical solutions for the industry and Europe.

B.3 Reasons for the Action

This Action is timely, as it addresses the need to exploit the latest understanding and knowledge of the GIT ecosystem of the pig and its pivotal role in health and disease. A COST Action is the most appropriate tool to build a fruitful synergy at the European level, providing added scientific, practical and economic value and ensuring that the excellence of European research is translated into practical guidelines for sustainable livestock production. Importantly, PiGutNet will contribute to mitigating risks for consumers' health by the uncontrolled spread of zoonotic bacteria and bacterial resistance. A coordinated effort involving scientists with complementary expertise and interests from several countries and institutions in a larger network will provide a framework to facilitate advancements and organize the exchange of knowledge between and within specialized fields. This "stimulating environment" will favor the emergence of new challenges and stimulate the coordinated research of innovative approaches to disentangle the relationship among the various factors affecting the microbial community in the GIT of pigs.

This COST Action will establish a collaborative network of experts and Early-Stage Researchers (ESRs) and a forum for regular meetings, workshops, Short-Term Scientific Missions (STSM) and

Training Schools. The latter will be targeted to young scientists, enhancing their scientific carriers towards an international top level. These activities will provide improved communication between existing European and national research projects and provide for more effective integrated responses to future challenges. Moreover, this COST Action is not only aimed at scientific/technological advance but also at economic/societal needs towards the enhancement of the competitiveness of the European swine industries that have already expressed interest in participating in and contributing to this COST Action.

This COST Action will provide advantages to the European research community at different levels:

- avoiding duplication of research activities in Europe;
- reducing the fragmented research efforts on the swine microbiome by coordinating methods and strategies;
- standardising experimental, analytical and bioinformatic methodologies;
- improving exchange between fundamental and applied research areas focused on pig;
- improving dissemination of acquired advances and training for ESRs and the dissemination of the advances acquired to ESRs
- providing advice to policy makers and administrators.

B.4 Complementarity with other research programmes

No other parallel project by EU Framework Programme or by other European organizations exists that covers the objectives of PiGutNet.

However, the following COST Actions could be complementary:

- COST Action FA1201 (EPICONCEPT) deals with epigenetic mechanisms in livestock, which are also relevant for large animal models of diseases.
- COST Action FA1002 (Farm Animal Proteomics) will be helpful for the molecular analysis of large animal models.
- COST Action BM1006 (SeqAhead) helps deal with Next Generation Sequencing (NGS), which may arise from the molecular analysis of large animal models, using state-of-the art bioinformatics.
- COST Action BM1308: Sharing advances on large animal models (SALAAM)

- COST Action FA1005: Improving health properties of food by sharing our knowledge on the digestive process (INFOGEST)
- COST Action FA1202: an European network for mitigating bacterial colonization and persistence on foods and food processing environments

PiGutNet closely aligns with objectives of the strategic research agendas and vision documents of the Sustainable Farm Animal Breeding & Reproduction Technology Platform (FABRE-TP; <http://www.fabretp.info/Home.aspx>) and European Technology Platform for Global Animal Health (ETPGAH; <http://www.etpgah.eu/>). Moreover, the aims of this COST Action fit well with some topics of the EU program HORIZON2020, such as the new call SFS-01a-2014: “Genetics and nutrition and alternative feed sources for terrestrial livestock production”.

C. OBJECTIVES AND BENEFITS

C.1 Aim

The main objective of PiGutNet is to establish an integrated network of experts in fundamental and applied areas of porcine research and in pig production who are interested in a) optimizing diagnostic tools and standardizing operating procedures to understand the role of the GIT microbiota in pig health; b) developing strategies to capitalize on the resulting new knowledge and thus improving the risk management associated with antibiotic resistance in pig production; and c) defining areas with research needs, summarizing present knowledge for the industry, and disseminating knowledge to stakeholders on the topic of the network.

