CCH Fever FINAL REPORT

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1. Final Publishable Summary Report

1.1 Executive Summary

Over the last several years we have experienced an increase of large outbreaks of Crimean Congo Hemorrhagic Fever (CCHF), in several European countries and neighbouring areas. CCHF is a human tick-borne disease with a fatality rate of up to 30%. The disease is enzootic and asymptomatic in domestic animals. This disease poses a great threat to public health due to its high mortality rate in man (5-30%), modes of transmission (tick-to-person/animal, animal-to-person, person-to-person) and geographical distribution. CCHF Virus is widely distributed throughout large areas of sub-Saharan Africa, South-Eastern Europe, Middle-East, Central Asia down to Pakistan and as far east as the Xinjiang province of Northwest China, almost 30 countries. In fact, of all medically significant tick-borne diseases, CCHFV is the geographically most widespread pathogen. The complexity of the scientific and technical issues to be addressed requires a broad multidisciplinary network of experts. The CCH Fever project has built on existing leading-edge expertise in Europe, Asia, USA and Africa, and has brought together new constellations of scientists to work towards solving important public health and medical problems related to CCHF that are not tractable by individual groups. This project aimed to investigate and integrate basic virology, antiviral and vaccine development, epidemiology, genetic analysis, field diagnostics and medical training. The major objective of this joint initiative has been to create a multidisciplinary research activity that spans over the following subjects concerning CCHF: i) Improvement of Field Diagnostics, ii) Studies on epidemiology, immune response, phylogeny and evolution of the virus, iii) Vaccine candidate development, iv) Development of strategies for the discovery of new effective CCHF therapeutics, and v) Education of individuals involved with public and health policy in endemic regions and capacity building in these regions.

Within this program we have achieved following goals:

- Developing several diagnostic tools, such as lateral flow kit for point of care diagnostic,
- Evaluating several commercially available diagnostic kits,
- Performing for the first time uniformed extensively sero and molecular epidemiological studies covering endemic and non-endemic area in endemic countries in Europe,
- Developing several vaccine candidate with very promising preliminary results,
- Developing strategies for screening of antiviral compounds against CCHFV,
- Organising several technical workshops and courses treating diagnostic, clinical aspects of CCHF and outbreak management,
- Initiating a platform for medical healthcare workers from north and west Europe, to be trained in endemic area during epidemic periods,
- Linking the CCH Fever project to other networks and FP7 supported research program such as ERINHA, ArboZoonet, ENIVD, Quandip, EDEN NEXT, EVA, etc.,
- Establishing collaboration with National Public Health Agencies in endemic European countries and also with european (ECDC) and international organizations (WHO),
- Creating a new consultation of scientist and establishing a collaboration platform which resulted to new initiative such as EsCential (InfectERA), EbolaMoDRAD (IMI2), and EVA g.

We have also developed several new tools and knowledge beyond the scope of this project. These tools and basic knowledge were not available at the start of this project. These new knowledge and tools will undoubtedly contribute to and facilitate future research efforts.

1.2 Summary description of project context and objectives

In the 21st century, outbreaks have become more frequent in Europe (cases or outbreaks have been recorded in Kosovo, Albania, Greece and Bulgaria). Very recently, we faced large outbreaks in Turkey with more than 4 000 cases.

The understanding of CCHFV migration, transmission and recombination has been extremely limited. Except for ribavirin, there has been no effective therapy available. However, whether or not patients with CCHF disease actually benefit from being treated with ribavirin is still unclear. To date no vaccine for prevention of the disease is available.

The research activities concerning this disease have been restricted to very few institutes/laboratories, for several reasons such as i) the handling of the virus requires high containment laboratories (BSL-4), ii) sporadic outbreaks in endemic countries which have no facilities for performing a basic and/or applied research program, iii) lack of clinical specimens from the patients, animals and ticks.
It has been obvious that the basic knowledge on CCHFV biology, pathogenesis, vaccine development, therapeutic and integrated control measures has been highly limited for this important bio-threat.

It has, thus, been imperative to build a **multidisciplinary research activity with focused goals**. To achieve an effective program, multidisciplinary research activities proceeding from different specialties were required to produce the knowledge that could contribute to develop improved diagnostics and control measures, effective prevention, and therapy strategies. The complexity of the scientific and technical issues to be addressed required a broad multidisciplinary network of experts. The CCH Fever project aimed to build on existing leading-edge expertise in Europe, Asia, USA and Africa, and to bring together new constellations of scientists to work towards solving important public health and medical problems concerning CCHF that were/are not tractable by individual groups. This program aimed to investigate and integrate basic virology, antiviral and vaccine development, epidemiology, genetic analysis, field diagnostics, and medical training.

The field of CCHF has been a narrow research field with few top experts around the world and a limited number of capacity hubs. Altogether, it is clear that multidisciplinary research efforts need to be focused into a program on this virus. The CCH Fever project has succeeded to bring together selected competitive advantages: 1) operative capacity with appropriate facilities, 2) experienced researchers, 3) authorities and entities from endemic areas in Europe, 4) clinical samples from endemic countries and 5) an international network. The objectives proposed in this multidisciplinary research program were highly specific and measurable by the proposed program.

The CCH Fever project targets the following objectives:

- **Improvement of the field diagnostic**

CCHF is a neglected European deadly emerging vector-borne disease and poses a great threat to public health, due to its epidemic and transmission potential. There has been an urgent need for the development of novel, robust, highly sensitive and rapid diagnostic tests to detect the virus and disease. Within this project, we have focused to develop several diagnostic tools and SOPs, which in turn can contribute for better and safer diagnostic.

- **Variability, molecular evolution and migration patterns of CCHFV circulating in endemic area**

During the 21st century, CCHF outbreaks have become more frequent in the countries of former Yugoslavia, Turkey and Iran. Since CCHF is endemic in these countries, recent outbreaks are not likely the result of new CCHFV types. Instead, it is proposed that climate changes, in combination with extended and increasing use of land for agriculture and farming are partly responsible for increased CCHF incidence. The knowledge of variability, evolution and migration pattern of CCHFV is highly limited. Using statistical and advanced bio-informatics analysis from the sequences obtained in this program, we have aimed to contribute building essential tools for monitoring and predicting the movement of virus lineages over time, geographic locations and outbreaks in Europe and neighbouring countries.

- **Design of vaccine candidates**

Today, there is no effective vaccine available for CCHFV. In 1970, an aluminium hydroxide-adjuvanted, formalin-inactivated mouse brain-derived vaccine becomes available in former USSR. The weak immunogenicity of this inactivated vaccine, with difficulty in preparing this vaccine candidate together with high risk in preparation and validation of inactivation explain the need for an effective vaccine for CCHFV. Within this project, we have used several approaches for developing new vaccine candidates for CCHFV.

- **Design of new effective antivirals**

The establishment of new screening methodologies created a potential for screening of available antiviral libraries and even designing new compounds. Results and knowledge of this effort will have a big impact on designing strategies to counter CCHFV infection and other infectious diseases focused on in other research program and activities. CCH Fever project aimed to develop new screening methodologies by approaching several different strategies.

- **Mobilizing of the capacity and infrastructures of the participating groups from different areas**

There is a general public concern regarding emerging zoonotic diseases which has gained new relevance in the light of global warming. This is especially true regarding the spread of vector-borne diseases, such as CCHF, in Europe. Natural epidemics and outbreaks of emerging infectious diseases are growing problems internationally, like the Ebola outbreak of 2015 recently reminded us.
There is huge need for mobilization of the capacities and dissemination of the existing, as well as new gained knowledge in this field. This program have aimed to generate a large amount of scientific data which are of huge interest for public health,
In parallel to the enhanced activities mentioned in the objectives above, the planned training courses and exchange activities immediately aimed to provide the scientists with enhanced skills and SOPs to enable them to implement the most advanced techniques. The exchange activities as well as training courses facilitated the integration of scientists from Europe (Southern, Eastern and Northern Europe), Africa and USA. The CCH Fever program aimed also to contribute to mobilizing capacity also by Dissemination and linking this consortium to national and international organizations, initiatives and authorities.

1.3 Description of the main S&T results/foregrounds

The project is organised into six work packages (WP): the first one is dedicated to the management of the consortium and the organisation of annual and Executive Committee (ExCom) meetings. The Project Management Team is acting as a helpdesk for all partners to answer all their questions about EU project, finances and help for the reporting. The other WPs are for the development of the scientific aspects of the project and its dissemination.

