Risk Assessment Framework for Lumpy Skin Disease and African Swine Fever

Lumpy Skin Disease [LSD] of cattle and African SwineFever [ASF] in pigs are both internationally-spreading, viral animal diseases that damage livestock production in Europe. LSD causes nodular skin wounds, fever, potentially generalised illness and death in some exposed cattle. ASF causes a severe illness in pigs, leading to fever, leakage of blood vessels and fast killing of most infected pigs, including wild boar. Whilst LSD and ASF viruses do not cause illness in humans, control options for both diseases in animals are limited. Therefore, identification and minimisation of LSD- and ASF-associated risk factors is critical for disease prevention.

As part of the EU Horizon 2020 DEFEND project, the German Federal Research Institute for Animal Health (Friedrich-Loeffler-Institut) leads work package 1 [WP1] to develop a risk assessment framework for LSD and ASF. In a first step, the WP1 has gathered a large spectrum of potential risk factors by systematically searching scientific literature databases. The scientists found virus-, environmental-, biosecurity-, husbandry-, transport-, network-, society-, surveillance- and disease response-related risk factors for both diseases, arthropod-, cattle breed-, cattle and insect movement-related factors specifically for LSD, as well as pig breed-, pig and pig product movement- and wildlife management-related factors for ASF.

The identified portfolio of potential risk factors will guide control and prevention strategies for LSD and ASF.

Beyond borders: the role of conflicts and insecurity in animal disease spreading

The EU and neighbouring countries have experienced outbreaks of many animal diseases over the last 5 years including lumpy skin disease (LSD) and African swine fever (ASF). Progression of these diseases may have been facilitated by war or civil unrest. DEFEND Work Package 3 “Conflict, migration and virus spread” is investigating if and how civil wars, insecurity, conflicts and human migration act as drivers for animal disease emergence and spread. Special attention is focused on LSD and ASF, and other animal diseases including foot and mouth disease and peste des petits ruminants are also considered.
The objectives of the research are:
• to generate sound and consistent data/knowledge around how human and animal migration routes and dynamics are affected by conflict, insecurity and unrest
• to identify risk factors for the spread of animal diseases related to conflict-associated changes in mobility and trade patterns
• to tailor and test methodology for data collection on disease emergence and spread in conflict areas
Data regarding human and domestic animal migration will be collected using surveys and participatory methodological tools, such as Participatory Rural Appraisals and Participatory Epidemiology based on focus groups discussions, interviews of key informants, participatory

maps, and field inspection of strategic points. Data collected will be merged with official data from Animal Disease Notification Systems (ADNS) and migratory data obtained from migration platforms (IOM, UNDESA, IDMC, UNHCR etc.).
Activities will focus on three macro areas: i) border zones in the Middle East-Syria/Turkey and Syria/Lebanon; ii) border zones in the Balkan region; iii) border zones between Ukraine, Romania, Moldova and Hungary

African swine fever prevention and management

The task focuses on the risk factors associated to the transmission of African Swine Fever (ASF) at farm level. The outcome of the task is achieved by an extensive literature review on the epidemiological drivers relevant to the spread of ASF in domestic pig and wild boar at the interface with domestic pigs.
ASFV is a highly virulent, highly resistant virus affecting both domestic pigs and wild boars. Searching the most recent literature relative to the diffusion of ASFV into the eastern European Countries and to Belgium, we identified critical risk factors for the disease transmission at farm level, summarized as follows:
• Animal movements (direct pig-to-pig contact). Presence of infected wild boars which can transmit the virus in the wild and to domestic pigs. Boar to pig contacts are a major risk factor in free-ranging systems and in the backyard sector. Dead animals remain infectious for a long period.
• Carriage of the virus on fomites, clothing and footwear is a risk factor associated with the activities of hunters, mushrooms and berry collectors, veterinarians and paraveterinarians, vehicle movements from and to the farm as well as proximity to a slaughterhouse or refuse dump.
• In the backyard sector the main risk factors are human induced: low biosecurity measures, illegal movements of infected pigs, swill feeding and underreporting associated with “emergency sales” of infected pigs.
In order to mitigate the above risk factors it is imperative to improve the general biosecurity measures at farm sites, put under control free ranging systems, forbid swill feeding as well as promoting preparedness of farmers and technicians relative to all the necessary precautionary measures to adopt to protect their own herd from ASFV transmission.

Improving research tools for the study of neutralising antibodies to lumpy skin disease virus.