C.2 Objectives

The COST Action will have the following secondary objectives:

1. Develop and standardize metagenomic tools to define the composition and functionality of the microbiota in the GIT of pigs, characterizing the potential role of genomics, including the approach of genome-wide association studies (GWAS), to assess the link between host genotype and the pig gut microbiome. This approach may provide the opportunity to include the GIT microbiome in genomic indexes as phenotype.
2. Coordinating the methodological approaches to define the baseline for an “optimal” microbial gut colonization at various age of the pigs, and define a priority list of factors

able to determine the occurrence of dysbiosis in the GIT of pigs and consequently create a roadmap to develop novel tools for improving health status.

3. Develop a continuity concept in which the chain from sows to piglets to fattening pigs is considered and the GIT microbiota is optimized at each stage of production.
4. Evaluate the risks associated with long-term antibiotic exposure to the variability of the gut microbiota and to quantify its effect on the health status of pigs throughout life.
5. Exchange of experiences and knowledge, protocols, experimental design and data acquisition and analysis strategies between the PiGutNet partners.

C.3 How networking within the Action will yield the objectives?

The PiGutNet network will achieve its aims by setting up a collaborative framework that facilitates the exchange of expertise among internationally recognized experts in pig research in different areas and in complementary research fields (e.g. microbiologists, nutritionists, geneticists, physiologists, immunologists and pig industry experts). This COST Action will provide a vibrant and constructive interface between these disciplines and a framework for coordinating the various scientific tasks.

Specifically PiGutNet will:

1. Suggest suitable tools to assess the microbial community in the GIT, to harmonize analytical methods increasing the possibility to compare results obtained in different laboratories;
2. Facilitate the transfer of knowledge and flow of information between researchers working with different experimental models by combining complementary concepts and methodologies;
3. Use novel analytical tools and skills to promote linkages between scientist working in the areas of microbiology, genomics, proteomics, metabolomics, physiology, nutrition, immunology and bioinformatics in the context of the subject of the network;
4. Offer easy access to research infrastructure facilities for network member via STSM and the dissemination of advanced technologies among groups;

5. Organize workshops and Training Schools for sharing know-how mainly involving ESRs, to promote advances in the understanding of the contribution of microbiota to maintenance of gut health in the pig;
6. Disseminate results and best practices to farmers, scientists, industry, policy makers and the consumers across Europe and beyond.

Means needed:

Man-power

1. Most Action members are leaders of research groups that include several researchers, students and technicians. This will permit an active exchange of the information and a conduit for spreading of knowledge.
2. Specialist members will be responsible for conducting distinct tasks within the Action (e.g. validate methods, compile information, identify stakeholders and dissemination targets).
3. Action members will train incoming ESRs.

Equipment:

1. Research equipment and experimental facilities will be provided by each Action member;
2. All necessary analyses will be performed in the laboratories provided by Action members;
3. All Action members will share their own technical expertise provided by their specialized backgrounds.

C.4 Potential impact of the Action

This COST Action will bring together international experts in pig research areas and in complementary fields (microbiology, nutrition, genetics, physiology and immunology).

Understanding the factors inducing and affecting intestinal dysbiosis in pigs will ultimately provide pig producers with better tools to make a risk assessment and consequently improve health, welfare and efficiency in pig production, reducing the use of antimicrobials in the pig industry. The synergy provided by this multi-disciplinary network will lead to new hypotheses, research approaches and a significant increase in knowledge. This will have an extremely high translational potential and “lay

the foundations” for European companies to develop new strategies in the areas of genetics and breeding, feed additives, feed production and animal husbandry (including the promotion of natural pig behavior and adaptive capacities) and to exploit the genetically diverse heritage of pig breeds in Europe through innovative selection schemes. This coordinated approach and joint analysis will result in new cooperation and project groups, offering a competitive advantage for the European agriculture sector.

