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Autor: Thiel, Volker / Zimmer, Gert / Summerfield, Artur

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manure and birth material in open piles. This allowed spore-like particles to be spread to humans living in cities via the wind. Even though the dimensions of goat and sheep farms in Switzerland differ substantially from the situation in The Netherlands, our research had the objective to identify a potential risk of Q fever for humans in Switzerland. Five percent of the Swiss sheep farms and 11% of the goat farms were infected with the pathogen. The study concluded that while an outbreak of the same magnitude as in The Netherlands was extremely unlikely, a substantial risk of smaller outbreaks due to this disease remains. The proof that this estimation was correct was delivered shortly after, through an outbreak with 14 identified human cases in Lavaux.

By presenting and interpreting data on the incidence, prevalence and geographical distribution of zoonoses and their impact on animals and humans, veterinary epidemiology and VPH provide decision makers with the basis for effective disease control and prevention. This can only be achieved by interdisciplinary collaboration with clinicians, pathologists, microbiologists, virologist, biostatisticians and other specialists. In conclusion, veterinary epidemiology and VPH can bridge the gap between basic research and clinical sciences, as well as the one between human and veterinary medicine. This translational research has a great potential to contribute to a better health for all – animals as well as people. ■

Being prepared against viral zoonoses

Artur Summerfield*, Gert Zimmer**, Volker Thiel***

Emerging and re-emerging viral zoonoses pose a particular threat to both animals and man as they cannot be controlled by antibiotics and in most cases no vaccines are available. The frequency at which such viruses have emerged appears to have increased in recent years and is likely to continue to do so. This is caused by many factors favoring transmission amongst animals, between animals and man and between man. Amongst them are increased animal densities in farming, increased areas with high density of the human population, globalization with increased travelling and trading activities and climate changes with associated expansion or change of arthropod vector distribution. In addition to the health burden and losses of lives zoonotic outbreaks have caused, their impact on economies can be disastrous. An example for this is the recent outbreak caused by Ebola virus in West Africa. In addition, reverse zoonosis such as transmission of human influenza virus to pigs cause suffering and economic losses in swine farming.

For these reasons the Institute of Virology and Immunology (IVI) have started several research programs on viral zoonoses with the aim to increase preparedness against such infections and to provide knowledge and tools required to control outbreaks. Here we present our efforts focusing on influenza virus, Japanese encephalitis virus, a mosquito-borne flavivirus, and coronaviruses.

1. The Institute of Virology and Immunology (IVI)

Since January 2014 the IVI has been from a merger to the Institute of Veterinary Virology of the Vetsuisse-Faculty Bern and the former Institute of Virology and Immunology, a research facility of the Federal Administration. The present IVI still represents a Federal Research Institute but is now integrated into the Campus of the Vetsuisse-Faculty with both, the divisions of Immunology and Virology headed by university professors. The IVI is responsible for teaching and research in immunology and virology. The laboratory in Mittelhäusern operates at the Biosafety level 3 and BSL-3-Ag, the latter offering maximum protection of the environment for working with the most dangerous livestock pathogens.

2. Influenza A virus

Influenza A viruses are characterized by a genome consisting of 8 segments of single-stranded, negative-sense RNA. Each RNA segment is tightly associated with the nucleoprotein and the three components of the viral RNA polymerase complex thereby forming a ribonucleoprotein. Because the RNA polymerase is devoid of proof-reading activity viral offspring is genetically highly diverse (so-called quasi-species). As different influenza A viruses can exchange gene segments when infecting the same cell diversity of these viruses may become even more complex. Thus, influenza A viruses are highly mutable viruses which can easily evade the host's immune response and can adapt to new hosts.

*Prof. Dr. med. vet., **Prof. Dr. rer. nat., *** Dr. rer. nat., Vetsuisse Faculty Bern, Federal Department of Home Affairs (FDHA), Institute of Virology and Immunology IVI, and Department of Infectious Diseases and Pathobiology (DIP), <http://www.ivv.unibe.ch/>

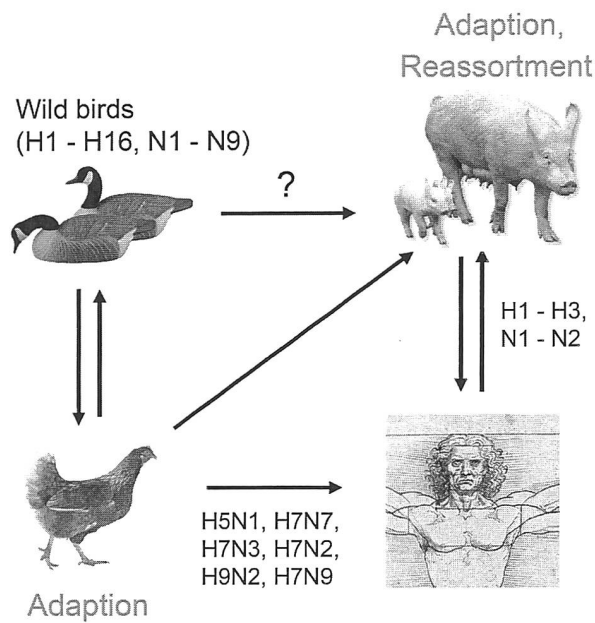


Figure 1. The zoonotic potential of avian influenza viruses.

