Proposal for EMIDA-ERA-NET

Project Name:

Molecular epidemiology of epizootic diseases using next generation sequencing technology

Project Acronym:

Epi-SEQ

Consortium Coordinator:

Van Borm

stevenvanborm@coda-cerva.be

Total Requested Funding	Total Costs
€ 1814139	**€ 2008639**

This is the SUBMITTED State.

Project Summary

Next-generation sequencing (NGS) techniques offer an unprecedented step-change increase in the amount of sequence data that can be generated from a sample. NGS technologies can determine complete viral genomes with a resolution allowing the quantification of RNA quasispecies variation within samples and can economize the sequencing of large numbers of samples or larger DNA virus genomes. Focusing on important epizootic viral diseases that threaten livestock industries in Europe, the aim of this project is to exploit NGS to generate improved tools that can be used in real-time during epidemics. This work will be undertaken by a multidisciplinary team of scientists from Belgium, Germany, Italy, Sweden, and the United Kingdom with expertise in molecular virology, bioinformatics, mathematical modeling, and evolutionary biology. RNA viruses evolve rapidly and quickly adapt to different environmental pressures escaping host immune defenses, altering their pathogenicity and host range, and evading diagnostic tests. Current methodologies limit the resolution at which we can study the evolutionary dynamics of the complex genomic mixtures (quasispecies) that are typical for these viruses. Archived sample collections representing epizootic outbreaks of pathogens such as foot-and-mouth disease virus (FMDV), avian influenza virus (AIV), Newcastle Disease Virus (NDV) and classical

swine fever virus (CSFV) will be used to monitor the evolution during field outbreaks of disease. Spatio-temporal data collected in the field will be integrated with this genetic data to produce robust models that can be used to reconstruct transmission trees during viral epidemics. Furthermore, in vitro experiments will be performed using FMDV (ss+RNA genome) and AIV (ss segmented-RNA genome) under strong selection pressures. Modeling of the resulting NGS data will provide a framework to describe the wider scale evolutionary patterns that are measured during these field outbreaks. Linking genetic data to viral phenotype will be undertaken by studying DNA viruses with large genomes (ASFV and poxviruses). Although the viruses have relatively stable genomes, their large size poses challenges for sequencing using traditional Sanger approaches. Specific work packages will focus on the improvement and dissemination of technical protocols and on data analysis and dissemination of bioinformatics and modeling tools. Insights from this project will result in: a) Novel information on viral evolution and (sub)populations; b) comparative evolutionary data between viruses with different genome organisation (+ vs. - sense RNA, segmented RNA, c) Improved diagnostic assays, based on an improved recognition of suitable sequence motifs; d) Powerful tools for molecular epidemiology; e) Enhanced capacity to optimize the strain composition of vaccines, and match to emerging virus variants; f) More effective tools to control epidemic and endemic infectious diseases.

Project Description

Epizootic viral diseases continue to pose threats to livestock industries in Europe. These diseases are caused by RNA viruses such as foot-and-mouth disease virus (FMDV), avian influenza virus (AIV), Newcastle Disease Virus (NDV), and classical swine fever virus (CSFV) or DNA viruses such as African swine fever virus (ASFV) and exotic poxviruses. Molecular epidemiology is widely used to monitor transboundary movements of these viruses, and to reconstruct transmission pathways over the course of disease outbreaks. Furthermore, genome data can also be used to identify the molecular determinants that underpin important phenotypic traits such as virulence and pathogenicity of viral strains and better understand how these traits evolve.

The aim of this proposal is to exploit next-generation sequencing (NGS) technologies to generate improved tools that can be used in real-time during epidemics of important viral diseases. NGS has the capacity to generate massive volumes of data, outstripping our ability to efficiently analyze and interpret the outputs. The proposed 3-year research project (Epi-SEQ) seeks to close this gap by bringing together a multidisciplinary team of scientists from Belgium, Germany, Italy, Sweden, and the United Kingdom with expertise in molecular virology, bioinformatics, mathematical modeling, and evolutionary biology. This project will target important RNA viruses that cause sporadic epidemics (FMDV, AIV, NDV), or are endemic (CSFV) in wild boar populations in some member states of the EU, as well as two DNA viruses that pose a threat to Europe (ASFV and poxviruses). Integrative studies encompassing this spectrum of epidemiology will stimulate the development of innovative tools and provide broad insights into the evolutionary ecology and epidemiology of viruses of human and veterinary importance.