WP2: Novel, biosafe methods for outbreak diagnostics

Crimean Congo Haemorrhagic fever virus (CCHFV) is a BSL4 organism, which makes diagnostics difficult, as only few specialised centres offer BSL4 infrastructure. Centres of this type are not available in countries where Crimean Congo haemorrhagic fever (CCHF) occurs. To improve this situation, we wanted to develop diagnostic tools that allow safe and reliable handling of patient material for diagnostic purposes and to develop assays for the detection of CCHFV-RNA, CCHFV-antigen and antibodies directed against CCHFV. Successfully tested assays have the potential to greatly improve and facilitate testing for CCHF at the point of care. This should make early detection and treatment available to larger populations in infrastructure-poor settings. The activities within this Work Package are divided in several tasks:

Task 2a: Development of nucleic acid detection by padlock technology

An Isothermal Padlock Probe assay (IPP) for CCHFV RNA detection in cells was developed and published at beginning of the CCH Fever project. This assay was eligible for use in centralized laboratories. A first format of a fluorescent IPP assay for use on a mobile and small footprint fluorescence reader was tested however its sensitivity was too low. We have applied several strategies to improve sensitivity of this assay. Within the final period of the project, an additional format of a fluorescent Rolling Circle Amplification (RCA) assay for use on a mobile and small footprint fluorescence reader were tested however their sensitivity was still either too low or produced signals in the negative control. The major problem consisted of having to produce circularized probes to amplify the initial linear RCA signal. Details of the purification were complex and the reproducibility was too low to allow for consistent testing. It emerged that remaining impurities in this process caused the false positive signals. The RCA protocol can therefore not be finalized in the frame of this project.

Task 2b: Development of VLP-ELISA

A CCHFV minireplicon system was established during early post start of the project and used for the production of CCHFV virus-like particles (VLPs). The VLPs was used for developing new tools for antibody detection and for detection of neutralizing antibodies. A VLP-ELISA assay for the detection of IgM and IgG has been established for CCHFV. The final draw back was a high background signal in the BSA control, which was used as a specificity and background control. Analysing means of reducing this background signal e.g. by addition of milkpowder in the inactivation step at 56°C/30min eventually led to the surprising discovery that the brand of BSA actually used caused the high background. The BSA routinely used in ELISA for fish sera which have a high unspecific reactivity turned out to produce a much reduced background signal to a good acceptable level. ***The final protocol detailing all reagents used was delivered to partner RNSPHA for validation*** by testing with patient sera and comparing with other in-house methods. ***It has also been shown that VLPs can be used in neutralization assays, which***

makes it possible to perform this diagnostic assay at BSL-2 level, a big success for the CCHFV research and diagnostic field.

Task 2c: Development of Fiber-optic immunosensor-assay (FOIA)

A final version of the fibre optic ELISA as well as the final chemistry to immobilise the CCHFV nucleocapsid on the glass surface of a fibre were developed. It was used to establish an IgG-Elisa in which a secondary horseradish peroxidase coupled antibody initiates a chemiluminescent reaction. In a proof of concept study the developed assay was more sensitive compared to plate based colorimetric and chemiluminescent ELISAs. ***The limit of detection in a diluted patient serum was 10-fold greater compared to colorimetric ELISA (manuscript submitted).***

Task 2 d: Development of a Lateral flow Dipstick

CCHFV-N was expressed and purified for the development of a fibre-optic immunosensor-assay (FOIA) and lateral flow dipsticks (LFD) respectively. A lateral flow strip was developed by partner 11 Coris BioConcept using an Ig capture reagent on the strip and recombinant CCHFV-nucleocapsid coupled to gold particles as a tracer. Optimisation included increasing the purity of recombinant CCHFV nucleocapsid protein. The lateral flow test was shown to work with 5µl serum or 10µl whole blood as a sample. Initial testing at the National Public Health laboratory in Ankara using 42 sera indicates a satisfactory performance. It appears however that there may be a lack of sensitivity with some IgM sera (false negatives). An improved second-generation prototype test has been developed with very promising data. This new prototype detects specifically IgM antibodies and shows increased signals intensities. ***This rapid test will be evaluated and validated in partners' labs in Turkey and Slovenia during 2015.***

Task 2e: Validation trial and field trial

Since the development of the assay formats took longer than expected, field validation trials were not performed during the period of the project, however, the VLP-ELISA and the lateral flow assay are now being evaluated by RNSPHA during spring and summer 2015.

Task 2f: Establishment of inactivation procedures

Finally, regarding the safe inactivation procedures, several approaches to inactivate the samples have been tested. However, work on the biosafe inactivation procedure was stalled by the workload the team at NICD experienced due to the Ebola outbreak in West-Africa. However, there are promising preliminary data available, which will be followed in 2015.

WP3: Epidemiology, immune response and virus evolution

Results of the seroprevalence and molecular epidemiology study aimed to give an insight into the endemic foci of the Balkan countries and Turkey. Analysis of the answered questionnaire aimed to give evidence about the risk factors for acquiring the infection. This information can be used by public health authorities to strengthen the preventive measures for the disease. Results of the host immune response analysis aimed to give insights into the pathogenesis of CCHF, while phylogenetic analysis of the circulating CCHFV strains enabled the studies on the evolution of the virus. The studies on immune response gave also important knowledge on the way that humans respond to the CCHFV infection, and the indentified biomarkers can be used for further studies for treatment design.

This knowledge, together with the availability of the samples from the BioBank with all related information from the DataBase, are the best material for further studies on new diagnostic methods, vaccine development and anti-CCHF drug design.

WP3 includes several tasks requiring strong collaboration among the 11 partners involved.

Task 3.a: Collection of samples and construction of a BioBank

A BioBank (collection of samples of confirmed CCHF cases) and the related DataBase, was constructed during the CCH Fever project. Two BioBanks are ready: one consisting of serum/blood samples of confirmed CCHF cases (n=65), and a second one containing IgG positive serum samples collected during the seroprevalence studies (n=120). Following the standardized protocols for collection of human samples and SOPs for storage and transportation of samples, the samples from confirmed clinical CCHF cases were sent and stored in a BioBank. Currently the first BioBank consists of 65 samples of CCHF confirmed cases: 28 from Turkey (partner 7 RSNPHA), 10 from Albania (partner 8 IPH-CIDD), 6 from Bulgaria (partner 9 NCIPD), 21 from Kosovo (provided by partner 12

UL). All samples included in the BioBank were thoroughly tested by serologic and molecular methods, and nucleotide sequences of partial S RNA segment with same primer sets. All available demographical, epidemiological and clinical data related to these samples were added in the DataBase. The second BioBank and the related DataBase (containing CCHFV IgG-positive samples obtained from the seroprevalence studies) has been used for validation of several new diagnostic assays as well as commercially available kits.

Task 3.b: Testing of clinical and ticks samples

The samples have been tested for serology (ELISA and IFA), RT-nested PCR and Real time PCR. The positive clinical samples have been sequenced from Turkey, Bulgaria, Kosovo and Albania. All samples were tested by the same primer sets. The final phylogenetic analysis of the obtained sequences and constructed phylogenetic trees for the molecular epidemiological studies in Albania, Bulgaria and Turkey has been preformed (see below).

Task 3.c: Detection of reassortment/recombination, estimation of evolution rates, and determination of migration routes by using full-genome sequences

An evolutionary study was performed on CCHFV partials RNA segment sequences. Whole genome sequencing finished for few CCHFV strains; Whole genome of Zahedan CCHF virus was completed and published. Two more CCHFV strains were sequenced (strains from Dakar and Turkey). Some segments were determined completely. To determine the terminal sequences of the segments, we ligated the RNA to form circularised segments and designed primers to amplify across the ligation site. In a first approach primers for PCR fragments 600bp in length were designed. Four out of nine PCRs did amplify correct fragments. Commercial cycle sequencing of these fragments however yielded unsatisfactory results arising especially from contaminant sequences apparently due to unspecific amplification. Several approaches to deal with these impurities did not solve the issue. In a second approach primers for fragments of 200-290bp in length were designed. Here 6 of 9 PCR amplificates yielded results. Partial sequence of the Dakar strain covering the missing ends (219 bp) was obtained using Sanger sequencing. Altogether the sequences of only 2 of 11 segments could be resolved. It is now planned to ligate all obtained 600pb and 200bp amplificates for the remaining 9 segments into pCRII to sequence directly from the recombinant plasmids generated. Work is ongoing and will extend beyond the project period.

The delay was due to several reasons (change of work position of partner 4 UMG-GOE going to partner 16 UoS, leaving of the PI who started the study at partner 13 NF-CDC while the Ebola outbreak highly affected partners 10 NICD and 13 NF-CDC). These partners will continue the ongoing studies and finalize the testing for reassortment/recombination, estimation of evolutionary rates and determination of migratory routes of CCHFV after the end of the project.