Influenza A virus is surrounded by a lipid membrane which contains two major antigens, the hemagglutinin (HA) and the neuraminidase (NA). The HA is a primary target for the humoral immune response of the host. Antibodies directed to HA may have virus neutralizing activity by interfering with HA functions that are important for early steps in the infection process, i.e. binding to sialic acid residues on cellular glycoconjugates and membrane fusion in endosomes. The second antigen, NA, is a glycosidase (sialidase), which is crucial for the release of progeny virus particles from the infected cell surface. Accordingly, anti-NA antibodies may efficiently block virus dissemination. However, the immune system of the host permanently drives the evolution of new antigenic HA and NA variants, which are not recognized by antibodies directed against the parental antigens. This process is known as «antigen drift» and represents a major hurdle for the generation of efficacious vaccines providing long-lasting protective immunity. The situation is even more complex as several subtypes of HA and NA exist (H1-H18 and N1-N11), which are serologically distinct. Swapping of RNA segments between viruses containing different subtypes may lead to viruses with new antigenic features, and this process, known as «antigen shift», is believed to facilitate zoonotic transmission of influenza viruses.

Wild-living birds, in particular waterfowl, represent a natural reservoir for influenza A viruses. Almost all HA and NA subtypes have been detected in several different combinations in avian influenza viruses (AIV). AIV are perfectly adapted to their natural hosts and do usually not cause severe disease. AIV primarily replicate in the gastrointestinal tract of waterfowl and in this way are shed into the environment at large quanti-

ties. This facilitates transmission of AIV to other species including marine mammals (e.g. seals), domestic poultry (chicken, turkey), pigs, and humans (Fig. 1). In domestic poultry, infection is often subclinical and may proceed unnoticed. However, AIV of subtypes H5 and H7 may evolve to highly pathogenic AIV, which cause a systemic infection in poultry leading to fatality rates of about 100%. For this reason, AIV of subtypes H5 and H7 are classified as notifiable agents. It is presently not known why highly pathogenic AIV are exclusively associated with subtypes H5 and H7. In addition, it remains an enigma where these viruses evolve, in wild-living birds or domestic poultry? Interestingly, H5 highly pathogenic AIV have been repeatedly detected in apparently healthy wild-living ducks. It is therefore likely that these viruses first evolve from low pathogenic ancestors in wild-living ducks before they are transmitted as highly pathogenic AIV to domestic poultry. Scientists at IVI want to decipher the molecular mechanisms explaining why highly pathogenic AIV cause a fatal hemorrhagic disease in domestic poultry while being less harmful to wild ducks.

AIV are potentially zoonotic agents, which can cause disease in humans. In 1918, the Spanish Flu claimed about 50 million human deaths worldwide. The virus responsible for this first influenza pandemic of the 20th century has been reconstructed from viral RNA recovered from tissues of victims who have been buried in permafrost in Alaska. Sequence analysis of the viral RNA genome revealed that the Spanish Flu originated from an avian influenza virus. This pandemic virus showed enhanced virulence in experimental infection of animals compared to seasonal human influenza viruses. It is nevertheless believed that coinfection with bacterial pathogens contributed significantly to the huge death toll of the pandemic. Because of this experience the more recent human infections with AIV of subtypes H5N1 and H7N9 have raised concerns about the pandemic potential of these viruses. H5N1 and H7N9 have been directly transmitted from infected domestic poultry to humans. However, transmission of these viruses between humans did not occur because these AIV were not perfectly adapted to the human host. In order to identify the determinants for airborne transmission of these viruses in a mammalian host, scientists from The Netherlands and the USA performed virus adaptation experiments in the ferret model. These gain-of-function experiments were a matter of controversial debate because of the potential dual use of these viruses. Nevertheless, this and other work have demonstrated that AIV require adaptive mutations in HA that affect receptor specificity, pH stability, and glycosylation of the molecule. Some of these changes might have been already acquired when AIV from wild birds adapt to domestic

poultry. This adaptation process is currently investigated at the IVI in Mittelhäusern.