Lumpy skin disease (LSD) is an emerging, global transboundary disease of economic importance. The causative agent, lumpy skin disease virus (LSDV), infects cattle causing systemic disease that can lead to substantial production losses. Improved laboratory tools are required in order to develop better vaccines and diagnostic tools.

Limited cell lines are available for the propagation of LSDV which impacts on subsequent research efforts. As part of the Horizon 2020 DEFEND project we have used a host-specific cell line, Madin-Darby bovine kidney (MDBK) cells. MDBK cells were shown to be permissible to LSDV infection forming distinct foci-type plaques.

From these results, we were able to develop an immunofluorescent virus neutralisation test (IFVNT) for the detection and quantification of LSDV neutralising antibodies (nAbs) - a key component of protective immunity to LSDV. Using serum from experimentally LSDV-infected cattle and labelling with a fluorescent secondary antibody, the assay was able to accurately detect nAbs in infected and uninfected animals. Visualisation of the fluorescent foci improved the

sensitivity of the assay making it easier to interpret results and semi-quantify nAbs in cattle throughout the study period.

Improving assays for the detection of nAbs is essential for evaluating vaccine efficacy against LSDV. The methods described from our research provide additional tools for this purpose and can be implemented across LSDV research laboratories. Overall, these tools contribute to LSD control and prevention programmes.

A Review of Risk Factors of African Swine Fever Incursion in Pig Farming within the European Union Scenario

Over the past decade African swine fever (ASF) spilt out of its original area of endemicity and spread widely in Europe and Asia. This evolution of ASF has caused serious economic damages to the affected countries, reshaping the pig farming sector and the commercial pork network around the world. We carried out a literature review to identify risk factors associated with introduction of ASF on farms.
The most critical risk factors were:
1. Low biosecurity measures for commercial, non-commercial and outdoor farming systems
2. Free-range or outdoor farming systems are considered, per se, critical risks
3. Contact with infected pigs purchased from infected areas
4. Contact with wild boars, free range pigs or pigs from other farms
5. Feeding kitchen leftovers or feedstuffs from untrusted vendors
6. Contact with contaminated fomites including footwear or clothing of the farmer, hunters, veterinarians and para-veterinarians
7. Failure to report symptomatic animals or illegal emergency slaughtering on farm
Since no effective vaccine or therapy for ASF is available, all stakeholders should tailor their management practices and in particular their biosecurity measures to avoid exposing their farms to these critical risks.

Estimating the risk of environmental contamination by forest users in African Swine Fever endemic areas

African Swine Fever (ASF) affects different species of wild and domestic suids, and is both an ecological and an economic concern, which causes large direct and indirect economic losses to the pig industry. The virus can survive for long periods in the environment, and humans can unintentionally act as vectors through infected fomites, thus contributing to disease spreading. To assess the magnitude of this issue, we ran a simulation study, in which we estimated the ASF contamination probability related to different types of human forest activities.

Our study revealed that ASF environmental contamination is as a rather unlikely event. When scaling up the contamination process to a whole year and to large geographic areas, though, the accumulation of the same forest activities in time and space produced the expectation that thousands of contamination events would occur each year. Wild boar supplemental feeding and hunting emerged as the riskiest activities in terms of contamination probabilities.

The risk of ASF environmental contamination should not be disregarded when planning management actions to reduce ASF circulation. Supplemental feeding should be strongly reduced or avoided in ASF affected areas. Wild boar hunting as a management and surveillance tool should be carefully evaluated, considering both its benefits and the associated contamination risks. It is essential to improve and enforce strict biosecurity measures for all forest-based human activities in ASF endemic areas.

Immune response to experimental LSDV challenge

Lumpy skin disease virus (LSDV) causes severe disease in cattle and water buffalo, and is transmitted by blood-feeding arthropod vectors. Information about the immune response to LSDV is limited which hampers the development of tools to control the disease.

We experimentally inoculated calves with LSDV via either needle-inoculation or arthropod-inoculation using virus-positive Stomoxys calcitrans and Aedes aegypti vectors. Seven out of seventeen needle-inoculated calves (41%) and 8/10 (80%) of the arthropod-inoculated calves developed clinical disease, defined as the development of multiple cutaneous lesions.

We found a variable LSDV-specific cell-mediated immune (CMI) response in the needle-inoculated calves which was indistinguishable between clinical and nonclinical calves. However the CMI response in arthropod-inoculated calves was very consistent with a clear difference between clinical (strong CMI response) and non-clinical (weak CMI response) cattle.