The objectives of the project will be delivered through the following work-packages (WPs):

WP1 Improvement of technical protocols will focus on technical aspects of NGS platforms. Using technology available to the consortium partners, work will be undertaken to harmonize sample processing steps to maximize and homogenize data quality for data generated from field and experimental material. This WP will also include a pilot evaluation of a novel sequencing platform (P3; IonTorrent).

WP2 Epidemic and endemic patterns of virus epidemiology will be undertaken for selected RNA viruses using field material (FMDV, AIV, NDV, and CSFV) already available in partner archives. In common with other RNA viruses, the replication machinery of these viruses introduces errors when it copies the genome. As a consequence, these viruses evolve very rapidly and can quickly adapt to different environmental pressures. This process helps viruses escape host immune defenses, alter pathogenicity and host range, and evade diagnostic tests. Genome sequence data can be used to study within-host dynamics, and (in real-time) to reconstruct transmission trees at high resolution. However, existing Sanger sequencing approaches are time consuming and expensive for large numbers of samples. A particular focus of the Epi-SEQ project will be to sequence samples from field outbreaks including the FMD epidemic that occurred in the United Kingdom during 2001 and the LPAI and HPAI H7N1 and H7N3 viruses collected during the respective 1999-2001 and 2002-2004 epidemic waves in Italy. These archives represent a unique dataset to investigate the epidemiological dynamics of acutely acting viruses. Supporting contact tracing and spatiotemporal incidence data will integrate epidemiological and genetic information to dissect spread of a RNA virus during an epidemic.

WP3 Linking genetic data to viral phenotype will be undertaken by studying DNA viruses with large genomes (ASFV and poxviruses). Although these viruses have relatively stable genomes, their large size poses challenges for sequencing using traditional Sanger approaches. The project will access and

sequence archived material (at P2 and P3) from different hosts and with different pathogenicity. This will allow phylogenomic analysis and identification of specific genetic elements associated with evasion of persistence and pathogenesis.
WP4 Quasispecies characterization of viruses and how they respond to selection will be investigated using in-vitro experimental model systems with FMDV (ss+RNA) and AIV (ss segmented-RNA). Starting from plaque purified (clonal) viral populations; selection pressure will be applied using either antiviral compounds or monoclonal antibodies. The dynamics and subsequent changes to the structure of the viral population variants in the samples will be defined at high resolution using next generation sequencing approaches. Modeling of these datasets will provide a framework to describe the wider scale evolutionary patterns that are measured during field outbreaks (WP2).
WP5 Data analysis, bioinformatics and modelling tools: will utilize, improve and validate the analytical pipelines previously generated by the project partners (reinforced by external expertise from the Linnaeus Centre for Bioinformatics, Sweden; Prof Jan Komorowski) to discriminate process-introduced artifacts from true sequence variants. NGSs power lies in its capacity to reveal and quantify viral population polymorphism at very high resolution. The analytical challenge is to infer which particular transmission events most likely relate these polymorphic populations to each other. WP5 will develop tools to infer these events using data generated from other work packages but particularly WP2.
WP6 Dissemination will include: submission of the raw (and annotated) sequence data to publically accessible databases; scientific and targeted presentations; distribution of relevant results and tools via the global networks of OIE, FAO and IAEA; a workshop during the last year of the project.
WP7 project management.

