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3 **2019 meeting of the Global Virus Network**
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6 **Ramesh Akkina^a, Robert Garry^b, Christian Bréchet^c, Heinz Ellerbrok^d, Hideki Hasegawa^e, Luis**
7 **Menéndez-Arias^f, Natalia Mercer^c, Johan Neyts^g, Victor Romanowski^h, Joaquim Segalésⁱ,**
8 **Anders Vahlne^{j,c}**
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10
11 ^aColorado State University. Microbiology, Immunology and
12 Pathology, USA
13
14

15 ^bTulane University, USA
16
17

18 ^cGlobal Virus Network, Baltimore, MD, USA
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20

21 ^dRobert Koch Institute. Center for International Health
22 Protection, Germany
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24

25 ^e National Institute of Infectious Diseases. Department
26 of Pathology, Japan
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30 ^fCentro de Biología Molecular Severo Ochoa, Spain
31

32 ^gRega Institute for Medical Research, University of Leuven,
33 Belgium
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35

36 ^hUniversidad Nacional de La Plata. IBBM, Facultad de
37 Ciencias Exactas, Argentina.
38
39

40 ⁱIRTA-CReSA, Spain
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43 ^jKarolinska Institutet, Stockholm, Sweden
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47
48 Corresponding author:

49 Anders Vahlne, Division of Clinical Microbiology, Karolinska Institutet, Stockholm, Sweden,
50 Anders.Vahlne@ki.se
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54 Authors have been ordered alphabetically.
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64 **Abstract**

65 The Global Virus Network (GVN) was established in 2011 to strengthen research and responses
66 to emerging viral causes of human disease and to prepare against new viral pandemics. There
67 are now 52 GVN Centers of Excellence and 9 Affiliate laboratories in 32 countries. The 11th
68 International GVN meeting was held from June 9–11, 2019 in Barcelona, Spain and was jointly
69 organized with the Spanish Society of Virology. A common theme throughout the meeting was
70 globalization and climate change. This report highlights the recent accomplishments of GVN
71 researchers in several important areas of medical virology, including severe virus epidemics,
72 anticipation and preparedness for changing disease dynamics, host-pathogen interactions,
73 zoonotic virus infections, ethical preparedness for epidemics and pandemics, one health and
74 antivirals.
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82 **1. Introduction to the GVN**

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85 The Global Virus Network (www.gvn.org) is a global coalition of leading virologists. Founded in
86 2011, the not-for-profit organization GVN has grown to 52 Centers of Excellence and 9 affiliate
87 institutions in 32 countries throughout the world.
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89 The GVN mission is to strengthen research and response to current viral causes of human
90 disease and to prepare for new viral pandemic threats through the collaboration of a global
91 network of expert virologists. The GVN activities are mainly based on virology research,
92 training and advocacy.
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95 The GVN International meetings is one way to support its mission, providing a framework
96 where global junior and senior virologists can connect, discuss and establish new
97 collaborations and advance the field.
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100 Past GVN International meetings have taken place in Washington DC, USA and Dublin, Ireland
101 (2011), Naples, Italy and Baltimore, MD, USA (2012), Munich, Germany and Moscow, Russia
102 (2013), Beijing, China (2015), Sapporo, Japan (2016), Melbourne, Australia (2017), and France
103 (2018).
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106 The 2019 GVN International meeting took place in Barcelona, Spain, June 9-12 and was jointly
107 organized with the Spanish Society of Virology. The meeting had 311 delegates from 22
108 different countries. A common theme throughout the meeting was globalization and climate
109 change. The 11th GVN International meeting gave GVN participants an opportunity to discuss
110 recent findings in virology, thus providing a platform for collaboration and networking,
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119 particularly for the high number of participating young Spanish virologists. The GVN Sessions
120 will be outlined in the present report.
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124 In addition to plenary lectures and workshops that were held at the SEV-GVN joint meeting,
125 contributions in the format of short oral presentations were arranged to include a wide
126 spectrum of issues addressed by member-scientists of GVN. Poster sessions allowed an
127 interesting discussion among essentially younger scientists from Spanish laboratories and
128 participants from other countries. With this perspective for the first time GVN established
129 travel grants that consisted of partial financial help to encourage participation of younger
130 members of GVN centers. In contrast to previous GVN meetings (4-5 oral presentations on
131 related subjects in each block) sessions were arranged as a series of talks that covered a quite
132 diverse repertoire of issues of interest to virologists around the world.
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141 **2. The 2019 Robert C. Gallo award for scientific excellence and leadership.**

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143 Criteria for the selection of this award include: 1. The candidate has published important
144 scientific information on virology in the areas of interest to the GVN, including but not limited
145 to: basic science, clinical aspects, pathogenesis, epidemiology, diagnostics, antivirals, and
146 vaccine development. 2. The candidate has made a consequential and meaningful contribution
147 to the GVN and has furthered the mission of the GVN, including but not limited to;
148 development of the network of Centers of Excellence, participation in training programs,
149 contributions to meetings and other GVN activities, and contributions to advocacy and public
150 communication activities.
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156 The 2019 Robert C. Gallo award for scientific excellence and leadership was awarded to Dr.
157 William Hall, GVN Co-founder.
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160 **3. Scientific presentations**

161 **3.1 Anticipation and preparedness**

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166 **Scott C Weaver** (University of Texas Medical Branch, USA) gave an overview on
167 the history and the distribution of Zika virus (ZIKV). He summarized the recent
168 epidemic of ZIKV in the Americas and depicted the association between ZIKV and
169 microcephaly that was established late in 2015. He then illustrated the reaction of
170 GVN with the assembly of a global Zika Task Force that was established within
171 three months after the association between ZIKV infection and microcephaly was
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180 recognized. This task force gathered experts on flaviviruses, arboviruses, and viral
181 congenital diseases from 13 GVN centers from 13 countries.

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183 Besides communication of the latest information on ZIKV and the distribution of
184 expert reviews (Weaver et al., 2016) the task force, which was supported by a
185 private donation, established the GVN ZIKV Serum Bank. This serum bank
186 collected and characterized more the 100 sera from donors who had been
187 exposed in different countries all across South and Latin America. They made this
188 collection of highly characterized sera available to international expert labs by
189 providing lyophilized aliquots. They further provided a collection of ZIKV strains,
190 different virus antigens, and two cDNA infectious clones.

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192 Weaver also reported on ZIKV vaccine strains that were attenuated through
193 deletions in the 3'UTR. As a DNA-launched ZIKV live-attenuated vaccine, having a
194 20-nucleotide deletion, this mutant virus gave rise to high antibody titers after a
195 single immunization and was highly protective in a ZIKV mouse model, as well as,
196 in a rhesus macaque infection model (Zou et al., 2018).