Task 3.d: Immune response studies

Seroprevalence studies in all countries are finished and the results from the studies in Greece, Bulgaria, Turkey, and Kosovo are already published, while the results from Albania were presented in IMED conference in Austria (15-18 February 2013). A good insight into the endemic foci of the countries was gained, and significant differences between endemic and endemic foci were observed regarding the risk factors. Since the estimated seroprevalence in some regions of Greece was found high (during the studies of the previous periods), but only 1 case of disease has been reported, 118 IgG positive samples were re-tested by 4 additional methods (two in house ELISAs, one commercial IFA and neutralization assays) and confirmed the positive results, suggesting that a nonpathogenic or low-pathogenicity strain may be circulating in Greece (article was published: Crimean-Congo hemorrhagic fever virus, Greece. Papa A, Sidira P, Larichev V, Gavrilova L, Kuzmina K, Mousavi-Jazi M, Mirazimi A, Ströher U, Nichol S. Emerg Infect Dis. 2014 Feb;20(2):288-90). Several countries expended the studies on specific risk groups and in animals, while a comparison among methods for IgG detection was performed. The study on neutralization assays from CCHFV patients demonstrated that CCHFV induces high level of neutralizing antibodies early after the symptoms onset, and these antibodies cross react with other viral strains was published. These data demonstrate high degree of cross reactivity of neutralization antibodies between different CCHFV strains, which is promising for development of new vaccine candidates.

However, we have been unsuccessful to identify and produce human neutralizing antibodies (for treatment purposes) derived from recovered CCHF patients. We could not receive any signals after isolation of RNA from the collected and stored samples at Kosovo from recovered patients. We found that the concentration of isolated RNA was very low. This was most probably due to the bad condition of stored PBMC at -70°C.

WP4: Prevention: Development of the new vaccine candidates against Crimean Congo Hemorrhagic fever virus
Today, there is no effective and safe vaccine against CCHFV available. Within this work package, we expected to obtain candidates for a safe and efficient CCHFV vaccine by using: (i) recombinant viral antigens, ii) DNA Plasmid expressing most important viral proteins and iii) also aimed to develop a virus liked particles (VLP) based on a minireplicon system. Establishment of a robust minireplicon system has been useful for antiviral drug discovery against CCHFV, and the determination of viral immunosuppression strategies. The VLP system has also been tested in as antigen spotted on ELISA plates for detection of antibodies in patients.

Task 4.a: Production of recombinant CCHFV antigens
We tried to express CCHFV N, Gn, codon-optimised Gn, and codon-optimised Gc in E.coli, using the DNA constructs. Only the virus-codon Gn plasmid allows proper expression of the respective protein (which was however insoluble). Attempts were made to express the proteins into a mammalian expression system (HEK293, BHK-21), but only the N plasmid allows proper expression of the respective protein (which was however insoluble). ***Since VLPs are a most promising avenue for a vaccine, it has been decided that Task 4a was not a priority anymore.***

Task 4.b: Production of expression constructs for DNA vaccination
Within this task, we have developed DNA constructs for bacterial expression of N, Gn, and Gc proteins of CCHFV (subunit vaccine), and DNA constructs for mammalian expression of N, Gn, Gc proteins, both with and without a fused ubiquitin domain (for DNA vaccination). Complete list of DNA vaccine plasmids is the following: pCMV_CCHFV_N (encodes CCHFV N), pCMV_Ub_CCHFV-N (encodes ubiquitin-tagged CCHFV N), pCMV_CCHFV_Gc (encodes CCHFV Gc), pCMV_Ub_CCHFV-Gc (encodes ubiquitin-tagged CCHFV Gc), pCMV_CCHFV_coGc (encodes CCHFV Gc, codon-optimized sequence), pCMV_Ub_CCHFV-coGc (encodes ubiquitin-tagged CCHFV G, codon-optimized sequence), pCMV_CCHFV-Gn (encodes CCHFV Gn), pCMV_Ub_CCHFV-Gn (encodes ubiquitin-tagged CCHFV Gn), pCMV_CCHFV-coGn (encodes CCHFV coGn, codon-optimized sequence), pCMV_Ub_CCHFV-coGn (encodes ubiquitin-tagged CCHFV Gn, codon-optimized sequence).

Task 4.c: Establishment of a virus-like particle system for CCHFV
We have established and optimized the CCHFV minireplicon system. This system was then used to develop a VLP assay for CCHFV. We tested 9 cell lines for production and detection of VLPs, 3 transfection agents, and we optimized minigenome reporter gene, plasmid backbone, viral promoter, plasmid ratios and time courses. We then determined the optimal conditions for VLP purification, preservation and long-term storage (> 1 year at -80°C).
VLP titers at beginning were low, but we developed an amplification technique, which allow reaching titers of 10E5 – 10E6 active particles/mL. Those VLPs are capable of autonomous transcription and are sensitive to type I interferons. We also showed their specificity to CCHFV in a neutralization assay, using human anti-CCHFV sera. Detection of protein content of VLPs by western blot confirmed their similarity to authentic CCHFV particles.
The VLP system is rather robust and highly active, and was used to develop ELISA and neutralization tests. We were also able to optimize production of VLPs with a viral polymerase mutated in its OTU domain, with the hope to increase immunogenicity. The VLP system was instrumental in identifying the CCHFV endonuclease domain, which has brought new insight on the CCHF virus biology.

Task 4.d: Testing immune responses to vaccine candidates
The vaccine candidates (plasmids and VLPs) were tested in wt mice and mice deficient in interferon systems of type I (IFNAR KO), and type II (IFNGR KO). Both, the DNA plasmids (Gn, Gc, and N expressed separately) and the VLPs, provoked a CCHFV-specific immune response that was largely IFN-dependent and could be improved by boostering. Although the immune responses to the vaccine candidates were comparatively low, neutralization antibodies are produced. Based on these data, we optimized the immunization protocol. The results were encouraging enough to proceed to challenge studies.

Task 4.e: Testing of the various vaccine candidates in the mouse model for challenge studies
A mouse model for performing CCHFV pathogenicity studies had been established. This *in vivo* model was used to inoculate vaccine candidates and test their capability to protect from a lethal CCHFV challenge. Immunizations with VLPs delayed the onset of CCHF symptoms, immunizations with ***plasmids expressing Gc, Gn, and N even resulted in a 100% protection.*** This shows that full protection can be achieved. The result with the mouse model

lacking the IFN system most likely underestimates the potency of VLPs. Further experiments to test wt mice are ongoing.

WP5: Towards drugs for Crimean Congo Hemorrhagic Fever Virus (CCHFV)

Currently, only ribavirin has been approved for the treatment of patients with CCHFV infection. However, the mechanism, by which this drug acts, is still poorly understood and discussion is still ongoing whether or not CCHFV-infected patients actually benefit from this treatment. WP5 aimed to set up at least one assay system suitable for the discovery of novel inhibitors of CCHFV replication outside a BSL-4 setting. Ideally, if an existing drug, which already has been approved for use in humans, also would show activity against CCHFV, it potentially could be used off-label for the treatment of CCHFV-infected patients. In addition, the identification and fundamental study of novel small-molecule inhibitors should catalyse a better understanding of the function of specific proteins in the replication cycle of CCHFV and could spur further drug development projects, ultimately increasing the number of drugs available for the treatment of this disease. During the course of the project, T-705 (Favipiravir) was shown to selectively inhibit the replication of CCHFV by other group. The same observations were made by another research groups and published. T-705, although first developed for the treatment of influenza virus, will prove to be a valid alternative for ribavirin for the treatment of patients with CCHFV. It was the scope of the CCH Fever consortium to support the clinical use of T-705 for the treatment of CCHFV infections beyond the end of the project.

Three different but complementary strategies have been pursued in WP5 to set up at least one system for drug discovery and development purposes against CCHFV that can be deployed outside a Biosafety Level 4 containment facility. (1) a minigenome system, (2) a virus-cell-based assay employing a model virus, and (3) a reporter cell line. In the end, WP5 aimed to constitute a knowledge platform that would drive the discovery, development and study of novel selective small- molecules inhibitors of CCHFV replication. As soon as either one of the assay systems to be developed within WP5 is validated for drug discovery and development purposes, and is ready to be deployed for a small-scale drug discovery effort, the main objective of WP5 will have been reached.

Task 5.a: Selection of a representative model virus

To standardize the drug discovery effort, a representative model virus had to be selected, that represents the taxonomic clade of the species CCHFV, genus Nairovirus, and family Bunyaviridae. We selected and agreed on this model virus based on relevant phylogenetic, taxonomic, epidemiologic and clinical evidence. A reference virus stock was generated and distributed to the participants of the consortium that had the necessary facilities and permissions to handle this virus.

Task 5.b: Development of quantification tools for this model virus

To detect and quantify this model virus in cell culture, two different assays at least need to be developed and validated. Based on the genome sequence of the virus, a primer and probe set was developed for real-time quantitative RT-PCR. Furthermore, available antisera were evaluated for affinity with this virus. The results of both techniques have been compared. The necessary materials and protocol were distributed to the participants of the consortium that had the necessary facilities and permissions to handle this virus.