In addition to adaptation to the human host, individual RNA segments from AIV may be introduced to human influenza viruses by reassortment, a process that led to the emergence of pandemic viruses in the past, e.g. the Asian Flu in 1957 and the Hong Kong Flu in 1968. It has been postulated for a long time that pigs play a role in the emergence of reassortant viruses as pigs are susceptible to infection by avian as well as human influenza viruses. Evidence for the correctness of this hypothesis was provided in 2009 when a new pandemic virus came up in pigs that turned out to be a triple reassortant containing gene segments from avian, porcine and human influenza viruses.

It is evident that vaccination of domestic poultry and pigs would reduce the risk of transmission of influenza viruses from animals to humans. However, conventional vaccines based on inactivated viruses may protect animals from severe outcome of disease but may not prevent virus shedding and transmission. In addition, it turned out to be difficult to distinguish between infected and vaccinated animals (DIVA) by serological means if animals have been immunized with inactivated virus vaccines, thus complicating surveillance of influenza in animals. For this reason, the general prophylactic immunization of domestic poultry against AIV of subtypes H5 and H7 is not allowed in Europe.

To overcome these obstacles, scientists at IVI have developed a generic vaccine platform based on recombinant virus replicon particles (VRP). VRPs are disabled viruses which lack the genetic information for an essential structural protein and therefore have to be propagated on helper cells providing the missing viral protein *in trans*. Infection of non-helper cells with the complemented VRPs leads to high level expression of the antigen while infectious progeny is not produced. This very safe single-cycle vector system efficiently triggers the humoral and cellular immune response without relying on adjuvants. In addition, VRPs are fully compatible with the DIVA principle and represent a very versatile platform, which allows us to rapidly respond if a new virus emerges. IVI scientists will further develop the VRP-based vaccines in particular with respect to mucosal immunization, as this route would facilitate mass vaccination of poultry.

3. Japanese encephalitis virus

Japanese encephalitis virus (JEV) and West Nile virus (WNV) represent two related encephalitic flaviviruses transmitted by mosquitoes. JEV is responsible for approximately 50,000 encephalitic infections annually, with a 30% case fatality rate. Furthermore, 30% of the

surviving patients remain suffering from serious neurological sequelae. These viruses are antigenically closely related and form the so-called «JEV serocomplex» with other viruses such as Usutu virus (USUV), which is circulating in Europe. Wild living birds represent an important reservoir for the JEV serocomplex viruses and together with distinct mosquito species (predominantly but not exclusively *Culex* spp) they represent the main pillars of the ecological system supporting the regional presence and persistence of these viruses. Occasionally, JEV is transmitted to large mammals including man. From those, pigs play an important role and are considered to represent an important amplifying host of the virus, resulting in a high rate of JEV-infected mosquitoes, which transmit the virus to man (Fig. 2). In contrast to pigs, humans are considered to be dead end host, in which the virus can cause severe disease but does not further spread. So far, JEV was restricted to South-East Asia including India and Pakistan. However, it is considered to represent a threat for many other parts of the world, in which a favorable ecosystem would support the cycle of transmission between birds, mosquitoes and eventually mammals.

At the IVI we are studying the interaction of JEV with the porcine host with the aim to understand the reasons for the pig being an amplifying host of the virus. This includes *in vitro* studies describing the ability of the virus to infect and replicate in various cell types and to induce innate immune responses. Our results show that in contrast to other members of the JEV serocomplex, such as WNV and USUV, JEV has a pronounced tropism for many types of porcine cells including macrophages and epithelial cells. We have confirmed those results *in vivo* and demonstrated that the virus replicates well in pigs causing high levels of viraemia (virus in the blood), which is a prerequisite for mosquito-mediated transmission. An additional very important observation was that the virus can transmit from pig to pig in absence of mosquitoes,

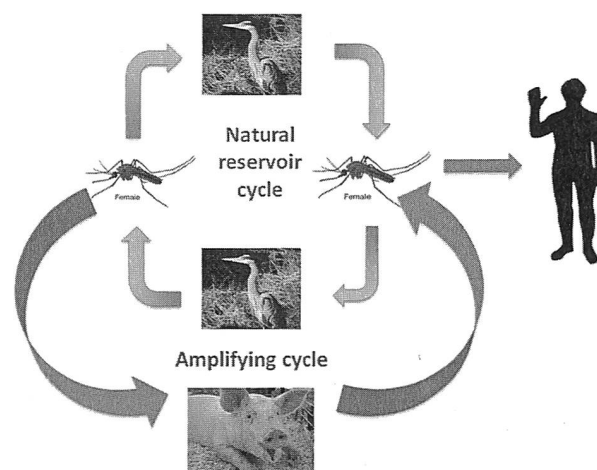


Figure 2. Japanese encephalitis virus life cycle.