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204 **Núria Busquets** (Centre de Recerca en Sanitat Animal, Spain) highlighted the
205 importance of arboviruses as pathogens and focused on the surveillance of
206 vectors and animal reservoirs. She gave a short overview on the emergence of
207 arboviruses which over the last 40 years has become an important global public
208 health threat causing significant morbidity and mortality among humans and
209 animals. While for Chikungunya, Dengue, and Zika virus carrying mosquitos easily
210 can spread also other arboviruses, e.g. Rift Valley Fever virus (RVFV), and West
211 Nile virus (WNV), there is a critical role for vertebrate hosts as animal reservoirs.
212 Dr. Busquets stressed that good surveillance data are the base for intervention
213 strategies thus helping to protect public health and to save money. Thus, detailed
214 knowledge on which mosquito species are competent hosts for the virus and
215 where and when these mosquitoes are present, as well as, on which the
216 vertebrate hosts are the reservoir and their presence are important. So is the
217 knowledge on the kinetics of infection, viremia and virus shedding, the immune
218 response and last but not least good, reliable, and rapid diagnostic tools. She
219 pointed out that virus circulation in vectors and in animal reservoirs precedes
220 human exposure and infection. During an arbovirus outbreak in humans, clinical
221 signs might only be detected when the peak of an outbreak has already been
222 passed. However, virus circulation in vectors and the animal reservoir will precede
223 human infections (Reusken et al., 2018). Therefore, early detection in vectors and
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239 animal reservoir hosts can serve as an early warning system to mitigate economic
240 and human health impact. How important the interplay between different
241 information sources is was demonstrated in 2015 – 2016 when the combination
242 of weather forecast and syndromic animal surveillance during El Nino rains in
243 Kenya resulted in an effective early warning for a major outbreak of RVFV, thus
244 allowing precautions to prevent spill over to humans (Oyas et al., 2018).

248 As a second example she presented the WNV outbreak on the American continent
249 starting in New York City in 1999 (CDC, 1999) and the emergence of WNV lineage
250 2 in Spain in 2017 (Napps et al., 2019). In both cases dead bird surveillance and
251 identification of equine infections was an important for WNV surveillance as an
252 early warning system. Dr. Busquets concluded that arbovirus surveillance provides
253 a realistic picture of the epidemiological situation and allows for monitoring the
254 effectiveness of intervention measures. Surveillance in vectors and in vertebrate
255 hosts enables detection of arbovirus circulation before clinical onset in humans.
256 However, countries need to adapt their surveillance scheme to their
257 epidemiological situation, surveillance objectives and capacities. Effective early
258 warning requires the interaction between multiple disciplines like entomologist,
259 veterinarians, biologists, clinicians, epidemiologists, etc.

267 **Masao Matsouka** (Kumamoto University, Japan) gave a detailed insight into
268 aspects of the strategy and pathogenesis of Human T-cell leukemia virus type 1
269 (HTLV-1). In his presentation he focused on two viral proteins, the transactivator
270 of viral gene expression (Tax) and the HTLV-1 bZIP factor (HBZ). HTLV-1 is the
271 causal agent of adult T-cell leukemia (ATL) and HTLV-1-associated
272 myelopathy/tropical spastic paraparesis (HAM/TSP) in adults. It has close relatives
273 in several non-human primates and is an important human pathogen with a global
274 distribution of infection foci and an estimated 10 million people are infected
275 (Sonoda et al., 2011). HTLV-1 is transmitted only through cell-to-cell contact
276 passing the virus from mother to infant through breast milk and from male to
277 female through semen. Therefore, living infected cells are essential for
278 transmission and in order to enhance transmission the virus increases the number
279 of infected cells. While the cellular receptor for HTLV-1 is ubiquitous (Manel et al.,
280 2003) the provirus is mainly detected in CD4⁺ effector/memory T cells *in vivo*.
281 However, it was not clear if HTLV-1 preferentially infects these cells or if infected
282 precursor cells differentiate into these cells. Analyzing tax expression as a marker
283 for viral replication in HTLV-1 infected Japanese macaques as a model for HTLV-1
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298 infection in humans, Matsouka and colleagues showed that elevated tax
299 expression is mainly seen in the bone marrow suggesting *de novo* infection of
300 hematopoietic stem cells (HSCs). Further, in HAM/TSP patients they detected
301 identical integration sites of HTLV-1 proviruses in neutrophils, monocytes, B cells
302 CD8⁺ T cells and CD4⁺ T cells indicating that these cells are all derived from HTLV-1
303 infected HSCs *in vivo*.
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307 While Tax has an important role in the upregulation of anti-apoptotic genes and
308 the maintenance of cell populations it is only expressed transiently, most likely
309 because the Tax protein is highly immunogenic and a major target for CTL. In
310 leukemic cells Tax is expressed only in a minor fraction of the cells and only for a
311 short period of time (Mahgoub et al., 2018). Matsouka then concentrated on the
312 important role of HBZ in HTLV-1 pathogenesis. HBZ is encoded on the antisense
313 strand of the provirus and has a low immunogenicity. In contrast to Tax it is
314 expressed constantly in all ATL cells. HBZ regulates several different pathways in
315 infected cells, enable immune escape, trigger inflammation through the induction
316 of IFN γ expression, and enable the infection to overcome immune suppressive
317 effects. HBZ also induces expression of the chemokine receptor CCR4 allowing
318 infiltration of infected T-cells into tissues. Seen that HTLV-1 is transmitted through
319 cell-to-cell contact he suggested that HBZ-induced CCR4 expression enables HTLV-
320 1 infected cells to migrate into breast milk and into semen, thus allowing efficient
321 transmission of the virus between humans.
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331 **Marion Koopmans** (Erasmus MC, Netherlands) elaborated in her presentation on
332 the complex interplay between man-made disturbances of ecosystems and the
333 resulting increase in number and amplitude of emerging viral disease outbreaks.
334 She presented several examples to underline how changes in environmental
335 conditions and in human behavior can create conditions that result in an increase
336 of pathogen transmission, spill-over to new hosts, broad dissemination of
337 infection, and changes in viral properties. The recent Ebola outbreak in West
338 Africa and the ongoing outbreak in the Democratic Republic of Congo illustrate
339 how demographical and political changes can affect spreading of diseases.
340 Dr. Koopmans emphasized the important role and the potential of surveillance by
341 presenting preliminary results on arbovirus surveillance from the ARBO study and
342 data from an influenza market surveillance in poultry in China (Bai et al., 2019).
343 She also pointed out a potential role for co-circulation of different viruses, e.g.
344 Zika and dengue for new pathogenesis pathways (Langerak et al., 2019). Shifting
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357 the burden of disease detection from clinicians to early detection and forecasting
358 of spillover through surveillance of reservoir hosts would mean a paradigm
359 change in the surveillance of emerging infectious diseases from reactive to pro-
360 active measures. Early detection allows timely control and thus mitigates the
361 number of human cases (Karesh et al., 2012). Matching these surveillance data
362 with data on demographic development, global travel and trade, and climate
363 change might allow to predict potential international spread of pathogens. The
364 utility of such approaches will very much depend on a switch from the detection
365 of single pathogens to an “open view” surveillance with multiplex assays, broad
366 antibody profiling, metagenomics approaches, and an efficient way of data
367 sharing.