Task 5.c: Validation of the CCHFV a minigenome system as drug discovery tool

In 2009, Habjan and colleagues (beneficiary 15 PUM) reported the development of a minigenome and infectious virus-like particle (VLP) system for RVFV (Virology 385 (2009) 400–408) and validated this system for the study of RVFV replication. A minigenome system for CCHFV has been developed. The assay was validated with ribavirin as reference compound. Subsequently, this assay was used to evaluate the antiviral activity of other known inhibitors on the replication of CCHFV (a collection of around 50 reference compounds with known mechanism of action is available at beneficiary 6 KU Leuven for this purpose such as S-adenosyl-homocysteine hydrolyse inhibitors or IMP dehydrogenase inhibitors) and to initiate the discovery of novel selective small-molecule inhibitors of CCHFV replication.

Task 5.d: Assessment of existing cell lines for replication efficiency

The consortium evaluated the susceptibility of cell-lines they have available in their laboratories for replication efficiency to the representative model virus selected in task 5.a, using the quantification tools described in Task 5b. The purpose of this task was to identify the cell-line that most efficiently supports replication (i.e. that produces the highest titre of the model virus) and that displays signs of virus-induced cell death.

Task 5.e: Clonal purification of cell line(s) that efficiently support model virus replication

Most cell-lines, whether or not they have a long passage history, consist out of a heterogeneous cell population of several subtypes. The cell-line that most efficiently supports model virus replication and in which signs of virus-induced cell death are observed, is clonal purified after which the different clones are again evaluated for susceptibility to viral replication as described in task.5.d. The selected clone has been amplified and sent to the participants of the consortium having access to Biosafety Level 4 facilities to evaluate this cell-line for replication efficiency of other CCHFV virus types. It is beyond the scope of the project to characterize the selected clone as well as the other clones, but this material is made available to other beneficiaries of the consortium that wish to study host factors that determine susceptibility to CCHFV replication.

Task.5.f: Adaptation of the model virus to the selected host cell line

Due to the lack of proof-reading activity of RNA-dependent RNA polymerases, RNA viruses intrinsically exist as a quasi-species i.e. the virus population is composed of virions that contain genome segments with minor differences. These small genetic differences however may give rise to significant phenotypic properties with respect to replication efficiency. So, in addition to the selection of a host cell-line that most efficiently supports model virus replication and is sensitive to virus-induced cell death, the model virus itself will be further adapted to the host cell-line by both virus passaging as well as virus purification. The first one implies serial passaging of the virus in the selected cell clone and verifying alterations in replication efficiency of the entire population (enrichment of the most replication-competent viruses). The latter technique implies clonal purification and phenotypic characterization of individual virus clones. The genotype of the most replication-competent clone will be characterized. It is beyond the scope of this project to characterize the genotype of other than the most replication-competent genotype, but the materials has been made available to the other participants of the consortium to study properties of the virus that have an impact on replication efficiency.

Task.5.g: Development and validation of a virus-cell-based antiviral assay

The most replication-competent model virus and most replication-supporting cell clone have been used to develop a virus-cell-based assay for the identification of selective inhibitors of CCHFV replication. This assay has been validated with ribavirin as reference compound. Subsequently, this assay has been used to evaluate the antiviral activity of other known inhibitors (similar approach as in task 5.c). Subsequently, it was used for the discovery of novel selective small-molecule inhibitors of CCHFV replication.

Task.5.h: Development of a reporter cell line for CCHFV replication

A cell-line that is susceptible to infection with the model virus supposed to be stably transformed to produce a reporter RNA consisting of a [CCHFV_leader]-[anti-coding_reporter_gene]-[CCHFV_trailer] sequence. Upon infection of the reporter cell-line with CCHFV, the reporter RNA will be transcribed by the viral RNA-dependent RNA polymerase into a coding mRNA which results in the expression of the reporter gene. Depending on the selection of the reporter gene, quantification will be possible either by fluorescence or luminescence.

Due to personnel-related issues at Partner 14 BGU, the task was transferred to Partner 15 PUM. Partner 15 PUM first determined the hygromycin threshold different cells lines that could be used to develop this system needed to be subjected too to allow selection of transfected cells. Subsequently, transfections were performed, but every time a clone of cells was selected, no compelling evidence could be obtained that the transfection was stable enough to be used for the development of a reporter cell line assay. Clones that showed some promise were sent to partner 1 FoHM to be infected with wild-type CCHFV, but the expected results were not obtained. As a consequence, Deliverable 5.6 (due Month 36) has not been met in time. With this, also Milestone 18 (due Month 30) was also not met.

WP6: Knowledge dissemination

The WP6 aims at disseminating the foreground within and outside the CCH fever consortium about CCH fever disease. Various means were used like exchanges of students and technicians between partners; organization of several courses/w-shops with ArboZoonet (Fp7 project) and WHO/Europe for partners, other interested networks and organizations, research community, laboratory staff and health workers; participation and presentation in national/ international workshops and congresses and publications in peer reviewed journals. We have also organized the 1 st international Congress on CCHF in Thessaloniki, and also co-organised a conference on Emerging and Re-emerging Epidemics Affecting Global Health in Orvieto, Italy with ArboZoonet, the Society for tropical veterinary medicine, Provect and Prostics.

26 publications and 48 dissemination activities has been achieved during the four years of the project. Thanks to these actions, we have disseminated the knowledge generated by the CCH Fever consortium to scientific researchers, encouraging new collaborations with other European consortia (such as ENIVD, EVA, ERINHA and ArboZoonet) and new networks, increasing visibility and awareness of the disease in endemic areas of Europe and encouraging capacity building in these regions.

Task 6.a: Training and exchange activities within the consortium

During this task, we had opportunity to exchange students and technical staff between our laboratories to not just exchange the new technologies but also initiate new links within this field.

Summary of all the exchanges within the CCH Fever consortium:

Date	Participant	Host	Activity
July 2011	**partner 12** , microbiologist Luka Fajs	National Institute of Publish Health of Kosovo in Pristina	Training their staff in molecular diagnosis and sequencing of CCHF. During the stay in Kosovo, a field trip was organized which included training in tick collection in CCHF endemic areas
April 2012	Partner 14 BGU, BSc Lina Konts	Partner 4 UMG-GOE	Doingfeasibility work on the immunosensor
May – June 2012	Partner 6 KU Leuven, technician Stijn Delmotte	Partner 15 PUM	Learning how to run the CCHFV minireplicon system and perform a pilot test with antivirals. Dr. Stéphanie Devignot, postdoc in the lab of Professor Friedemann Weber at PUM allowed Stijn to master how to grow and isolate the plasmids from bacterial cultures, transfect cells and collect data. In addition, the first validation antiviral experiments with a small collection of reference compounds was performed to check whether the minigenome system would be a suitable alternative for the live virus in medium-to-high throughput antiviral screening
October - December 2012	partner 14 BGU, technician Amna Abo Alkbash	partner 15 PUM	Training on cell culture and on the CCHFV minireplicon system (growth of plasmids, transfection, acquisition and analysis of data)
August 2012	partner 10 NICD, Scientist Antoinette Grobbelaar	partner 4 UMG-GOE	Training on in sequencing of CCHF virus isolates using 454 genome sequence platform
June 2013	Partner 10 NICD (Dr Jacqueline Weyer), Partner 1 FoHM (Dr Mehrdad Mousavi), Partner 5 AUTH (Dr Anna Papa, Elpida Papadopoupou) and Partner 12 UL (Luka Fajs)	Partner 6 KU Leuven	CCH-fever Antiviral Workshop: during this workshop, a theoretical introduction was given on the development of antiviral assays followed by a wet lab exercise in which the antiviral activity of compounds was evaluated on wild-type and compound-resistant virus

Task 6.b and c: Organizing courses in CCHF clinical, diagnostic and outbreak management designed for European and endemic countries

Course/W-shop 1: Clinical and epidemiology of CCHF. Today there are major gaps in experience and knowledge on clinical diagnostics, prognostic factors, barrier nursing and treatment of CCHF patients in non-endemic areas in Europe. In contrast, huge experience of CCHF from a clinical perspective exists in endemic areas, especially in Turkey, Kosovo and South Africa, where there is a total of more than 1000 cases per year.