Consortium Coordinator (Partner 1)

Title	Dr.
Login Name	stvan
Contact Name	Van Borm
Contact First Name	Steven
Organisation Type	non-profit organisation
Organisation (please specify)	
Name of Organisation	Veterinary and Agrochemical Research Center (CODA-CERVA)
Department	Operational Directorate Viral Diseases
POB	
Street	Groeselenberg, 99
Zip/Postal Code	B-1180
Town/City	Ukkel
Country	BE
Email Address	stevenvanborm@coda-cerva.be
Telephone Number	0032 2 3790505
Mobile Phone Number	0032 473923931
Fax Number	0032 2 379 04 01
National Regulations checked	YES

Consortium Coordinator Project Contribution

CODA-CERVA-VAR (Steven Van Borm, Kris De Clercq) will be the consortium coordinator and will be involved in WP1, WP4, and WP5. Within WP1, VAR will actively participate in the discussions and optimizations of sample preparation methodologies in direct and active collaboration with other partners. Especially our expertise in random access amplification protocols will be shared with all partners. WP4: Selection of FMDV in presence of antiviral compounds starting from purified clonal population. The resulting viruses will be sequenced in collaboration with P4. Additionally virus collections will be produced of avian influenza viruses from experimental evolution in presence of antiviral compounds or monoclonal antibodies. DNA libraries will be constructed (sample preparation methods from WP1) for these viruses and sequenced either in collaboration with partner 2 or using outsourcing via

an existing collaboration with a university core facility in Belgium. In WP5, VAR will participate in the discussion about optimal data management and analysis procedures and will handle the data it produced for its work in WP4.

Partners

Partner # 2

Title	Dr.
Login Name	BeerM
Contact Name	Beer
Contact First Name	Martin
Organisation Type	non-profit organisation
Organisation (please specify)	
Name of Organisation	Friedrich-Loeffler-Institut, Bundesforschungsinstitut für T iergesundheit (FLI)
Department	Institut für Virusdiagnostik
POB	
Street	Südufer 10
Zip/Postal Code	17893
Town/City	Greifswald - Insel Riems
Country	DE
Email Address	martin.beer@fli.bund.de
Telephone Number	038351 71200
Mobile Phone Number	
Fax Number	
National Rules checked	YES

Partner # 2 Project Contribution

FLI (Martin Beer, Bernd Hoffmann, Dirk Höper) will be involved in WPs 1, 2, 3, and 5. W ithin WP1, FLI will actively participate in the optimizations of sample preparation methodologies in direct and active collaboration with other partners. There is broad experience with preparation of virus nucleic acids for random and targeted sequencing approaches. Especially sample preparation methods for Poxviridae, Classical Swine Fever Virus and Avian Influenza A Virus will be made available to partners. In WP2, we will use pesitiviruses in order to analyze the differences between chronic disease (CSFV) and persistence (BVDV) with regard to evolution and quasispecies within the host. CSFV will also serve as a model for analysis of viruses evading molecular

diagnostics. For this part on pestiviruses, we have a collection of viruses and samples from animal experiments at hand. In WP3, poxviruses will serve as a model for analyses of virulence and pathogenicity evolution and in addition will be used to analyze host range determinants. For this purpose, a collection of poxviruses from different hosts exists and first experiments for investigation of pathogenicity and virulence have been conducted. In WP5, FLI will contribute its long term experience in NGS data management and analysis and will handle the data generated within WPs 2 and 3. We will further develop methods for viral full-length genome assemblies and analyses of viral quasispecies.

Partner # 3

Title	Prof.
Login Name	BelakS
Contact Name	Belák
Contact First Name	Sándor
Organisation Type	non-profit organisation
Organisation (please specify)	
Name of Organisation	Swedish University of Agricultural Sciences (SLU)
Department	Biomedical Sciences and Veterinary Public Health
POB	
Street	Box 7028
Zip/Postal Code	750 07
Town/City	Uppsala
Country	SE
Email Address	Sandor.Belak@slu.se
Telephone Number	(46)18 674135
Mobile Phone Number	46 70 552 16 64
Fax Number	(46)18 674669
National Rules checked	YES