373 374 375 **3.2 Host-pathogen interaction.**

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379 **Thomas Rasmussen**, a Senior Researcher at the National Veterinary Institute of Virology in
380 Australia, presented an update on the potential use of latency-reversing agents (LRAs) for HIV
381 elimination. The great success of antiretroviral therapy (ART) for HIV Infection is tempered by
382 the fact that treatment must be maintained for life. Rasmussen reviewed work showing that
383 HIV can remain in a latent state where the immune system or ART cannot eliminate the virus.
384 Rebound viremia occurs when ART is stopped even in individuals with no detectable virus in
385 cells or plasma. An experimental approach to clear latent HIV and thus cure HIV infection has
386 been dubbed “shock and kill.” LRAs are used to activate latent HIV allowing the reactivated
387 cells to be targeted and killed by ART and/or the immune system. Administration of LRAs alone
388 have thus far not demonstrated an effect on the frequency of latently infected cells or the time
389 to virus rebound following interruption of ART (“shock, no kill”). For example, in the DIORR
390 trial (Dolutegravir Intensification Effect On Residual virus Replication on ART) lead by
391 Rasmussen there was no change in levels of HIV DNA or RNA. Negative results where
392 mechanisms for failure are carefully examined are important because they can demonstrate
393 which strategies will not work and provide guidance to strategies that do work. Elimination of
394 latently infected cells (particularly those that are long-lived) may require HIV-specific CD8+ T
395 cell effector functions similar to those of so-called elite controllers of HIV-1 infection. Various
396 strategies to boost anti-HIV immunity currently under development including therapeutic
397 vaccines, toll-like receptor agonists, broadly neutralizing antibodies, immune checkpoint
398 inhibitors, interferon- α and interleukin therapy should be investigated. Immune checkpoint
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416 blockade could also play a role in developing an HIV cure by re-invigorating exhausted T cells
417 and potentially reversing HIV latency in CD4+ T cells.
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421 Infection by mosquito-borne viruses has increased over the past few decades due to
422 globalization, increase in societal mobility and climate change. **Juana Diez** from the Universitat
423 Pompeu Fabra in the lovely host city of Barcelona continued the session by exploring how
424 viruses such as Chikungunya virus (CHIKV) are able to express their genomes at high levels in
425 cells from diverse species. The four bases of the genetic code create 64 codons, of which 61
426 are translated into 20 amino acids and 3 are stop codons. Synonymous codons differ
427 principally at the third base or wobble position. Different species, including mosquitoes and
428 humans, vary widely in which codons are used for protein translation. Dr. Diaz and her team
429 integrated subcellular fractionation and transcriptome-wide analyses of translation in human
430 cells, to show that CHIKV infection induces a host adaptation to viral codon usage into the
431 endoplasmic reticulum (ER), the preferred site of CHIKV protein expression. The tRNA
432 modification enzyme methyltransferase 9 (KIAA1456) methylates uridine in the wobble
433 position and enhances speed of translation of certain mRNAs, including CHIKV mRNAs.
434 KIAA1456 mRNA is translationally activated up to 40-fold by CHIKV infection. Overexpression
435 of KIAA1456 increases CHIKV replication and KIAA1456 mRNA silencing decreases replication.
436 These findings demonstrate an unexpected interplay of viruses with the host tRNA
437 epitranscriptome that favors viral protein expression. This mechanism appears to be conserved
438 among viruses that replicate effectively in more than a single host species, which opens the
439 intriguing possibility of future exploitation of a broad-spectrum antiviral target.
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451 **3.3 Ebola virus, viral protein corona and arboviruses**

452 The ebolaviruses cause highly lethal disease with sporadic and unpredictable outbreaks. An
453 ongoing outbreak in Democratic Republic of the Congo is the second largest in history. While
454 the genome of Ebola virus encodes just seven proteins, the deleterious effects on the human
455 body are immense causing severe pathology and fatalities. Thus, basic research is needed to
456 understand the viral protein conformations and their role in viral replication. **Sara Lenderas**
457 **Bueno** from the Scripps Research Institute explained that several of these proteins are
458 multifunctional in the viral cycle, and some of these are also multi-structural, adopting
459 different forms at different times to mediate different, essential functions. Using
460 crystallography, electron microscopy and biochemistry, it was found that two essential
461 proteins in infection; the nucleoprotein NP and the matrix protein VP40 display different
462 conformations. NP is involved in viral nucleocapsid formation and facilitates viral transcription
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475 and replication. To avoid premature self-polymerization and non-productive binding to cellular
476 RNAs, NP is chaperoned by the viral phosphoprotein VP35. NP complexing with the amino-
477 terminal portion of VP35 was delineated at 2.3 Å resolution and electron microscopy indicated
478 the importance of this binding interaction to control NP polymerization. Structure-directed
479 mutagenesis studies identified conserved critical residues in the NP-VP35 interface which
480 could be targeted by broadly effective antivirals. With regard to the matrix protein VP40,
481 another multi-structural protein, it rearranges into three alternative structures, each with its
482 corresponding function at different stages of the life cycle. How these structural changes are
483 triggered has remained unclear. New data revealed that the binding of the VP40 dimer to
484 specific nucleotide sequences is enough to form the octameric structure/form responsible for
485 regulation of viral RNA replication. These findings provide the molecular basis for the
486 multifunctionality of these proteins and reveal attractive targets for therapeutic intervention.
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488 **Kariem Ezzat** from Stockholm University presented tantalizing data on the role of viral protein
489 corona in viral pathogenesis and amyloid aggregation. Viruses rely on the intracellular host
490 machinery for replication, production of viral proteins and assembly. However, outside cells,
491 viruses share many biophysical properties with nanoparticles. Based on these features, viruses
492 have the capacity to accumulate a host-derived protein corona layer in extracellular
493 environments similar to nanoparticles. To evaluate this possibility and its implications, protein
494 corona layers of respiratory syncytial virus (RSV) and herpes simplex virus 1 (HSV-1) in different
495 biological fluids such as human plasma and bronchioalveolar lavage fluid were analyzed using
496 proteomics, electron microscopy, infectivity, and dendritic cell activation assays. It was found
497 that RSV and HSV-1 accumulate rich protein corona layers that are unique for each biological
498 fluid and corona pre-coating differentially affects viral infectivity and immune cell activation. In
499 addition, like nanoparticles, viruses should be able to function as nano-surface catalysts that
500 enable accelerated extracellular amyloid protein aggregation. The authors also showed that
501 HSV-1, which has been implicated in Alzheimer's disease, catalyzes the nucleation and
502 accumulation of the A β 42 peptide both *in vitro* and *in vivo*. Results from Ezzat et al. 2019
503 showed that unlike the viral genome coded surface proteins, the viral protein corona is an
504 acquired structural layer that is dependent on the viral microenvironment resulting in different
505 viral identities based on the target tissue and the target organism. Additionally, the viral
506 corona-driven heterogeneous nucleation of amyloids illustrates convergence between viral
507 and amyloid pathologies suggesting a direct physical mechanistic link that warrants further
508 investigation.

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510 **Ana Isabel Nunez** from IRTA-CReSA, Barcelona, Spain, discussed novel findings on mosquito
511 molecular responses to arbovirus infection, particularly related to Rift Valley fever phlebovirus
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534 (RVFV) and *Culex pipiens*. In the literature, the *Aedes aegypti*-Dengue virus (DENV)
535 combination is the most studied in terms of gene expression. However, there is a general
536 paucity of information on mosquito genes involved in vector competence and immune
537 responses with regard to other important vector-virus interactions. RVFV causes an emerging
538 significant public health zoonotic disease which is commonly transmitted mainly by the *Culex*
539 and *Aedes* genus mosquitoes. In her talk, molecular responses of *Culex pipiens* to RVFV
540 infection were presented, particularly those related to genes implicated in the innate
541 immunity pathways (Toll, IMD, JAK/STAT) and RNAi. A total of 445 differentially expressed
542 genes (DEG) were identified. The gene expression profiles varied at different days post
543 infection. A total of 445 DEG were found wherein 42 DEG were immune function related.
544 Among these genes, some are involved in innate immunity pathways; Cactus or Defensin-A in
545 the Toll pathway or Piwi4 and Drosha in the RNAi pathway. Specifically, three immune
546 pathways Toll, IMD and RNAi and apoptosis were affected by RVFV infection. Conversely,
547 JAK/STAT pathway seems not to be involved in *Culex pipiens* response to RVFV. Toll and Imd
548 pathways are suppressed after infectious blood feeding, for example AMP (Defensin-A) was
549 down-regulated. The RNAi pathway was mainly down-regulated in the course of the RVFV
550 infection. All these immune system responses would allow the establishment of the RVFV
551 infection in *Culex pipiens* mosquitoes. These results form a basis for future in depth studies to
552 better understand the functionality of immune related DEG in relation to vector competence
553 to develop new strategies for vector control programs.