The transfer of knowledge between endemic and non-endemic countries is crucial for the success of clinical treatment and preparedness measures. By organising a course on clinical aspects of CCHF, we aimed to fill some of the gaps in this area. During the first day (workshop: CCHF in Europe, Asia and Africa), scientists and public health experts presented the most up-to-date information on epidemiology from the Balkans, Turkey, Central Asia, Middle East and Africa. The workshop started with a keynote by Dr. Nichol (CDC/USA). This followed general aspects of molecular epidemiology of CCHF by Dr. Papa and the situation of ticks in Europe by Dr. Vatensever. The second part of the Workshop was more dedicated on CCHF epidemiology in endemic area in Europe and the rest of the world by expert scientists. Specific attention was devoted to molecular epidemiology and evolution of the virus, as the genetic diversity has a high impact for research strategies on diagnostic tool development, the design of molecular tools for epidemiological and forensic analysis of outbreaks, and the development of effective vaccines. The second day (course: Clinical aspects of CCHF) treated clinical pathogenesis, features, infection control and prevention, case definitions, laboratory diagnosis, prognostic factors and treatment of the patients. A review of clinical features of CCHF was presented by Dr. Nazif Elaldi followed by review of several different and rare clinical cases presented by different clinics in Turkey. Later on Gul Ruhsar Yilmaz presented essential keypoint on infection control and prevention for CCHF. Dr Dilek Yagci Caglayik described the case definition which is used in Turkey for CCHF. The methods and Turkish experience of laboratory diagnosis of CCHF was presented by Dr. Yavuz. Prognostic factors of the disease were explanied by Esragul Akinci. Last speaker of the day was Dr. Hurrem Bodur who reviewed and discussed the treatment of CCHF patients. The event was attended by over 100 participants from the Middle East (Iran, Israel), Europe (Italy, Greece, Albania, Germany, Kosovo, Slovenia, Sweden, Turkey and France), North America (USA) and Africa (South Africa). We had about 90 participants from Turkey, Greece, Bulgaria, Slovenia, Kosovo, Iran, South Africa, Albania, Sweden, Germany, Italy, etc.

Course/W-shop 2: CCH Fever co-organised a course on diagnostic aspects of CCHF for microbiologist and scientist on 4th of September 2012 in Göttingen, Germany together with ArboZoonet. The event was a great success and was attended by 31 participants from the Middle East (Iran, Israel), Europe (Italy, Greece, Albania, Germany, Kosovo, Slovenia, Sweden, Turkey, France) and Africa (West Africa and South Africa). To encourage the exchange of experience and dissemination, key networks and organisations were also invited such as ERINHA, ENIVD, EDEN Next and ECDC. The firstExperts gave lectures on the following topics; i) clinical diagnostics, ii) molecular diagnostics, iii) serological diagnos tics, iv) sample collection, shipment, biosafety issues and waste management, v) commercially available kits and vi) results from an international EQA for CCHFV detection day, assays. On the second day, a technical workshop on CCHF Diagnostic was co-organized with ArboZoonet. The participants had the unique access to clinical samples from an outbreak in Turkey for diagnostic testing in a wetlab. The participant also had access to available kits for both PCR and serology. This is the first initiative to organise a technical workshop on diagnostics for participants from endemic countries with clinical samples. This event gave a unique possibility to expert scientist, physicians and laboratory staff to share their experience and disseminate most-up-to date knowledge.

W-shop/Course3: CCH Fever organised a third course on outbreak management of CCH Fever for epidemiologist, microbiologists and scientists within the field on 22-23rd October 2013 in Tirana, Albania, in collaboration with WHO Europe. The event was a great success and was attended by 30 participants from Europe (Greece, Albania, Germany, Kosovo, Slovenia, Sweden, and Turkey), USA (CDC) and Africa (South Africa). To encourage the exchange of experience and dissemination, key networks and organisations also invited such as ERINHA, ENIVD, EDEN Next and ECDC. Experts gave lectures in following topics: i) Investigation of an outbreak, ii) Risk assessment, iii) Risk matrix, iv) Treatment, v) Infection Control (field and Hospital), vi) Outbreaks versus endemic activities, vii) Crises Communication (Lecture and exercises).

Task 6.d: Sustainably and dissemination of (access to) resources generated by CCH Fever program

We have disseminated the knowledge generated by CCH Fever project thanks to participation to international conferences. The CCH fever consortium has been presented in several meetings, congresses, workshops.
CCH Fever has also organized the 1st International congress of Crimean Congo haemorrhagic Fever on 12-13th February 2015 at Thessaloniki, Greece. The general purpose of this initiative was to give the opportunity to scientists from all around the world to exchange information on all aspects of the CCHF virus. One of the most important tasks was to disseminate the results achieved within the CCH Fever consortium to other scientist. One main goal was also to establish a platform for scientist, health worker and epidemiologist with interest for CCHF both *in veterinary and human medicine* to meet, initiate new programs and collaborations, exchange information. Due to the success of this initiative, the participants agreed to organize the 2nd Conference on Crimean Congo Haemorrhagic Fever virus in September 2017 and the 3rd one in 2019 in Bulgaria. CCH Fever also co-organised a conference on Emerging and Re-emerging Epidemics Affecting Global Health, Orvieto, Italy with ArboZoonet, Society for tropical veterinary medicine, Provect and Prostick.
Within the CCH Fever we have also established a very strong collaboration with other network, International organizations and other European research program:

Network/research program	Activities
ArboZoonet	Joint technical workshop for CCHFV Diagnostic , Joint Conference with Italian society for Virology in Orvieto
ENIVD	Preparation of EQA for CCHFV and Joint action to validate the existing commercial kits for CCHFV
EDEN Next	Joint action to prepare Commercial Diagnostic
EVA	Participate to the W-shops organized by CCH Fever Program
ERINHA	Participate to the W-shops organized by CCH Fever Program
DG Home Affairs	Organization of Biosafety Courses in Göttingen and Barcelona
WHO	Joint Technical w-shop for Outbreak Response
ECDC	Invited as advisory board in CCH Fever meetings

CCH Fever have also linked the activities to the national level by visiting Public Health authorities and also participating to national scientific meeting,

Activities	Participant
Meeting with Minister of Health of Kosovo	CCH Fever steering Committee
Meeting with Head of Public health agency of Turkey	CCH Fever steering Committee
Meeting with Public health agency of Albania	CCH Fever steering Committee
Meeting with Institute for infectious Disease Control, Sweden	CCH Fever steering Committee
Dissemination of the CCH fever activities at National scientific meetings	Partners from Sweden, Germany, Belgium, Greece, Turkey, Bulgaria, Albania, etc.

1.4Potential Impact

The CCH Fever program aimed to build a multidisciplinary research network able to deliver methods and procedures eligible for pre-clinical studies with a special focus on prevention and therapy of this disease. The proposed initiative is designed to benefit society. Thanks to the background available among the consortium participants, we have delivered several tools for countering the threat of this infection in Europe and in endemic areas of the world. This work program attempted to fill gaps in CCHFV virus research on virus-biology, -migration, -evolution and -transmission. The outcome of this multidisciplinary research program have included novel and better diagnostic tools, new and effective screening methodologies for discovery of effective antiviral

compounds, and new vaccine candidates. One key impact of this program has been the integration of research activities between northern, southern and eastern European institutions. The program has also focused on strengthening the research potential, by promoting excellence of research, training and capacity building, and by increasing visibility and awareness of the disease in endemic areas of Europe.

- **Updating the advanced knowledge and creating new tools to counter this disease**

The primary expected impact of this program was an enhanced and upgraded scientific knowledge of various aspects of CCHF disease by developing and applying new scientific tools. By gathering the know-how and experiences from Europe and from endemic areas in the different WPs, the CCH Fever consortium bridged the gap between different disciplines and resources. This had an immediate impact on the achievement of the goals proposed in the program. Based on the outstanding and high quality-focused scientific activity, CCH Fever will ***undoubtedly*** contribute to upgrade the knowledge in this field.

- **Improvement of the field diagnostic resources**

There has been an urgent need for the development of novel, robust, highly sensitive and rapid diagnostic tests to detect the virus and disease. The CCH Fever program have provided methods for initial on-site (point-of care) diagnosis" (e.g CCHFV-antibody detecting lateral flow dipsticks). Using this "first-line" preparedness, spread of infection can be limited and all necessary activities for control and/or eradication can be implemented more quickly.

- **Variability, molecular evolution and migration patterns of CCHFV circulating in endemic area.**

Statistical and advanced bio-informatics analyses from the sequences obtained in this program have built essential tools for monitoring, predicting the movement of virus lineages over time and geographic locations and outbreaks in Europe and neighbouring countries. The outcome of this program should contribute to preparedness and it may also guide health policy decisions of the European community against this disease.

- **Sero-prevalence and neutralizing epitopes**

This program has gained insight into the circulation of CCHFV in European endemic areas (South-eastern Europe and Turkey). Altogether, the outcome of this program has benefited the new strategies to counter the infection.

- **Design of vaccine candidates**

The existing tools within the consortium and novel strategies established in this program has provide the unique possibility to gain an understanding of which vaccine candidates can trigger protective immune responses in animal models. During this program, we have studied several traditional and new vaccine candidate concepts (e.g. VLP) and choose the most promising for future vaccine development.