C.5 Target groups/end users

Researchers: animal nutritionists, geneticist, microbiologist, immunologist, physiologists, behaviorists, clinicians, bio-informaticians, statisticians involved in PiGutNet. This Action will generate new standardized procedures, guidelines, integrative approaches, and genome-based selection strategies available to all.

Advisors: animal breeding organizations and companies involved in animal feeding will get easy access to new knowledge and innovative tools, and can take a role in dissemination improved practice to those involved in pig production.

Policy makers/governments: from national and international bodies, and bodies from industry who are seeking information for policy development. Two end users of particular relevance could be European Food Safety Authority (EFSA) and EU Commission.

Industry: breeding organizations, feed industries and farmers will obtain perspectives and knowledge for future decisions on production strategies. Other companies giving services involved in animal production (animal monitoring, data managing, bio-analytical labs, bio-analytical platforms and quality certification) will also benefit.

Focus groups and scientific foundations:

- European Innovation Partnership (EIP) focus group on animal husbandry (reduction of anti-biotic use in the pig sector);(http://ec.europa.eu/agriculture/eip/focus-groups/animal-husbandry/index_en.htm)
- European Feed Technology Center (EUFETEC) (www.eufetec.eu);
- Farm Animal Breeding and Reproduction (FABRE) Technology Platform (<http://www.fabre.info>);
- Animal Task Forces (ATF) (www.animaltaskforces.eu);

- The European Network for Gastrointestinal Health Research (ENGIHR) (<http://www.engihr.eu/>);
- STAR-IDAZ, the Global Network for Animal Diseases Research (www.star-idaz.net).

Stakeholders and end-users will be directly involved in this Action. Some of them have already provided input for the content of the Action's activities and outputs.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

This COST Action has four distinct tasks that share the common aim to provide new and harmonized data to understand the cause and the consequences of microbial dysbiosis in the pig GIT. Each of these topics will be explored in detail within a separate research track that is briefly described below. Due to the quick evolution of the knowledge in these topics, PiGutNet will be flexible and opened to be implemented on the bases of new perspective and innovative approaches.

Molecular microbiology

Thanks to the next generation sequencing (NGS) techniques, in a few years the full genome of a number of bacteria will have been sequenced. These data will be highly relevant for bacterial characterization and will increase the knowledge on diversity, taxonomy and, where relevant the pathogenesis of a particular bacterial infection. Moreover, these databases will provide the opportunity to study changes in bacterial metabolism under different gut conditions (enterotype approach). Using this approach PiGutNet will deepen the interactions between the microbiota and all the factors that modify its composition and function. Numerous protocols have been published based on different techniques/instruments, and in order facilitate data sharing a standardized approach will be promoted. The metabolomic approach will be integrated, as compositional data may not always reflect the situation of the gut microbiota.

Environment, host genetics and epigenetic approach

The gut homeostasis is a delicate equilibrium. To improve the risk management linked with gut dysbiosis, it will be necessary to highlight all factors involved in the maintenance of an optimal microbial balance or, conversely, the factors that increase the destabilization of the gut homeostasis. For environmental factors there is a need to expand knowledge, whereas to date there is no consensus on the relevance of host genetic effects. Thus the network review the present knowledge and provide new models to integrate the host genomics into the studies on gut phenotypes. Furthermore, it can be suspected that some environmental effects can be replicated between

generations. Thus it would be relevant to focus on the mother-piglet relationship. Based on recent findings, it has been suggested that intrauterine growth retardation (IUGR) could play a central role in the proper development of gut function and gut tissues maturation. IUGR could be evaluated as a potential condition that affects the microbial ecosystem during the whole life of the animals.

Feeding strategy to manage the risk of dysbiosis

The pressure of the EU ban on the use of in-feed antibiotics stimulated studies on the development of feeding strategies to control gut microbiota, mostly focusing on other functional feed ingredients and additives predicted as having their main action through pro- or antibacterial activity. Less interest, however has been given to feeding strategies that could help the host to develop a more stable and favourable gut environment from birth and thereby exploit the natural ability of piglets to develop their gut environment, reducing the risks of dysbiosis in suboptimal rearing conditions.