565 **Ken Olson** from Colorado State University reviewed the RNAi and arbovirus interactions. Such
566 infections in *Aedes aegypti* allows transmission of yellow fever, dengue, Zika, and chikungunya
567 viruses throughout the mosquito's lifetime. The mechanisms of viral persistence in
568 mosquitoes, which involves the production of virus RNA-derived siRNAs and piRNAs, are not
569 well understood. The RNA interference pathways involve double stranded RNAs that degrade
570 target RNAs and mediate gene regulation. In his studies, siRNA and piRNA product depletion,
571 small RNA sequencing, piRNA product expression profiles, immunoprecipitation, and arbovirus
572 assays were used to dissect the viral and host-cell interactions. It was found that the Piwi-
573 family protein Piwi4 has antiviral activity in *Aedes aegypti* Aag2 cells and in mosquitoes
574 infected with arboviruses and insect-specific flaviviruses. Although these RNA viruses encode
575 no reverse transcriptase, circular episomal DNA in arbovirus-infected *Aedes aegypti* cells
576 consisting of hybrid sequences of arbovirus-derived cDNA (vDNA) and retrotransposable
577 elements were found. These episomal DNAs appear to be acquired during reverse-
578 transcription by a discriminatory process of vDNA recombination with retrotransposons.
579 Transcripts from vDNA may serve as precursors for antiviral vpiRNAs. Integrated viral-derived
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593 (vDNA) can also be detected in the mosquito genome as endogenous viral elements (EVEs)
594 that are often associated with piRNA clusters in the mosquito genome. EVEs are transcribed to
595 produce piRNAs that associate preferentially with Piwi4. Importantly, EVE-derived piRNAs can
596 inhibit the replication of a cognate virus. These findings suggest that the *Aedes aegypti* Piwi
597 family of proteins and episomal vDNA, and EVEs provide a means of moderating viral load in
600 mosquito cells and a potential mechanism for transgenerational virus tolerance in the
601 mosquito.

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603 **Richard Zhao** from the University of Maryland presented data on the virologic differences in
604 severity between historical and epidemic Zika virus mediated infection and neurocytotoxicity.
605 The 2015 Zika virus (ZIKV) outbreak in the Americas have had a severe impact as it in Brazil
606 alone left >3000 babies with ZIKV-associated neurological disorders (ZAND) including
607 microcephaly. Even though the causal relationship between the ZIKV and ZAND have been
608 confirmed, the reasons why the ZIKV suddenly became so pathogenic and caused ZAND in
609 humans remain largely unknown. To help answer this question, the virologic differences and
610 the underlying molecular mechanisms between the representative historical African MR766
611 ZIKV strain and the epidemic Brazilian BR15 ZIKV strain were examined. Glioma SNB-19 cell line
612 and 3-D neurospheres were used to evaluate both primary and chimeric viruses. Notable
613 differences were found between strains with regard to viral attachment, permissiveness and
614 replication, as well as, the induction of neurocytopathic effects in host neuronal cells. Chimeric
615 virus analyses suggested that the ZIKV E protein correlates with the viral attachment, and the
616 C-prM region contributes to the permissiveness and ZIKV-induced cytopathic effects.
617 Furthermore, the prM protein and its cleaved Pr product, but not the mature M protein,
618 induces apoptotic cell death in the SNB-19 cells. The Pr region, which resides on the N-terminal
619 side of prM protein, is responsible for prM-induced apoptotic cell death. Mutational analysis
620 further identified four amino-acid residues that have an impact on the ability of prM to induce
621 apoptosis. These findings suggest that functions of the structural prM-E proteins contribute in
622 part to the difference in ZIKV-mediated viral pathogenicity between the historic and epidemic
623 strains. Ongoing studies are likely to identify the role of other viral proteins with regard to
624 neuropathogenesis.

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627 **Ramesh Akkina** from Colorado State University reported on the ZIKV mediated pathology on
628 human hematopoietic cells. While many previous studies have focused on ZIKV viral effects on
629 the CNS, few have explored the viral effects on the human immune and hematopoietic system.
630 Dr. Akkina presented both *in vitro* and *in vivo* studies conducted on human cells. *In vivo* studies
631 involved humanized mice (hu-mice) that harbor a transplanted human immune system. These
632 mice generate human T cells, B cells, NK cells, monocytes and macrophages, as well as,
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652 dendritic cells that orchestrate both humoral and cellular immune responses and thus are ideal
653 models to examine viral effects and interactions with human immune cells (Akkina, 2013).
654 Since the hu-mice also harbor human hematopoietic stem cells in the bone marrow, viral
655 effects on these cells can also be ascertained. Recent studies (Schmitt et al, 2018) has shown
656 that ZIKV infection of hu-mice can generate a human neutralizing antibody response to ZIKV
657 and also that human B cells and CD34 HSC can be infected. In studies presented at the
658 meeting, further investigations were carried out on the ZIKV infection on human B cells *in vitro*
659 and *in vivo* to determine any adverse effects such as cell death or cell dysfunction, as well as,
660 viral persistence. Viral exposure of mature naïve B cells resulted in the loss of one subset and
661 aberrant proliferation of another. Upregulation of markers indicative of B cell progression into
662 the plasmablast stage was seen in the expanding subset. *In vivo*, results from ZIKV infected hu-
663 mice showed the presence of ZIKV+ B cells in the periphery during acute infection and later in
664 bone marrow during the chronic stage. It was also found that CD34+ HSC were susceptible to
665 ZIKV infection *in vitro* and could be detected in the bone marrow of infected hu-mice. B cell
666 loss can lead to delayed viral clearance, whereas aberrant B cell activation may have
667 implications in the pathogenesis of autoimmune diseases such as Guillain-Barré syndrome.
668 Infection of CD34+ HSC has implications for hematopoietic cell differentiation and viral
669 persistence.

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680 Currently several murine models of ZIKV neuropathogenesis exist. Since standard mice are not
681 susceptible to ZIKV, mostly IFN deficient mice that lack an innate immune system and thus
682 permitting ready viral infection are used. A significant drawback with these models is that viral
683 infection leads to rapid fatalities, which is not a common feature with ZIKA. In the studies
684 presented BALB/c RAG2-/- γ c-/- mice (also known as BRG mice) with intact IFN pathway were
685 used. Neonatal mice when exposed to ZIKV developed severe microcephaly and other
686 important CZS features such as eye deformations and stunted growth. This model is likely to
687 be useful as an experimental neonatal ZIKV infection model. With regard to human cell
688 infections, these findings highlighted the utility of hu-mice as valuable human surrogate
689 experimental system to directly assess ZIKV effects on these cells *in vivo*.

690 691 692 693 694 695 696 697 **3.4 Preparedness for zoonotic infections, emerging infectious diseases and ethical review,** 698 **and vaccine take in the elderly**

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702 **Christopher Kratochvil**, co-director of Global Center for Health Security (GCHS, University of
703 Nebraska) presented the mission and resources of the center, which is an initiative of the
704 University of Nebraska Medical Center (UNMC) with the purpose of leading US domestic and
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709 global preparedness for emerging infectious diseases (EIDs)

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711 (<https://www.unmc.edu/iexcel/global-center/index.html>). Since its foundation in 2017 it has
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713 become a primary biosecurity resource for training, education, research, and clinical care and
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715 for advancing international capacity and innovation to prevent and mitigate the effects of EIDs
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717 and other public health emergencies. The program's successes have been built upon a
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719 foundation of robust partnerships with diverse governmental and academic
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721 organizations (Kratochvil et al., 2017; Eitzen et al., 2019).