- **Design of new effective antivirals**

The establishment of new screening methodologies (e.g. reporter cell lines, Minireplicon system, etc.) will create a potential for screening of available antiviral libraries and even designing new compounds. Results and knowledge of this effort will have a big impact on designing strategies to counter CCHFV infection and other infectious diseases focused on in other research program and activities.

- **Creation of a BioBank, sharing and exchanging of clinical data and monitoring disease occurrence**

Access to an aggregated and storage database containing the reported case, clinical data and bio-bank from the patients with CCHF in Europe linked to sequence analysis data (established within the CCH Fever) have provide an opportunity to i) help physicians in their diagnosis and handling of information, ii) validation of new diagnostic tools and iii) design new innovative project by scientists.

- **Creation of tools and new basic knowledge beyond the scope of the project**

Within CCHF Fever project, new tools and knowledge has been generated beyond the scope of this project. These tools and basic knowledge are not available at present, but will ***undoubtedly*** contribute to and facilitate future research efforts (e.g. cell-lines for host factors related to susceptibility to CCHFV replication; new virus isolates; tools for determining the virulence factors).

- **Mobilizing of the capacity and infrastructures of the participating groups from different areas**

In parallel to the enhanced activities mentioned above, the training courses and exchange activities have immediately provided the scientists with enhanced skills and SOPs to enable them to implement the most

advanced techniques. The exchange activities as well as training courses have facilitated the integration of scientists from Europe (Southern, Eastern and Northern Europe), Africa and USA. This has also given scientists the opportunity to use the most advanced facilities (such as BSL-4 infrastructures) and expertise to promote development of new ideas inspired from the different disciplines.

- **Dissemination and linking this consortium to national and international organizations, initiatives and authorities**

Dissemination of the new and aggregated knowledge during the program have supported authorities and entities at national and international levels, to monitor and take action to reduce/prevent the spread of threats in endemic area as well as at the international level. An innovative approach of the CCH Fever project is to build on and integrate with other relevant national and internationally funded projects. The program aimed at integrating the other initiatives (within the neglected and emerging disease call), funded by the European Commission. This will optimized the resources already available at the partner centres and maximize the level of success.

- **Impact on EU-Preparedness**

Natural epidemics and outbreaks of emerging infectious diseases are growing problems internationally, but also within Europe. Events like SARS, Bird Flu, Swine Flu, Ebola in Congo, Foot-and-mouth disease in Great Britain, and Crimean Congo Hemorrhagic fever (CCHF) in Turkey, Greece and Albania are recent reminders.
The new knowledge generated by our activities such as novel prevention strategies, strategies to monitor and predicate virus migration, and field diagnostic will have an impact on the European preparedness to handle, treat and counter the spread of this disease.

1.5Public website and contact

http://www.cchfever.eu/

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2. Use and Dissemination of foreground

Section A (Public)

LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS										
Nº	**Title**	**Main Author**	**Title of the periodical or the series**	**Number, date or frequency**	**Publisher**	**Place of publication**	**Year of publication**	**Relevant pages**	**Permanent identifier** (if available)	**Open access**[2] (Yes / No)
2011										
1	A colorimetric nucleic acid testing assay for RNA virus detection based on circle-to-circle amplification of padlock probes.	Rongqin Ke, Anna Zorzet, Jenny Göransson, Gunnel Lindegren, Batol Sharifi, Sadegh Chinikar, Masoud Mardani, Dan Andersson, Ali Mirazimi and Mats Nilsson.	Journal of Clinical Microbiology	Nr 12, 12 times per Year	ASM press	Washington DC, USA	28/09/2011	4279-85		No
2	Current situation of Crimean Congo hemorrhagic fever (CCHF) in Anatolia and Balkan Peninsula	Uyar Y, Christova I, Papa A	Turkish Bulletin of Hygiene and Experimental Biology	68	Refik Saydam National Public Health Agency (RSNPHA)	Ankara, Turkey	27/09/2011	139-51	http://www.turkhijyen.org/jvi.aspx?pdir=turkhijyen&plng=tur&volume=68&issue=3	Yes

3	Seroepidemiological study of Crimean-Congo hemorrhagic fever in Greece, 2009-2010	Sidira P, Maltezou HC, Haidich AB, Papa A	Clinical Microbiology and Infection	18	Wiley-Blackwell		07/11/2011	E16-19		No
2012										
4	Regulatory, Biosafety and Safety Challenges for Novel Cells as Substrates for Human Vaccines	Hess R, Weber F, Watson K, Schmitt S	Vaccine	30	Elsevier	Amsterdam	17/02/2012	2715-2727		No
5	Healthy individuals' immune response to the Bulgarian Crimean-Congo haemorrhagic fever virus vaccine	Mousavi-Jazi M., H. Karlberg, A. Papa, I. Christova, A. Mirazimi.	Vaccine	Vol. 30, Sept, 2012	Elsevier		2012	6225-6229		
6	Current state of Crimean-Congo hemorrhagic fever in Bulgaria.	Kalvatchev N**,** I. Christova.	Biotechnol.& Biotechnol. Eq.	Vol.26	Diagnosis Press		2012	3079-3085		
7	Diagnostic Assays for Crimean-Congo Hemorrhagic Fever	Jessica Vanhomwegen, Maria João Alves, Tatjana Avšič Županc, Silvia Bino, Sadegh Chinikar, Helen Karlberg, Gülay Korukluoğlu, Miša Korva, Masoud Mardani, Ali Mirazimi, Mehrdad Mousavi,	www.cdc.gov/eid	Vol. 18, No. 12, December 2012			01/12/2012			

		Anna Papa, Ana Saksida, Batool Sharifi -Mood, Persofoni Sidira, Katerina Tsergouli, Roman Wölfel, Hervé Zeller, and Philippe Dubois								
8	Structure of Crimean-Congo hemorrhagic fever virus nucleoprotein: superhelical homo-oligomers and the role of caspase-3 cleavage	Wang Y, Dutta S, Karlberg H, Devignot S, Weber F, Hao Q, Tan YJ, Mirazimi A, Kotaka M	J. Virol	Nov;86	ASM	Washington DC	2012	12294-303		
2013										
9	Seroprevalence of Crimean-Congo Hemorrhagic Fever Virus, Bulgaria	Iva Christova, Teodora Gladnishka, Evgenia Taseva, Nikolay Kalvatchev, Katerina Tsergouli and Anna Papa	www.cdc.gov/eid	Vol. 19, No. 1, January 2013			12/01/2013			
10	Crimean-Congo hemorrhagic fever virus nucleoprotein suppresses IFN-beta-promoter-mediated gene expression	Luka Fajs, Katarina Resman, Tatjana Avšič-Županc	Archives of Virology	12 times per year	Springer	Austria	In press 2013		Arch Virol. 2014 Feb;159(2):345-8.	No
11	Hemorrhagic fever with renal syndrome and Crimean Congo hemorrhagic fever as causes	Christova I., R. Refaat, E. Taseva, T.	Vector-Borne and Zoonotic Diseases	Vo.13			2013	188-192		

	of acute undifferentiated febrile illness in Bulgaria	Gladnishka, I. Trifonova, V. Ivanova, E. Mohareb.								
12	Seroprevalence and risk factors of Crimean–Congo hemorrhagic fever in selected seven provinces in Turkey	Dilek Yagci-Caglayik, Gülay Korukluoglu, Yavuz Uyar	Journal of Medical Virology	DOI: 10.1002/jmv.23699			13 September 2013	http://onlinelibrary.wiley.com/doi/10.1002/jmv.23699/full		No
13	Factors associated with IgG positivity to Crimean-Congo hemorrhagic fever virus in the area with the highest seroprevalence in Greece.	Papa A, Sidira P, Kallia S, Ntouska M, Zotos N, Doumbali E, Maltezou HC, Demiris N, Tsatsaris A	Ticks and Tick Borne Diseases	Sep;4(5)	Elsevier	USA	2013	417-420	doi: 10.1016/j.ttbdis.2013.04.003.	No
14	Prevalence of Crimean-Congo hemorrhagic fever virus antibodies in Greek residents in the area where the AP92 strain was isolated.	Sidira P, Nikza P, Danis K, Panagiotopoulos T, Samara D, Maltezou H, Papa A	Hippokratia	Oct;17(4)		Greece	2013	322-325		Yes
2014										
15	Molecular epidemiology of Crimean-Congo hemorrhagic fever virus in Kosovo.	Fajs Luka, Jakupi X, Ahmeti S, Humolli I, Dedushaj I, Avšič-Županc T.	PLoS Negl Trop Dis	Jan 9;8(1)	PLoS	/	2014	e2647	doi: 10.1371/journal.pntd.0002647	Yes
16	Crimean-Congo hemorrhagic	Papa A, Sidira P,	Emerg Infect	Feb;20(2):			2014	288-90.		