This will be done by focusing more on piglet intervention measures immediately from birth, considering the integrated system piglet-sow-weaning-post-weaning, instead of concentrating only upon feeding during the post-weaning period. This could be obtained in PiGutNet 1) by collecting and discussing the new evidence about the ability of the host to develop an interplay with the developing microbiota, including all data about transcriptomic and proteomic at pig gut level; 2) by suggesting new dietary intervention techniques to exploit this new knowledge; 3) by considering viable strategies to induce precocious maturation before weaning in the GIT, to achieve the characteristics of the post-weaning condition. For example, specific lectin extracts could be used in early life to achieve this effect. Preliminary data indicate that this strategy is potent in inducing changes resembling weaning, but a collaborative approach with the expertise of PiGutNet could favour a more profound evaluation of how to achieve the objective; and 4) by incorporating other promising feeding practices present in different geographic regions of the COST countries.

Antibiotic resistance

This task aims to develop research targeted to assess: the development of antibiotic resistance (resistome) in pigs, the emergence of multidrug-resistant Enterobacteriaceae and possible dietary and management factors that reduce the risk of selecting for bacterial strains with anti-microbial resistance. PiGutNet will target the collection and generation of data to better understand this complex picture and contribute to the identification of the main factors characterizing farms using high amount of antibiotics from those that do not use antibiotics. This could help to provide more awareness of the large scale effect of the antibiotic free systems.

This COST Action will advance the knowledge across all the areas related with gut microbial variation, epigenetic modifications, and expression profiling for the enhancement and advancement in the sustainability of the livestock production. The active development of computer infrastructure

and analysis software will allow the integration of data from diverse platforms. Due to the scientific robustness of the network, interdisciplinarity will be guaranteed. Each partner will contribute to achieve the objectives of the COST by bringing in their scientific experience and expertise. This network takes advantage from the high level of human and technical resource exchanges during the 4-years period. The wide dissemination of the knowledge and the possibility for the groups to have access to the infrastructure of all the COST partners give added value to this project, creating the right conditions to increase the competency of the researchers (with special attention to the ESRs) and the level of the scientific research.

D.2 Scientific work plan methods and means

The experts that have already expressed their interest to join the network PiGutNet have collected data in many research projects. This will provide a database to enable addressing the issues raised in each of the Working Groups (WGs). Each WG will discuss recent developments in a specific area of this rapidly-advancing field to facilitate the progress. The 4-years PiGutNet program will be carried out within 5 WGs identified according to 4 main research areas, while one WG will be dedicated to the dissemination.

WG1. Functional and genetic characterization of microbial communities in the gastrointestinal tract of pigs. The ability to fully characterize the intestinal ecosystem is the cornerstone to understand the effectiveness of practical solutions to manage the intestinal homeostasis and to improve the health status and disease resistance of the animals. The main objectives of WG1 are to compare procedures to characterize the microbiome under the new light of the metagenomics approach in the GIT of pigs to define its composition and functionality at different production phases under experimental conditions (optimized housing and feeding conditions) and to define the status of eubiosis and dysbiosis.

Due to the complexity of this scientific approach, the following further tasks have been identified for WG1:

- To critically highlight the specificities of each of the available NGS methods for studying the bacterial profile, comparing also the available data provided by the COST partners.
- To standardize the microbiome sequencing techniques across laboratories and establish equivalence of different methodologies, without intention to restrict research to one

technique. Detailed discussions, opinion exchange and research are required to develop a common strategy as it is a practice in consortia for human studies and in the National Institutes of Health (NIH) in the USA.

- To harmonize the NGS data by the use of shared bioinformatics tools.
- To establish shared protocols suitable for determining the “standard” and determine the “optimal” microbiome in the GIT of pigs.
- To assess the microbiome across different pig production phases and experimental conditions, to obtain an overview in terms of composition and functionality, including analysis of metabolites on the gut ecosystem of healthy pigs.
- To exchange experience and knowledge, protocols, experimental design and data analysis with other WGs ESRs in an international forum.