Partner # 3 Project Contribution

The Joint R&D Division in Virology of SLU and the Swedish National Veterinary Institute (SVA) is the World Organisation for Animal Health's (OIE) Collaborating Centre for Biotechnology-based Diagnosis of Infectious Diseases in Veterinary Medicine and from this structure, SLU will be the partner. Within WP1, SLU will actively participate in the optimizations of sample preparation methodologies and share our expertise in random access amplification protocols with all partners. SLU will also contribute with a pilot evaluation of a 3rd generation sequencing platform by assessing the performance of the Ion Torrent PGM (available through our collaboration with SciLifeLab Uppsala). In WP2, SLU will investigate two related types of avian paramyxoviruses, NDV and pigeon paramyxoviruses (PPMV). This effort will also be supported by SVA with both additional expertise (Lena Renström and Siamak Z ohari) and access to samples collections. As leader for WP3, SLU will coordinate the

study of DNA viruses with large genomes and contribute with sequencing of African swine fever viruses. In WP5, SLU will join the collaborative effort to establish NGS analysis pipelines suitable for the challenges posed by the data generated within the project. SLU will also use the experiences gained from previous European projects (participant in CSF VACCINE&WILD BOAR, coordinator for Multiplex PCR and LAB-ON-SITE), as well as our direct connection with IAEA, to ensure proper dissemination of results to key stakeholders and other audiences. As part of this work, a final dissemination workshop will be organized during the last year of the project.

Partner # 4

Title	Dr.
Login Name	KingD
Contact Name	King
Contact First Name	Donald
Organisation Type	non-profit organisation
Organisation (please specify)	
Name of Organisation	Institute for Animal Health (IAH)
Department	
POB	
Street	Ash Road
Zip/Postal Code	GU24 0NF
Town/City	Pirbright
Country	GB
Email Address	donald.king@bbsrc.ac.uk
Telephone Number	44 1483 231131
Mobile Phone Number	44 7796 923927
Fax Number	44 1483 231142
National Rules checked	YES

Partner # 4 Project Contribution

IAH (Donald King, Nick Knowles). The Institute for Animal Heath (IAH) provides FAO-UN, OIE, European Community and UK National Reference laboratories for FMD and OIE Regional Reference Laboratories for ASF and poxviruses that are exotic to Europe. IAH will participate in all work-packages of the EPI-Seq project, and in particular will focus on the fine-scale evolution of FMDV using field samples (WP2) and material generated from in-vitro-experiments (WP4). Using the 454 platform (at TGAC, Norwich), the IAH will sequence all available samples (>1000) from the FMD epidemic that occurred in the United Kingdom during 2001. Consensus level sequence data will be integrated with spatiotemporal field data and will be used to robust models describing the transmission epidemiology of FMDV. Furthermore, viral population structures will be determined from these samples and also from in-vitro culture experiments of viruses under strong selection pressures. The results from this work will be linked to a BBSRC funded project: BB/I014314/1 (Beyond the consensus: defining the significance of foot-and-mouth disease viral sequence diversity) and will

provide tools for FMD-endemic regions of the world (such as Tanzania) where existing collaborative projects with the University of Glasgow are ongoing (BB/H009302: Towards the strategic control of endemic foot-and-mouth disease in Africa: new techniques for a neglected problem).

Partner # 5

Title	Dr.
Login Name	MonneI
Contact Name	Monne
Contact First Name	Isabella
Organisation Type	non-profit organisation
Organisation (please specify)	
Name of Organisation	Instituto Zooprofilattico Sperimentale delle Venezie (IZSVe)
Department	Research & Innovation Department
POB	
Street	Viale dell'Università
Zip/Postal Code	35020
Town/City	Legnaro (PD)
Country	IT
Email Address	imonne@izsvenezie.it
Telephone Number	390498084381
Mobile Phone Number	
Fax Number	390498084360
National Rules checked	YES

Partner # 5 Project Contribution

WP1: will actively participate in the discussions and optimizations of sample preparation methodologies in direct and active collaboration with other partners. Especially sample preparation methods for Avian Influenza will be made available to partners.

WP2: Avian Influenza strains causing distinct epidemics in poultry, (e.g. LPAI and HPAI H7N1 viruses collected during 1999-2001 epidemic in Italy and LPAI H7N3 viruses identified during epidemic waves occurred in Italy between 2002 and 2004) are available at IZSVe repository. Next generation sequencing will be applied in collaboration with partner 3 or using outsourcing via an existing collaboration with the Laboratory of Genomics; Dept. of Pediatrics ? University of Padova to contribute to understand the factors responsible for increased pathogenicity and host adaptation of these viruses at subpopulation level.