	fever virus, Greece.	Larichev V, Gavrilova L, Kuzmina K, Mousavi-Jazi M, Mirazimi A, Ströher U, Nichol S.	Dis.							
17	The microbial detection array for detection of emerging viruses in clinical samples--a useful panmicrobial diagnostic tool.	Rosenstierne MW, McLoughlin KS, Olesen ML, Papa A, Gardner SN, Engler O, Plumet S, Mirazimi A, Weidmann M, Niedrig M, Fomsgaard A, Erlandsson L.	PLoS One.	Jun 25;	California (US) corporation		2014	9(6):e100813		yes
18	Prevalence of Crimean-Congo hemorrhagic fever virus in healthy population, livestock and ticks in Kosovo.	Fajs Luka, Humolli I, Saksida A, Knap N, Jelovšek M, Korva M, Dedushaj I, Avšič-Županc T.	PLoS One	Nov 13;9(11)	PLoS	/	2014	e110982	doi: 10.1371/journal.pone.0110982	Yes
19	Development and evaluation of a real-time RT-qPCR for detection of Crimean-Congo hemorrhagic fever virus representing different genotypes.	Jääskeläinen AJ[1], Kallio-Kokko H, Ozkul A, Bodur H, Korukruoglu G, Mousavi M, Pranav P, Vaheri A,	Vector Borne Zoonotic Dis.	Dec;14(12)	Mary Ann Liebert, Inc.		2014	870-2		yes

		Mirazimi A, Vapalahti O.								
20	Structural insights into RNA encapsidation and helical assembly of the Toscana virus nucleoprotein.	Olal D, Dick A, Woods VL Jr, Liu T, Li S, Devignot S, Weber F, Saphire EO, Daumke O.	Nucleic Acids Res.	May;42			2014	6025-37		yes
2015										
21	Crimean-Congo hemorrhagic fever: CXCL10 correlates with the viral load.	Papa A, Yagci Caglayık D, Christova I, Tsergouli K, Korukluoglu G, Uyar Y.	Journal of Medical Virology	Feb 3, 2015	Wiley	USA	2015			
22	Recent advances in research on Crimean-Congo hemorrhagic fever.	Papa A.	Journal of Clinical Virology	64 March	Elsevier	USA	2015	137-143		
23	Fatal outcome of coinfection of crimean-congo hemorrhagic Fever and malaria.	Christova I.	Japanese Journal of Infectious Diseases	March 23;68(2)	J-STAGE	Tokyo, Japan	2015	131-134		
24	Crimean-Congo haemorrhagic fever replication interplays with regulation mechanisms of apoptosis.	Karlberg H, Tan YJ, Mirazimi A.	J. General Virology	Mar;96(Pt 3):	The Society for General Microbiology publishes online with the assistance of HighWire Press®.	Great Britanien	2015	538-46		no

25	Virus-like particle system identifies the endonuclease domain of Crimean-Congo hemorrhagic fever virus.	Devignot S, Bergeron E, Nichol S, Mirazimi A, Weber F.	J Virol.	June 1, 89	ASM	Washington	2015	5957-67		no
26	Molecular and serological findings in suspected patients with Crimean-Congo hemorrhagic fever virus in Iran.	Karlberg H, Sharifi-Mood B, Mousavi-Jazi M, Dilcher M, Lindegren G, Mardani M, Bereskly S, Weidmann M, Mirazimi A.	J Med Virol.	Apr;87(4).	John Wiley & Sons		2015	686-93		

A2: LIST OF DISSEMINATION ACTIVITIES

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
1	Presentation	SMI	General meeting of ArboZoonet: CCH Fever presentation	22-24/11-2010	Rabat, Morocco	Scientific community		International
2	Poster	SMI/ IT	CCH Fever Presentation Poster	06/05/2011	ICAR, Sofia, Bulgaria	Scientific community		International
3	Poster	SMI/ IT	CCH Fever Presentation Poster	07/05/2011	ECCMID Milano, Italy	Scientific community	1000	International
4	Workshop	RSNPHA	CCH Fever Workshop: CCHF in Europe, Asia, Africa	19/10/2011	Ankara, Turkey	Scientific community	100	International
5	Clinical Course	RSNPHA	CCH Fever Course: Clinical aspects of CCHF	20/10/2011	Ankara, Turkey	Scientific	100	International

A2: LIST OF DISSEMINATION ACTIVITIES

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
						community		
6	Poster	PUM	Complex anti-interferon action of the ovarian tumor domain of Crimean-Congo Hemorrhagic Fever virus	15/03/2012	22nd Annual Meeting of the Society for Virology, Essen, Germany	Scientific community		Germany
7	Poster	SMI/ IT	CCH Fever Presentation Poster	31/03/2012	ECCMID, London, UK	Scientific community	1000	International
8	Course	UMG-GOE /IT	CCH-Fever and Arbo-Zoonet: Joint course on Diagnostic	04/09/2012	Gottingen, Germany	Scientific community	35	International
9	Workshop	UMG-GOE /IT	CCH-Fever and Arbo-Zoonet: Joint workshop on Diagnostic	05/09/2012	Gottingen, Germany	Scientific community	30	International
10	Presentation	PUM	A virus-like particle system for Crimean-Congo Hemorrhagic Fever virus	20/09/2012	Joint Conference on Emerging and Re-emerging Epidemics Affecting Global Health, Orvieto, Italy	Scientific community		International
11	Presentation	SMI	Pathogenesis of CCHF	20/09/2012	Joint Conference on Emerging and Re-emerging Epidemics Affecting Global Health, Orvieto, Italy	Scientific community		International
12	Presentation	SMI	Replication of CCHFV	22/09/2012	Society of Italian Virology	Scientific community		Italy

A2: LIST OF DISSEMINATION ACTIVITIES

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
13	CCH Fever leaflet	Coris		09/2012	MEDICA Dusseldorf	Scientific community		International
14	Presentation	PUM	A virus-like particle system for Crimean-Congo hemorrhagic fever virus	28/11/2012	Virus-like particle and nanoparticle vaccines, Cannes, France	Scientific community		International
15	Presentation	PUM	A virus-like particle system for Crimean-Congo hemorrhagic fever virus	08/03/2013	23rd Annual Meeting of the Society for Virology, Kiel, Germany	Scientific community		Germany
16	Poster	SMI/ IT	CCH Fever Presentation Poster	30/05/2013	ECCMID, Berlin, Germany	Scientific community	1000	International
17	Poster	NCIPD	Crimean-Congo haemorrhagic fever in Bulgaria	30/05/2013	ECCMID, Berlin, Germany	Scientific community	1000	International
18	Poster	Coris	CCH Fever leaflet	05/2013	ECCMID Berlin	Scientific community	1000	International
19	Presentation	UL	Meeting of the infectious disease specialists of Kosovo, Albania and Macedonia: Genetic diversity of CCHF in Kosovo	30/05/2013	Pristina, Kosovo	Infectious disease specialists	200	International
20	Poster	AUTH/ IPH-CIDD / RSNPHA	Negative Strand Viruses meeting	16-21/06/2013	Granada, Spain	Scientific community	600	International
21	Presentation	PUM	A virus-like particle system for Crimean-Congo hemorrhagic fever virus	13/09/2013	5th European Congress of Virology, Lyon, France	Scientific community		International
22	Presentation	NF-CDC	CCH Fever presentation at the Negative	19/06/2013	Granada, Spain	Scientific	1000	International

A2: LIST OF DISSEMINATION ACTIVITIES

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
			Strand virus Meeting			community		
23	Workshop	Gulay FP7-EU CCHF project	The course in outbreak management of CCHFV	10.10.2013	Tirana	Workshop	50 persons	Albania
24	Congress	Gulay Turkish Microbiology Society	National Clinical Microbiology Association Congress	09-11.11.2013	Antalya	National Congress	500 persons	Turkey
25	Advertisement	Pascal Mertens	Advertisement on the project with the Leaflet as a support during the annual MEDICA fair ; Coris BioConcept Booth	20-23/11/2013	Dusseldorf Germany	Biologists, customers	>100 on our booth	worldwide (mainly europe)
26	Course	BuG	Sorbonne Universites. UMPC-LRS. UMR 7197. Laboratory of Surface reactivity. Peregrinacion around the world developing biosensors.	December 9, 2013.	Ivry-sur-Seine, France			
27	Poster presentation	Stephanie Devignot	Annual Conference of the "Gesellschaft für Virologie"	March 2014	Alpbach, Austria	Virologists	Ca. 600	Mostly from Austria, Switzerland, Germany
28	Oral presentation	Stephanie Devignot	European meeting on viral zoonoses	May 2014	St Raphael, France	Virologists	Ca. 400	Mostly european
29	Poster	FoHM/ IT	CCH Fever Presentation Poster	11/05/2014	ECCMID, Barcelona, Spain	Scientific community	1000	International
30	Course/W-shop	University of Göttinge	Biosafety Training	20-23.05.14	Göttingen/	Biosafety Officer and Scientist	10persons	Germany