WG2. Genetic and environmental factors to understand dysbiosis including their interaction (epigenetics). Dysbiosis is considered an essential cause of GIT diseases and must be discussed as a function of genetics and husbandry factors. Therefore, in this WG, geneticists, microbiologists, physiologists, nutritionists and immunologists will work together to manage the following tasks:

- To establish a multidisciplinary network to disclose the basis of intestinal dysbiosis in pigs.
- To improve the knowledge on the genetic potential of the most important European pig breeds, to increase the resistance against pathogens, with special emphasis on the immune response and gut maturation (novel molecular markers).
- To share biological samples of different pig populations and database based on genetic information (Single Nucleotide Polymorphism - SNPs) and traits related with the gut microbiome, heats and rearing conditions.
- To define the most important phenotypes related with the gut microbiota and the best ways to collect/register them.
- To share data and samples to improve the effectiveness of the GWAS in this field.
- To exchange experience, knowledge, protocols and bioinformatics tools, especially within ESRs in an international forum.

- To discuss on the environmental factors as causes of dysbiosis (mother imprinting, diet, housing, etc.) and their interaction with the physiological and genetic make-up of pigs' (epigenetics) parameters.
- To create a database based upon published literature and farm conditions to analyze the main environmental factors that in the field conditions modulate the gut microbiota.
- To define a priority list of factors determining the occurrence of dysbiosis and consequently create a road map to increase the resistance of pigs against the GIT infections.

WG3. Feeding strategy to maintain/restore the gut homeostasis. Diet composition and feeding strategy both play important roles in the development and establishment of the gut microbiota in terms of function and composition. They provide the opportunity to manage the risk associated with inheriting a genetic predisposition to a particular infection (e.g. adhesion determinant) without the use of antibiotics. There is a lack of understanding of the mechanisms that underlie these effects and this remains a pre-requisite if there possible benefits are to be developed in a practical “on-farm” solution. In this WG the attention will be focused on the tasks listed below:

- Reviewing the literature on the impact of different feeding strategies such as: use of protein sources, level of amino acids, dietary fibre sources and levels, application of feed technology, use of fermented liquid feed and the potential of established and new feed additives to control the gut ecosystem, and performing a meta-analysis on the available information. Pre- and probiotic interventions will also be evaluated considering their important role in immune/physiological/metabolomic responses.
- Discussing the experimental protocols and parameters to be considered during the study regardless of the manipulation of the gut microbiota using different categories of feed or additives.
- Providing data from the PiGutNet participants on new feeding strategies and their impact on gut homeostasis with special attention to the microbial profile and immune function of the gut as well as the effects on the production of metabolites.
- Discussing new approaches related to the induction of precocious maturation of the GIT mucosa, to improve GIT health and function.

WG4. Antibiotics as a factor of dysbiosis and spread of antibiotic resistance genes. Working Group 4 is especially dedicated to the causes of development of antibiotic resistance (resistome) in

pigs and in particular the emergence of multidrug-resistant *Enterobacteriaceae* and *Staphylococcaceae*. A second aim is to involve businesses in practice and to investigate the association between farming systems, genetics and feeding and veterinary care and use of chemotherapeutic agents. Aims are:

- To stimulate discussion about health conditions and how management decisions differ in farms with high use of antibiotics relative to farms that have been able to largely eliminate the use of antibiotics in piglet rearing.
- To analyse and discuss the impact of the spread of antibiotic resistance of bacteria in pig production around the world, with main attention on the areas with high livestock production.
- To highlight the impact of genetic make-up and varying hygiene conditions on the increase in antibiotic resistance.
- To open discussion on recent evidence that some dietary factors can favour antibiotic resistance (such as high dosed minerals or amino acids).
- To assess the impact of the reduction of bacterial variability due to the antibiotic resistance of some bacterial strains, on health and productivity in pig production systems.

