VETBIONET

Veterinary Biocontained facility Network for excellence in animal infectiology research and experimentation

Deliverable D9.6

Enhanced temporal and organ-specific readouts of infectious diseases models including more refined criteria for the application of humane end points in order to minimize animal welfare impact

Due date of deliverable: M66

Actual submission date: M72

Start date of the project: March 1st, 2017 **Duration:** 72 months

Organisation name of lead contractor: Erasmus MC

Revision: V1

Dissemination level	
Public	**X**
Confidential, only for members of the consortium (including Commission Services)	
Classified, as referred to in Commission Decision 2001/844/EC	

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This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°731014

1. Summary

Objectives:

Refined criteria for the application of humane end points in order to minimize the animal welfare impact of experimental infectious disease are aiming to match and combine scientific endpoints and humane endpoint criteria to ensure that animals studied will not suffer more than is unavoidable to obtain the scientific outcomes of the study.

Rationale:

Temporal and organ specific readouts of infectious diseases models include the dynamics of infection (which organs and tissues are involved) and host responses (such as inflammation), while the temporal readouts indicate how infection and infectious disease develop over time. This is typically an unknown when a novel model (agent-host combination) is set up, but retrospectively, an evidence-based approach can be adopted in subsequent studies to collect the scientific readouts in a timely and comprehensive manner, and to apply a humane endpoint if the animal's welfare is compromised.

Partners involved:

Erasmus MC, INRAE, WBVR, TPI, APHA, AU, ANSES, Noldus, FLI, INIA, IRTA, ISZVe, UNOTT

2. Results

2.1 In vivo studies

In the course of the project, many animal studies were set up based on existing models and novel ones, both in the context of JRAs (WP7, WP8, WP9) and TNAs. Additional models developed and/or used in the project (WP7) were taken into account in relationship with innovative technologies such as behavioral and physiological monitoring (WP9 Task 9.2). Innovation includes approaches that require special consideration when applied in high biocontainment facilities, as technical procedures and animal handling are minimized for biosafety reasons. Many partners have developed, piloted or implemented advanced approaches to monitor the course of disease over time (both infection and the host response) and thus contributed to the body of knowledge essential for managing read-outs for scientific purposes and for managing animal welfare. These combined studies included farm(ed) animals, purpose-bred laboratory animals, and wild fauna species, including birds, carnivores, ruminants and pigs, as well as a variety of life stages. Species studied were typical pathogen hosts, other species at risk, or natural reservoir species for the agent studied. Results from these studied are detailed in deliverables D7.2 “Novel reservoir host infection models for BSL3

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zoonotic pathogens", D7.4 "Improved fish model for relevant pathogens in aquiculture", D7.5 "Optimised pathogenesis and immunity models in genetic context for BSL3 pathogens", D7.6 "Novel avian/mammalian host models for airborne transmission and susceptibility", D8.6 "Pulmonar microbiome effects related to porcine influenza infections", D9.5 "Validation of integrated telemetry and behavioural monitoring" and D9.7 "Development and validation of molecular tags to study the interaction of class 3 viruses including organ and cell type tropism and host responses".

2.2 Approaches to humane endpoints

The European legislation on the protection of animals used for scientific purposes (Directive 2010/63/EU) requires Member States to implement, through national legislation, the principles of Replacement, Reduction and Refinement of animal uses, and, to this end, to implement requirements for the licensed establishments and defined requirements for their personnel (definitions of roles and competences), for harm-benefit analysis of project license applications, and reporting obligations. Building on prior experience in several Member States, a severity classification system and scale has been introduced to be applied beforehand (project license evaluation), in statistical reporting (of each animal use when concluded), and as a powerful tool for Refinement.

By definition, regulated animal use implies that animal welfare is affected to some extent, the puncture of the skin with a needle, or equivalent, being defined as the lower threshold of harms to be considered. The defined classes are mild, moderate, or severe, and 'non-recovery' is a separate class if the animal is only used once while anesthetized and will not recover from anesthesia. For Refinement, the development, validation, and application of humane endpoints allows for the complete avoidance of the "severe" category and the refinement of the "moderate" category. With this strategy in mind, infectious diseases research is to apply a risk-based approach, and to prioritize models in which animals can become severely ill or even die following experimental infection. The project included only a few models with such characteristics, and humane endpoints were applied (predefined or not), unless this was impossible for practical or scientific reasons, e.g. a peracute course of disease. Some types of readouts depend on the lethality of an infection, but even then, the animals do not need to die and they can be killed humanely when death is inevitable (evidence-based point of no return). Good science requires that all samplings, measurements, and observations are completed timely and completely, and that animals do not suffer beyond what's needed scientifically. To validate this approach, models should be evaluated (upfront and retrospectively) to identify readouts (clinical or other) that, in combination, would be proper criteria for defining a humane endpoint in a comprehensive manner, to be applied by all those with a direct responsibility. Such a set of criteria may include clinical signs (specific scoring sheet for each animal model

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to be developed) but can also incorporate measures such as body weight, body temperature, behavioral changes and outcomes of sample analyses. Some of the innovative methods developed in this project can inform or even alert about changes that prompt an intervention. In order to secure all scientific data, it should also be stated what should be done when the humane endpoint is met (e.g., full necropsy and the sampling of body materials). The consistent and carefully managed application of predefined human criteria allows for model refinement and optimization of scientific data acquisition.

3. Conclusions

The project used a wide range of animal models for the study of infectious diseases, including well-established models, natural host infection, and some new models in which the agent-host interaction was initially unpredictable. Risk-based approaches were discussed many times, as well as basic and more sophisticated approaches to assessing animal welfare when used in infectious disease research. In general, infectious disease studies in high-biocontainment facilities use minimal numbers of animals and their disease status is monitored longitudinally, so Reduction is a strength in this field. The same is true for Replacement, as most infectious agents can be grown in vitro and live host organisms are generally only used to study the interaction between agent and host, leaving Refinement as the primary alternative approach open to further development.

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