722 The GCHS has numerous resources, including a clinical biocontainment unit, a quarantine unit,
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724 a training and simulation center which includes a simulated biocontainment unit, as well as,
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726 diverse laboratory and clinical resources from across the academic health center.

727 Strategic partnerships with the Assistant Secretary for Preparedness and Response (ASPR), the
728
729 Centers for Disease Control and Prevention (CDC), the Department of Defense, and other
730
731 academic health centers, have resulted in multiple collaborative initiatives. Examples include
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733 the National Ebola Training and Education Center (NETEC), the Special Pathogens Research
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735 Network, the U.S. Air Force C-STARS training program, the Federal Quarantine Center, a
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737 National Disaster Medical System training program, and a central institutional review board
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739 (IRB) specializing in preparedness, as well as, rapid response (NETEC, 2019). The center is open
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741 to global collaboration, which already includes Singapore, Germany, South Korea, and China.
742
743 Among the relevant events Dr. Kratochvil mentioned recent workshops and training courses
744
745 and the anticipated 2020 FDA Course "Achieving Data Quality and Integrity in Clinical Trials
746
747 Involving High Consequence Pathogens" at the Davis Global Center

748 **Jordi Rodon** (Food & Agriculture Science and Technology Center IRTA; Center for Animal
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750 Health Research CReSA, Barcelona, Spain) presented a suitable a new model to study early
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752 events of Middle East respiratory syndrome coronavirus (MERS-CoV) infections (van
753
754 Boheemen et al., 2012; Cotton et al., 2014)

755 MERS, a previously unreported zoonotic disease emerged in 2012. As of May 2019, World
756
757 Health Organization has been informed of more than 2,000 laboratory-confirmed human cases
758
759 including almost 838 fatalities (Ramadan and Shaib, 2019). It is endemic in the Middle East and
760
761 human cases have been reported in 27 countries. Symptoms range from asymptomatic to very
762
763 severe pneumonia with acute respiratory distress syndrome (ARDS), septic shock and
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765 multiorgan failure. No vaccines are commercially available nor have specific treatments been
766
767 developed.

768 Dromedaries are the natural reservoir, but alpacas have also been reported as potential hosts
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770 for MERS-CoV (Chan et al., 2014). Since handling dromedary camels is not an easy option,
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772 especially under biocontainment conditions, and based on preliminary data showing that

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769
770 alpacas is a valuable model to study early MERS-CoV infection events, the Catalan scientists set
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772 out to develop an *ex vivo* model derived from the latter animals. Dr. Rodon described the
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774 isolation of respiratory tissues to assess the early local immune events elicited upon infection.
775
776 Isolated nasal mucosa and tracheal explants were maintained *in vitro* and infected with MERS-
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778 CoV/Qatar 2015. Samples obtained at different times from 0 to 96 hours post-inoculation were
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780 used to quantify viral RNA by RT-qPCR, detect virus antigen localization by
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782 immunohistochemistry (IHC) and to isolate virus.

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782 This work indicated that nasal mucosa and tracheal explants from alpaca are suitable *ex vivo*
783
784 models to study MERS-CoV replication and molecular mechanisms leading to viral infection
785
786 and/or virus clearance. The researchers hope to further develop the system to explore antiviral
787
788 therapeutic approaches (Stalin Raj et al., 2018).

787
788 **Wendy K. Jo**(RIZ, TiHo, Hannover, Germany) described a new flavivirus (genus *Pestivirus*)
789
790 detected in a toothed whales from the North Sea harbor porpoise *Phocoena phocoena* (Tautz
791
792 et al., 2015).

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793 High-throughput data obtained by using next generation sequencing (NGS) and analyzed using
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795 an *in-house* metagenomics pipeline, followed by *de novo* assembly and phylogenetic analyses,
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797 allowed the identification of a novel pestivirus in two out of three investigated harbor
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799 porpoises. The complete genome of 11,880 bp, were reconstructed by *de novo* assembly of
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801 sequence reads, and confirmed by Sanger sequencing of the full-length genome. Alignment of
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803 the complete genomes of pestivirus species showed that the newly identified virus is
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805 evolutionary closest to porcine LINDA virus and porcine Bungowannah virus with 60%
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807 homology at the amino acid level. *In situ* hybridization showed strong granular staining in the
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809 cytoplasm of multiple cell types. Based on the new sequence information RT-PCR screening of
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811 samples from more than 100 stranded harbor porpoises, collected from the North Sea
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813 indicated that about 9% of these animals were positive for the novel pestivirus.

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810 The identification of a novel pestivirus in harbor porpoises suggests that the host spectrum of
811
812 pestiviruses extends to members of the order Cetacea (whales, dolphins, and porpoises),
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814 which are considered to have evolved from artiodactyls (even-toed ungulates) (Jo et al., 2019).

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816 **Elena García Sánchez** (Center of Molecular Biology “Severo Ochoa”, Madrid, Spain) reported
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818 an outbreak of African swine fever (ASF) affecting wild boars and domestic pigs that started in
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820 the Caucasus in 2007 and spread across Russia and Eastern Europe. This more recent
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822 geographic expansion of ASF further increases the threat to the global swine industry (Karger
823
824 et al., 2019). Dr. García Sánchez presented information on genes involved in immune evasion
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829 and those hypothetically involved in attenuation of virulence of the genotype I parental ASFV
830 NH/P68. Based on the information obtained, the research team generated putative live
831 attenuated vaccines (LAV) prototypes by constructing recombinant NH/P68 viruses lacking
832 specific genes and containing markers for DIVA tests (Gallardo et al., 2018). A bottleneck for
833 the production of live attenuated virus vaccines has been the lack of permanent cell lines able
834 to sustain productive virus infection. In the studies presented, porcine alveolar macrophages
835 (PAM) were used to propagate the viruses. The results showed that naturally attenuated ASFV
836 NH/P68 strain induced full protection against both homologous (genotype I) Lisbon 60 (L60)
837 and heterologous (genotype II) Armenia07 virulent strains. The recombinant viruses carrying
838 specific deletions were all fully protective against parental homologous (genotype I) Lisbon 60
839 (L60) strain but only slightly protective against the heterologous (genotype II) circulating
840 Armenia07 strain. More studies are required to assess the basis for the lack of heterologous
841 protection of the recombinant vaccine strain, which could be related to the cell line used to
842 produce the vaccine (Sánchez et al., 2019).
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852 **Meagan Deming** (Institute of Human Virology; Center for Vaccine Development and Global
853 Health, Baltimore, United States) analyzed the response to hepatitis B vaccines in an aging
854 population and its dependence on route of injection (Weinberger et al., 2008; Williams et al.,
855 2012; de Lalla et al., 1988). Fifty-two healthy adults (65-82 years; mean age 72 years),
856 seronegative for hepatitis B (HBV), were recruited and enrolled by the SENIEUR protocol to
857 select a strictly healthy population (Ligthart et al., 1984). These seniors were randomized to
858 receive an alum-adsorbed recombinant HepB vaccine, either subcutaneous (SC) or
859 intramuscular (IM) injection, with the inoculum site guided by Computerized Tomography (CT)
860 imaging. The immunological response, expressed as anti-HBs antibody titers at day 210,
861 demonstrated that volunteers who received their vaccinations IM were over three-times more
862 likely to be responders to the HBV vaccine than volunteers receiving SC vaccinations (54%
863 versus 16%, $P=0.004$) (Ikeno D, et al.2010). The low seroconversion rate even in the IM group
864 showed a progressive decline with increasing age of the cohort and was associated with
865 significantly lower IgG2 and IgG1 isotypes suggesting a marked shift in Th1 responses.
866 Moreover, the percentage of seniors that showed T-cell mediated responses was significantly
867 reduced and also lower in intensity compared to young adults (Arnold et al., 2011). This study
868 confirmed that SC inoculation sites markedly impair seroconversion rates probably related to
869 the inoculation in the SC fat. These data show qualitative and quantitative deficits in B and T
870 cell responses to alum adjuvanted protein antigens, even in strictly healthy elderly cohorts
871 (Schillie et al., 2018).
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890 **Abigail Lowe** (University of Nebraska Medical Center, United States) turned the attention to
891 the institutional preparedness for ethical review in scenarios of outbreaks (WHO, 2014).
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893 Clinical research during epidemics is crucial to build an urgent response and, yet, requires
894 thoughtful oversight by research ethics committees to ensure the protection of vulnerable
895 subjects facing health uncertainties. To appropriately address the regulatory aspect should be
896 cultivated in anticipation of epidemics and pandemics, rather than in response to them (Alirol
897 et al., 2017). The University of Nebraska Medical Center (UNMC) institutional review board
898 (IRB) response during the 2014-2015 Ebola epidemic provided an example of research ethics
899 review under a rapid response model. This IRB provided rapid review of multiple protocols
900 during the epidemic, typically with 24 hours or less from submission to approval. Since then,
901 UNMC has been developing a centralized IRB for two national networks, both focused on
902 public health emergency research with a vision of establishing a rapid response resource for
903 these networks (Busta et al., 2017).
904
905 A new model is being developed to adapt it for research networks in order to pave a way for
906 clinical research including early drug development for novel pathogens that may emerge in
907 future outbreaks. This review model will be tested for multi-site research within the US;
908 however, there is also a need to explore models for international public health emergency
909 research. This process is complex from regulatory, as well as, operational perspectives and will
910 only succeed with close collaborations by broad stakeholders.
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922 **3.5 One Health: fact or fiction**