WG5. Knowledge and management exchange (KME). Working Group 5 will support KME to support exchange of experiences and knowledge, protocols, experimental design and data acquisition and analysis strategies across the WG1 to WG4, and dissemination towards external stakeholders. Exchange with stakeholders will be in both directions. The goals of this WG will be realized by the following methods: organized and specialized workshops and meetings, STSMs, dissemination and sharing of information via share points, social media, conferences, scientific papers and articles for the industry.

The following further tasks have been identified for WG5:

- Stimulate research, education, exchange of knowledge, technical experience, and mobility among scientists and PhD students.
- Manage and exchange knowledge with/to the scientific community, policy makers, primary producers, industries, etc.
- Provide feedback to pig farmers about the results originated during COST Action meetings can be achieved by not only a web-page but also newsletter that can be distributed by the mean of farmers associations.

E. ORGANISATION

E.1 Coordination and organisation

PiGutNet is incardinated in the “Food and Agriculture” domain and the organization of this COST Action will follow rules and regulations described in the “Rules and Procedures for Implementing COST Actions” (doc. COST 4154/11). The coordination of the COST Action will be assumed by a **Management Committee (MC)** consisting of a maximum of two designated members from each country that has joined the Action by accepting the Memorandum of Understanding (MoU). At the kick-off meeting, the MC will elect a Chair and a Vice Chair from two different participating COST countries by majority vote. Moreover, leaders of each of the 5 WGs will be also elected. A Core Group, composed of a subset of MC members will be established within the MC to support the Chair in his/her duties and to manage STSMs. The COST Action will begin with the kick-off MC meeting, followed by MC meetings once a year organized in association with the workshops and during other relevant meetings. Moreover, video-conference and exchange of e-mails between the MC members will provide further possibilities for discussions and decisions.

The scientific progress of the COST Action will be monitored by the WG reports including annual reports from the MC members. The COST Action will be open to any interested party with the main aim to constitute the largest network around the Action topic.

The MC will be charged with the following responsibilities:

- Appointment of the Working Group Leader, Vice Leader and website/publicity manager (Milestone MS1);
- Creation and maintenance of a dedicated website (MS2), that aims to maintain and enhance communication among the partners and to disseminate results obtained in the different WGs. The website will contain a restricted access page for registered participants of the COST Action in order to give access to relevant information, tools and databases;
- Planning and coordinating the different meetings/workshops, including MC meetings (MS3), and the final conference (MS8);
- Promoting collaborations and exchange of data, tools and results across WG, and maximizing interactions among partners;
- Establishing programmes for Short-Term Scientific Missions, (MS4);

- Organizing Training Schools in collaboration with the WGs (MS5);
- Monitoring and assessing the different activities (meetings, scientific exchanges, Training Schools, website, databases, publications) to ensure that they meet the objectives defined for this COST Action;
- Eventually propose changes in activities in order to better fulfill the objectives;
- Preparation of the annual (MS6) and final reports (MS7);
- Coordination of national research activities and programmes, creating a dedicated list hosted in the restricted password space of the website in order avoid duplication of research studies and identify possible synergies among groups;
- Coordination of the preparation of new proposals for application to European or multilateral calls;
- Establishment of collaborations between WG and other related actions and scientific programmes in Europe and world-wide;
- Increasing visibility of the Action and promoting and managing interactions with non-members;
- Promoting interactions with end-users and dealing with issues relating to exploitation of results.

Moreover, the MC will establish priorities for all activities in agreement with the objectives of the COST Action. All Action events will be carefully managed according to the available budget in order to avoid waste of funds and time. MC meetings will take place once a year, linked to WG meetings and workshops or other Action events, in order to maximize efficiency, reduce costs and ensure coordination activities.