Notifiable avian influenza viruses collected in presence of vaccination (e.g. Egyptian

HPAI H5N1 viruses collected between 2006 and 2010) will be used to evaluate the effect of immunological pressure on subpopulation dynamics and emergence of escape mutants.
WP5: IZSVe will participate in the discussion about optimal data management and analysis procedures and will handle the data it produced for its work in WP3. Especially our expertise in phylogenetic and evolutionary analysis will be shared with all partners.

Partner # 6

Title	Prof.
Login Name	HaydonD
Contact Name	Haydon
Contact First Name	Daniel
Organisation Type	non-profit organisation
Organisation (please specify)	
Name of Organisation	University of Glasgow
Department	Institute for Biodiversity, Animal Health and Comparative Medicine
POB	
Street	Graham Kerr Bldg
Zip/Postal Code	G12 8QQ
Town/City	Glasgow
Country	GB
Email Address	Daniel.Haydon@glasgow.ac.uk
Telephone Number	0141 330 5849
Mobile Phone Number	
Fax Number	
National Rules checked	YES

Partner # 6 Project Contribution

The University of Glasgow (UG) will contribute primarily to WP5 (Data analysis and dissemination of bioinformatics and modeling tools). This package is closely linked with data generation that will take place in WP2 and WP4. Outputs will comprise bioinformatic approaches to the analysis of NGS data, and modelling tools that will advance our understanding of viral quasi-species dynamics, generate epidemiological inferences from genomic data, and enable the integration of spatial and temporal data on incidence with genomic data and the reconstruction of transmission chains. Researchers at UG have worked closely with IAH-Pirbright in the development of early methods for the analysis of NGS viral genome data, focussing particularly on the identification and accounting of sequencing artefacts. Furthermore, researchers currently based at UG have considerable experience of models of FMDV epidemiology (Haydon, Kao, Matthews), and have already developed preliminary frameworks that have been applied on a local scale to reconstruct transmission trees

from the integration of genomic and epidemiological data. We are therefore ideally placed to scale this work up to that of the UK, where the full epidemiological/genomic data set will constitute a unique and powerful test-bed for developing and validating epidemiological inference methodology. UG will also contribute to the analysis of other data sets generated within WP2, where similar methodologies can be applied.

Project Data

Project is related to	H4: Molecular epidemiology of epizootic diseases using next generation sequencing technology (Next generation sequencing, molecular epidemiology, persistence of strains in populations, evolution of virus strains, phylogeny)
Project Title	Molecular epidemiology of epizootic diseases using next generation sequencing technology
Project Acronym	Epi-SEQ
Duration	36

Finances

	Requested Funding						
Organisation Name	**Personnel**	**Travel**	**Consumables / Equipment**	**Subcontracts**	**Requested Funding**	**Total Own Contribution**	**Total Costs**
Veterinary and Agrochemical Research Center (CODA-CERVA)	135000	2000	0	0	137000	112000	249000
Overhead	0	0	0	0			
Friedrich-Loeffler-Institut, Bundesforschungsinstitut für Tiergesundheit (FLI)	87500	6000	54000	0	147500	0	147500
Overhead	0	0	0	0			
Swedish University of Agricultural Sciences (SLU)	207000	10000	117000	0	434000	0	434000
Overhead	72000	0	28000	0			
Institute for Animal Health (IAH)	135019	6762	112706	0	593639	0	593639
Overhead	339152	0	0	0			
Instituto Zooprofilattico Sperimentale delle Venezie (IZSVe)	40000	10000	64000	0	125000	82500	207500
Overhead	4000	1000	6000	0			
University of Glasgow	187000	7000	12000	0	377000	0	377000
Overhead	155230	5810	9960	0			
TOTAL	1361901	48572	403666	0	1814139	194500	2008639

	Own Contribution					
Organisation Name	**Personnel**	**Travel**	**Consumables / Equipment**	**Subcontracts**	**Other**	**Total Own Contribution**
Veterinary and Agrochemical Research Center (CODA-CERVA)	67000	0	45000	0	0	112000
Friedrich-Loeffler-Institut, Bundesforschungsinstitut für Tiergesundheit (FLI)	0	0	0	0	0	0
Swedish University of Agricultural Sciences (SLU)	0	0	0	0	0	0
Institute for Animal Health (IAH)	0	0	0	0	0	0
Instituto Zooprofilattico Sperimentale delle Venezie (IZSVe)	82500	0	0	0	0	82500
University of Glasgow	0	0	0	0	0	0
TOTAL	149500	0	45000	0	0	194500

Veterinary and Agrochemical Research Center (CODA-CERVA)

Personnel Costs

A scientist with 2 years of experience in virus genome sequencing will be hired on own contribution in 2012. The requested Emida budget will allow prolongation of the employment for the full duration of the project.