A2: LIST OF DISSEMINATION ACTIVITIES

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
		n						
31	Congress	Gulay Turkish Microbiology Society	National Moleculer Microbiology Congress	4-7 June 2014	Ankara	National Congress	300 persons	Turkey
32	International Conference	Marks, R.S.	Peregrination across continents developing tools to monitor environmental water and create rapid medical diagnostics.	17-21 June 2014	Istanbul; Turkey	Nanoscience and Nanotechnology conference		
33	International Conference	Marks, R.S.	2nd Biennale of Influenza Research	22-23 October 2014.	Saint-Petersburg, Russia			
34	Advertisement	Pascal Mertens	Advertisement on the project with the Leaflet as a support during the annual MEDICA fair ; Coris BioConcept Booth	12-15/11/2014	Dusseldorf Germany	Biologists, customers	>100 on our booth	worldwide (mainly europe)
35	Course/W-shop	University of Göttingen	Biosafety Training	25-28.11.14	Göttingen/	Biosafety Officer and Scientist	10persons	Germany
36	Doctoral dissertation	Author: Luka Fajs, Mentor: Tatjana Avsic-Zupanc	Antagonism of type I interferon responses by Crimean-Congo hemorrhagic fever	2014	Ljubljana, Slovenia	Biotechnical faculty	/	/
37	Presentation	Marko Zivcec	PRODUCTION OF RECOMBINANT CCHF VIRUS BY REVERSE GENETICS	February 13, 2015	Thessaloniki, Greece	Scientist and clinicians	50-70	Most CCHF endemic countries
38	Presentation	Jessica Spengler	THE 5'-MONOPHOSPHORYLATED GENOMIC RNA OF CRIMEAN-CONGO HEMORRHAGIC	February 13, 2015	Thessaloniki, Greece	Scientist and clinicians	50-70	Most CCHF endemic

A2: LIST OF DISSEMINATION ACTIVITIES

Nº	Type of activities	Main leader	Title	Date	Place	Type of audience	Size of audience	Countries addressed
			FEVER VIRUS IS RECOGNIZED BY RIG-I					countries
39	Poster	Papa A.	AP-92 like CCHFV strain in ticks collected in Albania	13-14 Febr. 2015	Thessaloniki, Greece	1st Int. Conference on CCHF	80	European countries, USA, S. Africa, Asia
40	Lecture	Papa A.	Round Table: Current Epidemiology of CCHF	13-14 Febr. 2015	Thessaloniki, Greece	1st Int. Conference on CCHF	80	European countries, USA, S. Africa, Asia
41	Oral presentation	Stephanie Devignot	1st International Conference on Crimean-Congo Hemorrhagic Fever	February 2015	Thessaloniki, Greece	Virologists	Ca. 200	International
42	Oral presentation	Friedemann Weber	1st International Conference on Crimean-Congo Hemorrhagic Fever	February 2015	Thessaloniki, Greece	Virologists	Ca. 200	International
43	International conference	Iva Christova	Epidemiology of Crimean-Congo hemorrhagic fever in Bulgaria	13-14 February 2015	Thessaloniki, Greece	Medical doctors, public health specialists, PhD students	100	European countries
44	Course/W-shop	University of Göttingen	Biosafety Training	24-27.03-15	Barcelona	Biosafety Officer and Scientist	10persons	Spain
45	Oral presentation	Stephanie Devignot	Annual Conference of the “Gesellschaft für Virologie”	March 2015	Bochum, Germany	Virologists	Ca. 800	Mostly from Austria, Switzerland, Germany
46	Conference	Robert Marks	National Institute for Communicable Diseases. Special Pathogens Unit.	March 27, 2015.	Johannesburg. South Africa.			

A2: LIST OF DISSEMINATION ACTIVITIES								
Nº	**Type of activities**	**Main leader**	**Title**	**Date**	**Place**	**Type of audience**	**Size of audience**	**Countries addressed**
			Development of medical and environmental biosensors.					
47	International conference	Iva Christova	Investigations on Crimean Congo hemorrhagic fever virus circulation in Bulgaria	March 31-April 2, 2015	Izmir, Turkey	Medical doctors, public health specialists, PhD students	200	European countries
48	Doctoral dissertation	Katerina Tsergouli	Immune response in Crimean-Congo hemorrhagic fever and hemorrhagic fever with renal syndrome					

Section B (Confidential)

Part B1: List of applications for Patents, Trademarks, registered designs

B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS								
Type of IP Rights	**Application reference(s) (e.g. EP123456)**	**Subject or title of application**	**Confidential [1] (Yes / No)**	**Foreseen embargo date** (dd/mm/yyyy)	**Applicant(s) (as on the application)**	**URL of application**	**Status**	**Actions**

Part B2: Exploitable foreground

Type of exploitable foreground	**Exploitable Foreground (description)**	**Confidential YES/NO**	**Foreseen embargo date** (dd/mm/yyyy)	**Exploitable product(s) or measure(s)**	**Sector(s) of application**	**Timetable for commercial use or any other use**	**Patents or other IPR exploitation (licenses)**	**Owner & Other Beneficiary(s) involved**	**Status**	**Actions**
1- LFD for CCHF detection	Rapid test for CCHFV antibody detection	Currently yes	Needs further improvement/validation; no date available	Rapid test for CCHFV antibody detection	IVD (in vitro diagnostics)	Depends on validation results; 2016 or later	No (owning of reagent and manufacture secret)	Coris BioConcept	Late development stage	To be validat
2-VLP-ELISA	Sandwich ELISA for the detection of CCHFV IgM /IgG using VLPs as immobilized antigen	Not yet published, to be decided	Needs further improvement/validation; no date available	Could be developed into a commercial ELISA kit	Diagnostics of CCHFV patients		none	STIR PUM	Late development stage	To be validat
3-VLP	Vaccine	Yes	Needs further	Vaccine	Vaccine		No	Partner 13	Late	Needs furthe

[1] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

	candidate (PUM)		improvement/validation; no date available						development stage	improvemen no date avai
4-DNA Vaccine Plasmid	Vaccine candidate (PUM)	Yes	Needs further improvement/validation; no date available	Vaccine	Vaccine		No	Partner 13	Late development stage	Needs furthe improvemen no date avai

Exploitable Foreground 1 :	Lateral flow serological assay for detection of antibodies against CCHF virus. This assay is developed to fulfill the ASSURED criteria from WHO (affordable, sensitive, specific, user friendly, robust and rapid, equipment free, and deliverable to those in need). The aim of this test is to be performing in primary health care settings as well as directly in endemic villages for screening or diagnostic purpose.
Exploitable Foreground 2 & 3:	The VLP system allows studying virus particle attachment, entry, transcription, replication and particle assembly and release under relaxed biosafety conditions. Also, antiviral drug discovery, production of ELISA antigens, and testing of neutralizing antibodies can now be done under BSL2 conditions. This is of broad interest for the CCHFV and bunyavirus researcher community, and for developers of diagnostic tests.
Exploitable Foreground 4 :	DNA Plasmid expressing Gn, Gc and Np pCMV_CCHFV_N (encodes CCHFV N), pCMV_Ub_CCHFV-N (encodes ubiquitin-tagged CCHFV N), pCMV_CCHFV_Gc (encodes CCHFV Gc), pCMV_Ub_CCHFV-Gc (encodes ubiquitin-tagged CCHFV Gc), pCMV_CCHFV_coGc (encodes CCHFV Gc, codon-optimized sequence), pCMV_Ub_CCHFV-coGc (encodes ubiquitin-tagged CCHFV G, codon-optimized sequence), pCMV_CCHFV-Gn (encodes CCHFV Gn), pCMV_Ub_CCHFV-Gn (encodes ubiquitin-tagged CCHFV Gn), pCMV_CCHFV-coGn (encodes CCHFV coGn, codon-optimized sequence), pCMV_Ub_CCHFV-coGn (encodes ubiquitin-tagged CCHFV Gn, codon-optimized sequence). Our experiments demonstrate that 100% protection from a lethal CCHFV challenge is possible with DNA vaccines. We also show that VLPs elicit neztalizing antibodies and can delay the onset of infection in IFN-deficient mice. Ongoing studies will clarify whether wt mice produce protective levels of neutralizing antibodies, and whether the immunogenicity of VLPs can be increased using an OTU-deficient L polymerase.

3. Report on Societal implications

See on the Participant Portal.