E.2 Working Groups

- WG1. Functional and genetic characterization of microbial communities in the gastrointestinal tract of pigs.
- WG2. Genetic and environmental factors to understand dysbiosis including their interaction (epigenetics).
- WG3. Feeding strategy to maintain/restore the gut homeostasis.

- WG4. Antibiotics as a factor of dysbiosis and spread of antibiotic resistance genes.
- WG5. Knowledge and management exchange (KME)

MC members based on their expertise and interest will take part in the different WGs. Each WG will have a leader and a deputy leader to guide the scientific progress in their WG. WG leaders will report progress on WG objectives and deliverables to the MC.

E.3 Liaison and interaction with other research programmes

FoodSEG

Coordination and support action. FOODSEG has the overall objective to disseminate and transfer state-of-the-art research results in traditional food safety and quality topics through a series of symposia, expert Working Group meetings, an online platform with best practise examples and coordination of cooperation and a plan for the preparation of future activities

(<http://www.foodseg.net/>).

NEAT

Networking to enhance the use of economics in animal health education, research and policy making in Europe and beyond” and is a 3-year project co-funded by the Lifelong Learning Programme of the European Union (<http://www.neat-network.eu/>).

The Action will be done by organizing joint meeting and seminars on overlapping area of interest.

E.4 Gender balance and involvement of early-stage researchers

This COST Action will respect an appropriate gender balance in all its activities and the Management Committee will place this as a standard item on all its MC agendas. The Action will also be committed to considerably involve ESRs. This item will also be placed as a standard item on all MC agendas.

Specific actions to improve the representation of women in research are central to improving gender equity in the life sciences (e.g., as detailed in <http://eu.europa.eu.int/>). Specific rules will be observed for the constitution of the MC and for all relevant roles and activities in PiGutNet to favor the inclusion and the role of women. At least 50% gender balance will be considered for the nomination of WG coordinators and deputy coordinators and for any other key role in this COST Action.

Gender balance will be also observed in the workshops, teaching activities and STSMs. Early-Stage Researchers will be encouraged to join WG and to actively participate in workshops and conferences. PiGutNet will helping to plan and organise ESRs at events, and actively promote their participation in Short-Term Scientific Missions and Training Schools where they can improve their skills and where knowledge transfer from other colleagues can occur.

F. TIMETABLE

Action	Milestone	Year 1				Year 2				Year 3				Year 4			
Coordination		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Kick-off meeting	MS1	x															
Homepage and database	MS2	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
MC meeting	MS3	x				x				x				x			
WG1 meeting	MS3	x				x				x				x			
WG2 meeting	MS3	x				x				x				x			
WG3 meeting	MS3	x				x				x				x			
WG4 meeting	MS3	x				x				x				x			
WG5 meeting	MS3	x				x				x				x			
STSM	MS4			x	x	x	x	x	x	x	x	x	x	x	x	x	x
Training Schools	MS5			x				x				x				x	
Reporting	MS6/MS7				x				x				x				x
Final conference	MS8																x

STSM: Short-Term Scientific Missions to be initiated after the first MC and WG meetings

WG meeting: Working Group meeting/workshop

MC meeting: Management Committee meeting

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, BE, CZ, DE, DK, EL, ES, FI, FR, HR, IE, IT, NL, NO, PL, PT, RO, RS, SE, SK, UK. On the basis of national estimates, the economic dimension of the

activities to be carried out under the Action has been estimated at 84 Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

The target audience for the dissemination of the results of the Action will include:

- **Partners in PiGutNet**
- **Research community:** animal nutritionists, geneticists, microbiologists, immunologists, physiologists, behaviorists, clinicians, bio-informaticians, statisticians. The Action will generate new standardized procedures, guidelines, integrative approaches, and genome-based selection strategies available to all.
- **Advisors:** from animal breeding organisations and companies involved in animal feeding will get easy access to new knowledge and innovative tools, and can take a role in dissemination towards swine producers.
- **Policy makers/governments:** from national and international bodies, and bodies from industry who are seeking information for policy development. Two end users of particular relevance could be EFSA and EU Commission.
- **Industry:** breeding organizations, feed industries and farmers will obtain perspectives and knowledge for future decisions on production strategies. Other companies giving services involved in animal production (animal monitoring, data managing, bio-analytical labs, bio-analytical platforms and quality certification).
- **European focus groups, technology platforms and scientific foundations**

H.2 What?