923 **Jordi Figuerola** (*Estación Biológica de Doñana* – CSIC, Spain), **Albert Osterhaus** (Research
924 Center for Emerging Infections and Zoonoses (RIZ), University of Veterinary Medicine
925 Hannover, Germany) and **Amelia Nieto** (*Centro Nacional de Biotecnología* – CSIC, Spain)
926 presented their views on the One Health concept taking into account the fields of wildlife,
927 ecology, as well as, veterinary and medical infectious disease disciplines. Figuerola highlighted
928 the fundamental contribution of wildlife and environment (including climate change) to
929 emerging and re-emerging zoonotic diseases, including some examples on mosquito borne
930 flaviviruses like West Nile virus (Rizzoli et al., 2015), as well as, non-infectious diseases. He
931 emphasized the need to further research on how organisms interact and how they interact
932 with their environment as a cornerstone to understand infectious disease dynamics. Osterhaus
933 covered aspects of emerging diseases in wildlife, livestock and companion animals, and the
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947 role they play in emerging human infections. Subsequently he highlighted the need to
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949 prioritize syndrome surveillance and diagnostic platforms in humans and animals, as well as,
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951 the increasing role of novel molecular technologies in pathogen discovery (Kruppa et al.,
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953 2018). In addition, he stressed that these priorities must be coupled with platforms offering
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955 mathematical modelling capacity, animal models and pathogenesis studies for new infections.
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957 Moreover, all these aspects should be accompanied by investment in therapeutics discovery
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959 and preventive intervention strategies. He finally emphasized a key point of the One Health
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961 concept, which is communication among experts, politicians, stakeholders and society
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963 (Reperant et al., 2015). Nieto focused specifically on one of the most devastating zoonoses in
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965 human history: influenza. She presented recent results showing that the heart should be
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967 considered a new target of influenza A viruses in addition to lung, especially by strains of high
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969 pathogenicity. In addition, she discussed the need for novel developments of most effective,
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971 ideally universal, influenza vaccines, as well as, more effective antivirals with low escape
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973 potential.

969 After their presentations, the roundtable was open to all delegates and several interventions
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971 took place. Some focused on the issue of communication. Most contributors agreed that
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973 scientists in general are not the best advocates to communicate hazards associated with
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975 emerging and re-emerging diseases to animals and humans alike. Moreover, we are still in a
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977 reactive framework (acting once the disease is present) and should rather move towards a
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979 preventive and preparedness strategy, that ideally prevents interspecies transmission of
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981 pathogens to occur, and if not successful provide the tools for early detection and intervention
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983 strategies. Consequently, confronting emerging epidemics at the source and from the onset is
984
985 paramount to control global infectious disease prevention and mitigation scenarios.

983 Availability of adequate resources in 'peacetime' was another hot topic, since investment in a
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985 preventive and preparedness scenario should be done in preparation for emerging infections,
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987 and not 'when the house is on fire'. Unfortunately, both at national and the international
988
989 levels, current reality shows a generally reactive rather than proactive willingness to invest in
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991 preventive and preparedness scenarios. The title of the roundtable ("One Health: fact or
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993 fiction?") was intentionally provocative. The impact of deadly infectious diseases is far
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995 different when comparing developed and developing countries, which further emphasizes the
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997 lack of a global, coordinated and effective agenda on One Health. Interestingly, the concept
998
999 One Health has been highlighted regularly in the scientific literature (more than 1800 peer-
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1001 reviewed articles), but only few manuscripts propose methodologies to measure the true
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1003 impact of the implementation of this concept. Moreover, only few suggested quantitative
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1005 indicators to follow, but no common methodology proved to be available (Baum et al., 2017).

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1006 Therefore, and concluding the round-table discussion, much effort is needed by scientists,
1007 administration, politicians, stakeholders and citizens to truly implement the One Health
1008 concept at a global level.
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1011 1012 **3.6 Antivirals**

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1014 **Johan Neyts**, Rega Institute for Medical Research, University of Leuven, Belgium, started the
1015 session by giving an overview of the antiviral agents now available for the treatment of
1016 infections with HIV, HBV, HCV, influenza and RSV infections. Dr. Neyts pointed out that for
1017 many other viruses that cause life-threatening infections and many of which are considered
1018 emerging and/or neglected pathogens, there are no drugs available. He then described the
1019 robotized lab-in-a-box automated platformed in a BSL3+ environment for high-throughput
1020 screening now installed at the Rega institute.
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1022
1023 Dr. Neyts presented the current state of Rega Institute's development of potent antivirals
1024 against flaviviruses (such as dengue), alphaviruses (such as chikungunya) and enteroviruses as
1025 well as against noroviruses, the hepatitis E and the rabies virus. Several excellent molecular
1026 targets for the selective inhibition of viral replication (and that have remained largely
1027 unexplored) have been identified, such as the non-structural protein NS4B of flaviviruses, the
1028 capping machinery of alphaviruses and the 2C helicase of enteroviruses. The Rega Institute
1029 now has molecules that target the NSB4 of the dengue virus with pan-serotype antiviral
1030 activity in the low nM to pM range. Dr. Neyts also reported that the anti-flu compound
1031 Favipiravir (T705), also active against flavi-, arena-, bunya-, and filoviruses, protects mice from
1032 infection with chikungunya virus (CHIKV). A new class of CHIKV inhibitors, targeting viral
1033 capping active in the low microM range have now also been described (Delang et al., 2016;
1034 Gigante et al., 2014). As for entero/Rhinoviruses, besides the target for capsid binders like
1035 Pocopavir (Thibout et al., 2012), the researchers at the Rega Institute have found a novel
1036 druggable pocket formed by the viral proteins VP1 and VP2 in the virus capsid and have now
1037 identified analogs targeting this pocket with broad spectrum activity (Abdelnabi et al., 2019).
1038 Also described was a novel class of tryptophan dendrimers targeting the capsid five-fold vertex
1039 were found to inhibit EV-A71 replication at low nanomolar to high picomolar concentrations *in*
1040 *vitro* (Sun et al., 2019). A lead compound in the series (MADAL385) prevented binding and
1041 internalization of the virus but did not, unlike classical capsid binders, stabilize the particle. Dr.
1042 Neyts stated that also potent inhibitors of the enterovirus virus helicase are under way. Other
1043 viruses for which antiviral substances are tested for at the Rega Institute include diarrhea
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1065 causing viruses like human norovirus (for which zebra fish larvae has been found to be a
1066 replication model) and rota virus, rabies virus, and hepatitis E virus.