Travel Costs

for project meetings and research missions to partner laboratories

Consumables Costs

Molecular Biology and other consumables for genome amplification and sequencing, as well as the necessary consumables for in vitro experiments with viruses. Next generation sequencing services may also be acquired using this budget.

Friedrich-Loeffler-Institut, Bundesforschungsinstitut für Tiergesundheit (FLI)

Personnel Costs

PhD-Student for the full duration of the project

Travel Costs

Cost for travelling to the project meetings and to partnering labs for method exchange

Consumables Costs

Costs for reagents for sample preparation and sequencing

Swedish University of Agricultural Sciences (SLU)

Personnel Costs

A researcher (Fredrik Granberg) employed at 80 percent for the full duration of the project and a senior scientist with extensive experience of EU-projects (Sándor B elák) at 15 percent during the last two years (due to increased workload caused by f inal dissemination and arrangement of a workshop).

Travel Costs

Project and WP related meetings as well as travel cost associated with dissemination of results.

Consumables Costs

Molecular reagents, consumables and other material for sample preparation (nucleic acid extraction, enrichment, amplification, and purification) and sequencing.

Subcontracts Costs

Subcontractors, i.e. associated partners, will bear their own costs.

Institute for Animal Health (IAH)

Personnel Costs

The project will provide support for a Band C Research Scientist post (100 FTE, to be

appointed) at the IAH. This person will process the archived samples (nucleic acid extraction, quantification and normalisation of viral RNA using real-time RT-PCR, and genome amplification, library prepartion and clean-up) at the IAH prior to subsequent sequence analysis using 454 at TGAC (BBSRC Institute, Norwich) or Illumina-Solexa (UoG). We also request 2.5 % FTE for the Don King and Nick Knowles to manage and provide oversight on the project.

Travel Costs

IAH will participate in project planning meetings and exchange visits to other partner laboratories to contribute to the data acquisition, analysis, and dissemination of project outputs.

Consumables Costs

Since FMDV (and full length RNA) can only be handled in the high-containment laboratory at the IAH, we will ensure biosafety considerations are accommodated prior to shipment of material to TGAC (454 analysis) or UoG (Illumina-Solexa). Any material remaining at TGAC or UoG after sequence analysis will be destroyed. During the 2001 FMD epidemic in the United Kingdom, 2030 farms were designated as infected. From these cases, samples from approximately 1330 individual farms are available for analysis. Budget is requested to optimise genome amplification and sequencing methods for FMDV, and to support the sequencing of these samples using multiplex-tagging approaches. We have already developed a strategy to amplify the genome of FMDV in two overlapping fragments which will be used to process samples for Illumina-Solexa analysis of viral populations that arise from the in-vitro antiviral experiments.

Instituto Zooprofilattico Sperimentale delle Venezie (IZSVe)

Personnel Costs

6 person months for a senior scientist with experience in virus genome sequencing will be involved in the project on own contribution (37500 euro). 9 person months for two laboratory technicians (45000 euro) will be hired on own contribution. the requested Emida budget will allow hiring of laboratory staff (24 person months).

Travel Costs

for project meetings and research missions to partner laboratories

Consumables Costs

Molecular biology and other consumables for genome amplification and sequencing , as well as the necessary consumables for sample preparation.

University of Glasgow

Personnel Costs

The project will provide support for a Grade 8 Post-doctoral Research Assistant post (100 FTE, to be appointed) at the University of Glasgow (UG). Working in close collaboration with IAH-Pirbright and TGAC this person will undertake all of the bioinformatic and phylodynamic analysis of the data generated for all the Infected Premises from the UK 2001 outbreak from the 454 sequencing undertaken at TGAC. We also request 5 % FTE for Dan Haydon to manage and provide oversight on the project.