The objective of the dissemination will differ depending on the audience, but for all the main reference tool will be the website that will be dedicated for the COST Action.

For the broader audience the following information will be disseminated: the foundation of the new COST Action and of the relative website link; updates on the main activities (workshops,

conferences etc.) and on the impact of the works generated by PiGutNet. These will be posted on the website and on the most popular social media.

The “in progress activities” will be documented by interim reports posted on the website and on the institutional COST Actions website.

- The list of all the pertaining documents published by the PiGutNet will be also put on the website as well as the guidelines that will be generated.
- The members of PiGutNet will have a reserved access to the working documents in a restricted area of the website. Contributions to national and international conferences and symposia will be also presented as well as articles in peer-reviewed scientific and technical journals.
- Contribution to national seminars and workshops will be used to disseminate knowledge gained in this COST Action to pig breeders, farmers and veterinarians.
- Dissemination of the knowledge generated in the COST will be to the research community and industry.

H.3 How?

A website dedicated to the project will be created. Dissemination of general information about the project, in an easy understandable format suitable for public, will be posted on a public area of the Action’s website. The website area will contain contact details of those representing the COST Action. An area password protected will be available for COST Action members that could access and update working documents. In the protected area there will be an electronic communication network available for members and those with invitations to join discussions via forums, emails and blogs, helping to disseminate information between work packages to ensure effective integration. Publications related to the COST Action will be distributed using email distribution lists to networks partners and other interested organizations. WG reports, event proceedings, final reports and an annual newsletter (in electronic format) will be distributed to relevant bodies, and available on the website. The Newsletter will be written for scientists and non-scientists (e.g. manager in industries, regional associations of farmers and veterinarians) using a suitable language. Two COST leaflets will be produced: i) one in the early stages of the project to announce the project, highlighting project objectives to promote awareness and generate interest. ii) a second leaflet will be produced at later phases of the project. This leaflet will highlight project activities and key

results. The leaflets will be spread during relevant events e.g. international conferences on livestock production, reproduction and genetics.

Published guidelines will be available for recommendations. Large events organized by the MC, such as conferences, workshops and seminars, will be publicly advertised via the internet, e-mail lists and through relevant press, with the aim to attract the broadest audience possible. Every year a dedicated workshop will bring together experts from research, veterinary practice and industry to create awareness amongst users and stakeholders. Significant outcome of the COST Action will be presented at national seminars for veterinarians and farmers. The PiGutNet members will participate in international conferences on pig nutrition and physiology, applied microbiology, animal production and health, pig genetics, such as the European Association for Animal Production (EAAP) Meeting, the Digestive Physiology in the Pig (DPP) symposium and other, in order to disseminate the knowledge and data resulting from the COST Action activities. The Action partners attending such conferences will promote the novel technology and know-how generated by PiGutNet and will make efforts to organise satellite symposia in association with such conferences. One Training School per year will be organized for ESRs. The topics will i) Gut biology and health; ii) Microbiota and host interactions in pigs; iii) Pig genetics and genomics under the light of the Next Generation Sequencing techniques and bioinformatics tools to manage large datasets; iv) Prevention tools instead of antibiotic cure to maintain gut eubiosis in pigs.

The most significant articles that have a potential big impact for future research or management will be published as soon as possible, through peer-reviewed journals, posters and papers at conferences and lay articles as standard procedures. The COST Action will support the move towards Open Publication with peer-review process to open up the widest possible access to the new knowledge generated.