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1068 **Raymond Schinazi** of Emory University School of Medicine presented his recent efforts to
1069 develop HBV capsid effectors that may offer an option to aid in a combination therapy aimed at
1070 curing chronic hepatitis B virus (HBV) infections. This virus affects over 250 million people
1071 globally and causes 686,000 deaths worldwide per year. HBV persists due to the formation of
1072 covalently-closed circular DNA (cccDNA) – the viral minichromosome – in the nucleus of
1073 hepatocytes, and current nucleoside analogs and interferon therapies have a low rate of cccDNA
1074 clearance and require lifelong treatment (Schinazi et al., 2018; Schinazi and Asselah, 2017,
1075 Boucle et al., 2016). Dr. Schinazi's group has identified the compound GLP-26, a novel and
1076 potentially best-in-class glyoxamide derivative, affecting HBV nucleocapsid formation and
1077 replenishment of the cccDNA pool. The drug has an EC₅₀ in the low nM range with a
1078 therapeutic index of >10,000. In a humanized mouse model stably engrafted with human
1079 hepatocytes and infected with HBV, GLP-26 displayed a major effect on HBeAg secretion and
1080 HBsAg in addition to a promising pre-clinical profile. More interestingly, long term
1081 combination treatment with Entecavir (ETV) in this model induced a four ¹⁰log decrease in viral
1082 loads and viral antigens reductions that were sustained for up to 12 weeks after ceasing
1083 treatment. Dr. Schinazi also briefly describe the activity of novel norovirus protease inhibitors
1084 which were also interestingly effective against certain enteroviruses at nM levels. Enterovirus
1085 protease has some similarity with norovirus and coxsackievirus protease.

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1097 **María Jesús Pérez Pérez**, Instituto de Química Médica (IQM) Consejo Superior de
1098 Investigaciones Científicas (CSIC), Spain argued that antiviral drug development has particular
1099 characteristics when compared to other therapeutic fields in drug discovery. As an example, it
1100 is indicated that phenotypic screening is still one of the main strategies to identify novel
1101 classes of antiviral agents while for other therapeutic areas target-based or fragment-based
1102 screening has often become more relevant (Murcko, J., 2018; Brown and Bostrom, 2014). Once
1103 a hit with antiviral properties has been identified, its optimization to become a lead is a
1104 multiparameter process, that can also be determined by the mechanism of action of the
1105 compound. Dr. Pérez Pérez' group's own experience in triazolopyrimidines as inhibitors of
1106 CHIKV replication was presented to illustrate how proactive and collaborative efforts among
1107 academic groups can lead to the identification and optimization of antivirals exploring a new
1108 mechanism of action (Gigante et al., 2014; Delang et al., 2016; Gigante et al., 2017; Gomez
1109 SanJuan et al.).

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1124 **Esteban Domingo**, Centro de Biología Molecular Severo ochoa (CSIC-UAM), Spain, discussed the
1125 development of antiviral resistance. The evolution of viruses involves two major steps: (i) intra-
1126 host (short-term) and (ii) inter-host (long-term) evolution. Step (i) is influenced mainly by
1127 host (short-term) and (ii) inter-host (long-term) evolution. Step (i) is influenced mainly by
1128 random genome variations and quasispecies dynamics, and its description is based on mutant
1129 spectrum analyses. Step (ii) is guided by the multifactorial epidemiological fitness and
1130 transmission-associated random drift of genomes, and its description is based on
1131 phylodynamic approaches (Geoghegan and Holmes, 2018; Domingo and Perales, 2019). How
1132 events in step (i) influence events in step (ii) is an open question. Selection of mutants
1133 resistant to antiviral agents, one of the major problems in antiviral therapy, occurs at step (i)
1134 and its consequences are felt in steps (i) and (ii).

1135 Several mechanisms of selection of mutants resistant to antiviral agents have been identified
1136 including amino acid substitutions termed RAS (resistance-associated substitutions) at the viral
1137 protein targeted by the antiviral agent, and antiviral resistance mediated by high viral fitness,
1138 documented with hepatitis C virus (HCV). Both mechanisms affect standard inhibitors and
1139 mutagenic agents active in lethal mutagenesis (Perales et al. 2019). Intra-host selection of
1140 resistant mutants affects treatment efficacy and the choice of rescue treatments. Resistant
1141 mutants can acquire epidemiological relevance, therefore affecting step (ii) of virus evolution.
1142 Epidemiological dominance of resistant mutants may render ineffective the relevant antiviral
1143 agents, a problem with similarities to antibiotic resistance in bacteria. Possible approaches to
1144 minimize selection of escape mutants inspired in quasispecies dynamics will be suggested.

1155 3.7 Respiratory Viruses

1156 **Leo Poon**, (The University of Hong Kong), reviewed the emerging influenza viruses like H5NX
1157 and H7N9 circulating in southeastern China. These viruses continue to reassort with different
1158 avian influenza viruses and spread to other geographical locations via wild birds. Dr. Poon
1159 reported on a study of 96 low pathogenic avian influenza virus genomes detected from wild
1160 bird samples collected from 2010-2017. Their phylogeographic analysis indicated several
1161 independent trans-regional reassortment events during the period. All of these reassorted
1162 viruses acquired at least one segment from avian influenza viruses found in North/South
1163 America.

1164 **Yungmee Jee** (National Institute of Health, South Korea) reported on the Middle East
1165 respiratory syndrome coronavirus (MERS-CoV) outbreaks in 2015 and 2018 in South Korea. She
1166 emphasized the lessons learned from their experience, which were as follows: A single, missed
1167 case may trigger a huge, nationwide outbreak. The first line of defense is not the thermal
1168 scanner at the airport, but doctors in the community clinics/hospitals. Superspreading events
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1183 may occur in healthcare settings, especially at the emergency department. Early detection and
1184 isolation are critically important. Aggressive strategy for quarantine maybe necessary,
1185 especially when a large number of individuals are exposed in the health-care settings.
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1188 **Enric Mateu** (Department de Sanitat I Anatoia Animals, Universitat Autònoma de Barcelona,
1189 Spain), described the virulence of and immunity against Porcine reproductive and respiratory
1190 syndrome virus (PRRSV) as two sides of the same problem. He emphasized that the genetic
1191 basis for the differences in virulence is multigenic and is related to (i) the damage caused by
1192 the virus, (ii) the damage caused by the immune response of the pig and, (iii) the interaction of
1193 the virus with the functionality of the immune system.
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1196 Neutralizing antibodies, cell-mediated immunity, ability or disability to induce type I interferon
1197 from macrophage and plasmacytoid dendritic cells, make the complexity of the disease
1198 outcome.
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1202 **3.8 influenza surveillance in Cameroon, monitoring hepatitis B virus in rural districts of India,** 1203 **virus evolution, three-dimensional cell culture infection models and new targets for antiviral** 1204 **intervention** 1205