Travel Costs

We request a modest travel budget to support visits of the PI and PDRA to IAH and TGAC for the purposes developing data analysis pipelines and communicating and discussing results.

Consumables Costs

We request funds for a laptop, additional components for the computer cluster that will be used to undertake the analyses, and open access publishing costs.

Citations

[1] Balish AL, Davis CT, Saad MD, El-Sayed N, Esmat H, Tjaden JA, Earhart KC, Ahmed LE, Abd El-Halem M, Ali AH, Nassif SA, El-Ebiary EA, Taha M, Aly MM, Arafa A, O'Neill E, Xiyan X, Cox NJ, Donis RO, Klimov AI Antigenic and genetic diversity of highly pathogenic avian influenza A (H5N1) viruses isolated in Egypt., **Avian Dis.**, 2010; 54 (1Suppl), 329

[2] Blomström AL, Widén F, Hammer AS, Belák S, Berg M Detection of a novel ast rovirus in brain tissue of mink suffering from shaking mink syndrome by use of viral metagenomics. , **J Clin Microbiol.**, 2010; 48(12), 4392

[3] Capua I, Marangon S The avian influenza epidemic in Italy, 1999-2000: a review. , **Avian Pathol.** , 2000;29(4), 289

[4] Cattoli G, Fusaro A, Monne I, Molia S, Le Menach A, Maregeya B, Nchare A, Bangana I, Garba Maina A, N?Goran Koffi J-N, Thiam H, Bezeid OEMA, Salviato A, Nisi R, Terregino C, Capua I Emergence of a new genetic lineage of Newcastle disease virus in West and Central Africa?Implications for diagnosis and control. , **Vet. Microbiol.**, 2010; 142, 168

[5] Cattoli G, Milani A, Temperton N, Zecchin B, Buratin A, Molesti E, Aly MM, Arafa A, Capua I Antigenic drift in H5N1 avian influenza virus in poultry is driven by mutations in major antigenic sites of the hemagglutinin molecule analogous to those for human influenza virus., **J Virol.**, 2011; 85(17), 8718

[6] Chong YL, Padhi A, Hudson PJ, Poss M The effect of vaccination on the evolution and population dynamics of avian paramyxovirus-1, **PLoS Pathog**, 2010; 6(4), e1000872

[7] Cottam EM, Haydon DT, Paton DJ, Gloster J, Wilesmith JW, Ferris NP, Hutchings GH, King DP Molecular epidemiology of the foot-and-mouth disease virus outbreak in the United Kingdom in 2001., **J Virol.** , 2006; 80(22), 11274

[8] de Villiers EP, Gallardo C, Arias M, da Silva M, Upton C, Martin R, Bishop RP Phylogenomic analysis of 11 complete African swine fever virus genome sequences., **Virology** , 2010; 400(1), 128

[9] Domingo E, Martin V, Perales C, Grande-Pérez A, García-Arriaza J, Arias A Vi ruses as quasispecies: biological implications., **Curr Top Microbiol Immunol.** , 2006; 299, 51

[10] Enroth S, Andersson R, Wadelius C, Komorowski J SICTIN: Rapid footprinting of massively parallel sequencing data. , **BioData Mining**, 2010; 3, 4

[11] Gabriel G, Dauber B, Wolff T, Planz O, Klenk HD, Stech J The viral polymerase mediates adaptation of an avian influenza virus to a mammalian host, **Proc Natl Acad Sci U S A**