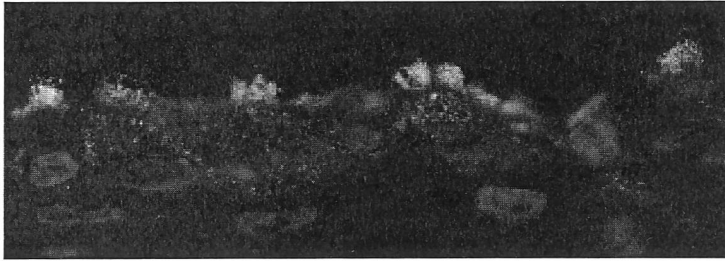


Figure 3 (colours see web version). Primary human airway epithelial cultures. The image shows a cross section of a fully differentiated human airway epithelial culture stained for cilia (green, top row brighter spots), nuclei (blue, bottom rows circular spots) and a coronavirus receptor (red, top row darker spots).

and persist in infected pigs for at least one month without any clinical symptoms in the infected animals. These are alarming results and indicate that controlling this virus in the pig population is of high importance. Our research is now focusing on understanding the virological and immunological features determining JEV tropism in pigs. We are applying this knowledge to various strategies to combat JEV reaching from providing knowledge required for risks assessments to vaccine development.

4. Coronaviruses

Coronaviruses have long been known as important pathogens of livestock and companion animals. Avian Infectious Bronchitis Virus (IBV) is a highly contagious respiratory disease of chickens, Porcine Epidemic Diarrhea Virus (PEDV) and Transmissible Gastroenteritis Virus (TGEV) can cause severe diarrhea and dehydration in pigs, often associated with considerable mortality in young piglets, and Feline Infectious Peritonitis Virus (FIPV), which arises through genetic changes of persistently infecting Feline Enteric Coronaviruses (FECV), causes a generally lethal disease in cats. In contrast, Human Coronaviruses (HCoV) have long been known to cause only mild respiratory symptoms (common cold). However, the appearance of the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) in China 2002/2003 exemplified that coronaviruses can also cause life-threatening disease in humans. Closely related viruses have been found in bats, suggesting a possible animal reservoir. Whether transmission to humans occurred directly through contacts with infected bats is controversially discussed, since SARS-like coronaviruses have been detected during the SARS epidemic in raccoon dogs and civet cats, which could serve as intermediate hosts to facilitate adaptation to the human host. A very similar scenario has just recently been observed in the context of the emerging Middle-East Respiratory Syndrome (MERS) coronavirus outbreak. MERS-CoV was first identified in the Middle East in 2012, and like SARS-CoV, there are related viruses known in bats. More importantly, MERS-CoV was also detected in

dromedary camels, which display seroprevalence of up to 90% and currently represent the most likely source for zoonotic transmission.

In ongoing projects at the IVI we are interested to shed light on how coronaviruses can cross the species barrier. Specifically, we are investigating the first events after coronavirus infection. For SARS-CoV, MERS-CoV and the human common cold coronaviruses, this takes place at the epithelium of the human airways. We have therefore established a culture system that allows us – in the laboratory – to study coronavirus infection of epithelial cells. This culture system is based on primary human epithelial cells that grow under so-called «air-liquid interface» conditions (Fig. 3). This means that a fully differentiated epithelial layer, comprised of various different cell types that are present in the human airways, receives growth medium from the basolateral side, while the cells are exposed to air at the apical side. This epithelial cell layer represents the entry port of human respiratory viruses and can be used to study molecular interactions of viruses with their natural host cells. Applying this technique to the generation of primary airway epithelial cells of animal species will furthermore allow us to study virus host tropism. For example, we have established primary airway cultures from bats, pigs and camelid species and can now investigate how efficient viruses originating for bats, pigs or camels will replicate on airway epithelia from different species, including humans. This technological platform will allow us to rapidly determine if viruses originating from bats or camels can replicate in the human airway epithelium, and if they do this with similar efficacy as in the epithelium of their natural host. Such analyses can then reveal the «zoonotic potential» of animal viruses, depending on how efficient they can replicate in human epithelial cells. In addition, we aim to use the airway epithelial cell culture system to elucidate basic parameters that impact on the ability of viruses to cross host species and cause zoonotic transmission.

As the examples of SARS-CoV and MERS-CoV have shown, we still have only rudimentary knowledge from where these viruses originate and how they can infect human cells and cross the species barrier. Do they use homologous cellular receptors in different species? Are there any other «intrinsic» host factors that contribute to the species barrier and prevent infection of viruses originating from a different host? These are important questions that we aim to tackle in the future at the IVI, and we hope to provide significant contribution towards a better understanding of how viruses can overcome the species barrier and cause epidemics in animals and humans. ■