1206 **Dr. Richard Njouom** (Centre Pasteur of Cameroon, Yaounde, Cameroon) showed data of
1207 influenza surveillance in Cameroon for the last 10 years. Although influenza virus strains
1208 circulate in the country all year round, larger prevalences were observed in the rainy season,
1209 with a major peak between September and December. Both types of influenza (A and B) were
1210 detected every year, although the A(H3N2) strain was the most prevalent when all data were
1211 considered. Unfortunately, the surveillance system revealed that in many seasons, the
1212 influenza vaccine compositions available were not a good match for the types and subtypes
1213 that circulated in Cameroon.
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1216 **Dr. Shyam Kottilil** (Institute of Human Virology, University of Maryland, Baltimore, USA)
1217 presented their impressive efforts in monitoring hepatitis B virus (HBV) in Western districts of
1218 Arunachal Pradesh (India). As part of this GVN-lead program, researchers interviewed >11,800
1219 patients and collected >11,500 samples for HBV testing. They found high HBV seroprevalences
1220 in most of the studied communities (4.8 to 12.9%), particularly in the Nyishi and Miji tribes. An
1221 interesting observation was the abundance of C/D recombinant genotypes, which had been
1222 previously found only in the neighboring Tibet, probably a consequence of migration across
1223 the Chinese border.
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1226 Two studies on virus evolution were presented by Drs. **Dieter Hoffmann** (Technische
1227 Universität München, Munich, Germany) and **Richard Scheuermann** (J Craig Venter Institute,
1228 La Jolla, USA). **Dr. Hoffmann** and colleagues studied norovirus evolution in chronically infected
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1242 patients using next-generation sequencing. They had previously shown that norovirus capsid
1243 gene sequences evolved quickly and accumulated non-synonymous mutations reflecting
1244 positive selection (Hoffmann et al., 2012). Sequentially appearing mutations correlated with
1245 structural changes that may lead to a decreased antibody binding. Interestingly, Dr. Hoffman's
1246 group has now reported that in infected individuals, progression of the infection associates
1247 with increased concentrations of capsid-specific antibodies.

1251 The presentation by **Dr. Scheuermann** concentrated on enterovirus evolution. An enterovirus
1252 (EV) D68 outbreak in the summer of 2014 coincided with a spike in the number of cases of
1253 polio-like acute flaccid myelitis/paralysis (AFM). Subsequent outbreaks in 2016 and 2018 raised
1254 concerns about the possibility of EV D68 being a new public health threat. Comparative
1255 genomics analysis showed the emergence of new EV D68 lineages in recent years. Cell culture
1256 and animal studies revealed that these emerging viruses have acquired the ability to infect and
1257 kill neuronal cells (Brown et al., 2018) and cause paralysis in mice. Virion binding and cell entry
1258 limit the neuronal infectivity of older isolates.

1264 Basic research was represented by the work of **Dr. Heinz Ellerbrok** (Robert Koch Institut,
1265 Berlin, Germany) who developed new three-dimensional cell culture infection models based
1266 on a biological extracellular matrix (decellularized equine pericardium) with primary human
1267 keratinocytes (Koban et al., 2018). Using these models for studying antiviral susceptibility in
1268 cowpox virus infection, Ellerbrok and colleagues showed that the inhibitory potency of host-
1269 directed epidermal growth factor receptor-blocking molecules such as gefitinib and cetuximab
1270 was considerably higher in three-dimensional cell culture models than in conventional two-
1271 dimensional models, suggesting that the classical monolayer cell cultures could underestimate
1272 the potential inhibitory effect of an undetermined number of antiviral drugs.

1278 **Dr. Luis Menéndez-Arias** (Centro de Biología Molecular Severo Ochoa, Madrid, Spain)
1279 presented new data on how HIV-1 reverse transcriptase (RT) connection subdomain mutations
1280 and non-nucleoside RT inhibitors modulate polypurine tract (PPT) removal during initiation of
1281 plus-strand DNA synthesis (Betancor et al., 2015). Using different HIV-1 and HIV-2 RT variants,
1282 this study showed that major determinants defining the correct cleavage site at the PPT/U3
1283 junction of the HIV genome reside at the connection subdomain between positions 342-351.
1284 These observations together with the fact that nevirapine, doravirine and efavirenz alter the
1285 efficiency of the PPT/U3 cleavage and impair the initiation of (+)-strand DNA synthesis, suggest
1286 that this step of reverse transcription could become a specific target for antiretroviral
1287 intervention.
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1301 **4. The Network in 2019**
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1304 In 2019, GVN welcomed the following Centers and Affiliates: University of Wisconsin-Madison
1305 Global Health Institute, U.S. Food and Drug Administration’s Office of Vaccine Research and
1306 Review, the Smorodintsev Research Institute of Influenza of the Ministry of Health of the
1307 Russian Federation, , Manipal Institute of Virology, The Tropical Medicine Institute
1308 “Alexander von Humboldt” of the Universidad Peruana Cayetano Heredia, Research
1309 Institute of Virology Ministry of Health of the Republic of Uzbekistan, Korea National
1310 Institute of Health’s Center for Infectious Diseases Research, Wyss Institute for
1311 Biologically Inspired Engineering at Harvard University, the Antiviral Pharmacology
1312 Laboratory and Clinical Trials Research Center Virology Program at the University of
1313 Zimbabwe.
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1323 The 6th GVN Short Course took place in Baltimore, July 29-August 2, training to date, a total of
1324 90 junior scientists from every continent.
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1327 The GVN is currently developing the “GVN Academy” initiative, which is an investment in a
1328 small group of outstanding mid-career virologist. For our pilot program, the idea is to match a
1329 selected number of outstanding early and mid-career from low-and-middle-income countries
1330 virology researchers with our senior leaders in the field to provide a series of mentoring and
1331 networking opportunities.
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1334 The GVN is also working on the development of Regional GVN Chapters to shift towards a
1335 flexible, global organization. Although the GVN is headquartered in Baltimore, it is believed
1336 that GVN presence needs to be truly global and therefore each GVN center needs to meet
1337 specific geographic challenges found particularly in Southeast Asia, South America, and Africa.
1338 This year, GVN has established the Africa GVN Regional Unit, in a meeting co-Organized by Dr.
1339 Pontiano Kaleebu, Director, UVRI and Dr. Glenda Gray, President, MRC South Africa. The
1340 meeting took place in Entebbe, Uganda and helped delineate collaboration as well as plan for
1341 future joint training initiatives.
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1347 During 2019 GVN has continued to provide public education and expert perspectives on
1348 current topics, such as the Ebola outbreak in the DRC.
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1350 Under the Anticipation and Preparedness Task Force umbrella, several Virus Watch Groups
1351 have been established, meeting regularly to discuss recent advances and findings in the field,
1352 monitoring viruses and virus research to ensure its efficacy and reinforce the GVN capacity.
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1362 **5. Plans for 2020**
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1364 We are in the process of organizing the GVN's 12th International meeting in Medellin,
1365 Colombia. This will be a unique opportunity to increase collaborations between the South
1366 American Regional GVN and GVN centers from other parts of the globe.
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1370 Plans are underway for the South East Asia Regional GVN kickoff meeting. This is an effort lead
1371 by Drs. Sharon Lewin and Linfa Wang.
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1373 GVN will continue fostering collaborations among its members, for example through joint
1374 grant applications and implementing various activities from the Anticipation and Preparedness
1375 Task Force and well as the Virus Watch Groups.
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1380 **Acknowledgements**

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1384 support.
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1390 **6. References**
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