, 2005;102(51), 18590
[12] Hensley SE, Das SR, Bailey AL, Schmidt LM, Hickman HD, Jayaraman A, Viswanathan K, Raman R, Sasisekharan R, Bennink JR, Yewdell JW Hemagglutinin receptor binding avidity drives influenza A virus antigenic drift., **Science**, 2009; 326(5953), 734
[13] Höper D, Hoffmann B, Beer M A comprehensive deep sequencing strategy for full-l ength genomes of influenza A., **PLoS One**, 2011;6(4), e19075
[14] Höper D, Kalthoff D, Hoffmann B, Beer M Highly Pathogenic Avian Influenza S ubtype H5N1 escaping neutralization: more than HA variation, **Submitted manuscript** , ,
[15] Roth SJ, Höper D, Beer M, Feineis S, Tischer BK, Osterrieder N Recovery of i nfectious virus from full-length cowpox virus (CPXV) DNA cloned as a bacterial artificial chromosome (BAC)., **Vet Res.** , 2011;42(1), 3
[16] Leifer I, Hoffmann B, Höper D, Bruun Rasmussen T, Blome S, Strebelow G, Höreth-Bö ntgen D, Staubach C, Beer M Molecular epidemiology of current classical swine fever virus isolates of wild boar in Germany. , **J Gen Virol.** , 2010;91(Pt 11), 2687
[17] Leijon M, Ullman K, Matyi-Tóth A, Zohari S, Kiss I, Thorén P, Belák S Advanced tec hniques for viral diagnostics, genotyping, pathotyping and surveillance, in connection to viral emergence and climatic changes: A novel assay for the rapid identification and screening of emerging and re-emerging RNA-viruses., **J Clin Microbiol.**, 2011; Sept. 7, Epub ahead of print
[18] Rosseel T, Lambrecht B, Vandenbussche F, van den Berg T, Van Borm S Identification and complete genome sequencing of paramyxoviruses in mallard ducks (Anas platyrhynchos) using random access amplification and next generation sequencing technologies., **Submitted manuscript**, ,
[19] Schadt EE, Turner S, Kasarskis A A window into third-generation sequencing. , **Hum Mol Genet**, 2010; 19(R2), R227
[20] Senne DA, Panigrahy B, Kawaoka Y, Pearson JE, Süss J, Lipkind M, Kida H, W ebster RG Survey of the hemagglutinin (HA) cleavage site sequence of H5 and H7 avian influenza viruses: amino acid sequence at the HA cleavage site as a marker of pathogenicity potential. , **Avian Dis.** , 1996; 40(2), 425
[21] Stech O, Veits J, Weber S, Deckers D, Schröer D, Vahlenkamp TW, Breithaupt A, T eifke J, Mettenleiter TC, Stech J Acquisition of a polybasic hemagglutinin cleavage site by a low-pathogenic avian influenza virus is not sufficient for immediate transformation into a highly pathogenic strain., **J Virol.** , 2009; 83(11), 5864
[22] Suarez DL, Senne DA, Banks J, Brown IH, Essen SC, Lee CW, Manvell RJ, Mathieu-Benson C, Moreno V, Pedersen JC, Panigrahy B, Rojas H, Spackman E,

Alexander DJ Recombination resulting in virulence shift in avian influenza outbreak, Chile. , **Emerg Infect Dis.** , 2004; 10(4), 693
[23] Ujvári D, Wehmann E, Herczeg J, Lomniczi B Identification and subgrouping of p igeon type Newcastle disease virus strains by restriction enzyme cleavage site analysis., **J Virol Methods.**, 2006;131(2), 115
[24] Wang J, Höper D, Beer M, Osterrieder N Complete genome sequence of virulent d uck enteritis virus (DEV) strain 2085 and comparison with genome sequences of virulent and attenuated DEV strains., **Virus Res.**, 2011;160(1-2), 316
[25] Wright CF, Morelli MJ, Thébaud G, Knowles NJ, Herzyk P, Paton DJ, Haydon D T, King DP Beyond the consensus: dissecting within-host viral population diversity of foot-and-mouth disease virus by using next-generation genome sequencing., **J Virol.** , 2011; 85(5), 2266

Keywords

- H:Epizootic diseases (general)
- H:Epizootic diseases (swine fevers)
- H:Epizootic diseases (AI)
- H:Epizootic diseases (FMD)
- H:[Molecular] epidemiology
- H:Host-pathogen interaction (virulence factors/determinants)
- H:Host-pathogen interaction (persistence factors)
- H:Host-pathogen interaction (viral genome/viral strains)

Optional Keywords

Keywords related to the broader thematic area of the project:
quasispecies
Keywords related specifically to the project:
Next Generation Sequencing