Neutralising antibodies against LSDV were detected in all inoculated cattle from 5-7 dpi. A strong IgM response in the nonclinical arthropod-inoculated calves suggested that this is a correlate of protection.

This study reveals differences in the immune response between clinical and nonclinical LSD cattle and highlights the importance of using a relevant transmission model. It also provides fundamental immunological details to guide development of novel diagnostic tests and improved vaccines for LSD.

Cellular and humoral immune responses after immunization

African swine fever virus causes a lethal haemorrhagic disease in domestic pigs and wild boar for which there is no vaccine currently licensed for use in the EU. Experimental African swine fever vaccines can be broadly divided into live attenuated vaccines, which are weakened versions of the fatal virus, or subunits vaccines similar to those developed for COVID. African swine fever virus is a complex pathogen that encodes for more than 150 genes, and our knowledge of the protective immune response against the virus is limited. This makes selecting which gene or combination of genes to include in a subunit vaccine technically challenging.

We experimentally inoculated pigs with a live attenuated strain of African swine fever virus and then challenged them three weeks later with a normally lethal isolate of the virus. We measured immune responses to the virus throughout the studies. Many of the pigs survived the experimental challenge and we found the pig produced both antibody and cell-mediated immune responses to the virus. In outbred farm pigs the ability of CD8 T-cells, a type of immune cell capable of detecting and killing virus infected cells, was most strongly correlated with protection after challenge with lethal African swine fever virus.

The immune assays developed for these studies, in combination with the samples collected from the animals, will now be used to screen the 150 plus African swine fever virus genes to identify potentially protective viral proteins. These will be incorporated into subunit vaccines and tested for their potential to protect pigs from disease.

What diffusion can tell us about African Swine Fever

African Swine Fever (ASF) is a severe viral disease of domestic and wild pigs that causes death in most infected pigs and has a massive socioeconomic and animal welfare impact. For controlling the disease, it is important to understand and anticipate how ASF spreads. As lead of work package 1 in the EU Horizon 2020 DEFEND project, the Friedrich-Loeffler-Institut,

Germany's Federal Research Institute for Animal Health recently developed a new risk assessment approach for ASF to help anticipate its spread among European wild boar.

Since there is no direct information on the details of ASF spread in wild boar populations, epidemiologists from WP-1 estimated disease spread indirectly: The scientists knew, how far one detected ASF case usually occurred away from the next one, and how much time normally passed between two successive cases. With this knowledge, they now used a trick to generate a large number of similar fictive ASF outbreak patterns, hypothesising that the pattern of early disease spread mathematically resembled the well-described process of diffusion (Brownian motion). The epidemiologists then used the statistical measures derived from their model to draw conclusions about the speed of disease spread and the area affected by ASF at various timepoints following disease incursion into a previously unaffected wild boar population.

Both estimates, speed of spread and ASF-affected area, are key metrics to anticipate, how ASF spreads. They can help to respond appropriately for controlling the disease or at least to minimise its damage.

What viral genomes can teach us about Lumpy Skin Disease and African Swine Fever

Although the genomes of DNA viruses like Lumpy Skin Disease virus (LSDV) and African Swine Fever virus (ASFV) evolve more slowly than RNA viruses like Avian Influenza, or Foot and Mouth Disease virus, they accumulate changes while replicating in animal populations. These can occur as the accumulation of single changes at different points in the genome sequence, but other processes like recombination can delete large amounts of genomic information from virus genomes and even swap sequences between virus strains. Carefully produced reference genome sequences are essential in the development of vaccines or antiviral treatments against viruses, they also allow the continuous verification of the efficacy of molecular diagnostic methods and provide completely characterized viruses for use in experimental research and assay validation. We provide high-quality genome assemblies of additional ASFV and LSDV genomes for this purpose. On another level, when viral genomes of sufficient animals in a population are sequenced, and provided sufficient genetic differences exist between viruses, phylogeographic methods can study the dispersal dynamics of the virus in animal populations in time and space. Providing detailed sampling of viral full genomes from well-chosen populations (LSDV: southeastern European epidemic 2015-2017; ASFV: local circulation in wildlife and swine in Lithuania) and combining this with publicly available genomes, we are investigating at what scale (global vs. regional vs. local) these DNA viral genomes provide sufficient resolution – i.e. show sufficient genetic differences between affected animals – to study virus dispersal dynamics.

How samples from LSD outbreaks can help breeding more resistant cows.

Lumpy skin disease (LSD) is endemic in Africa and a serious threat to cattle in Europe and Asia. The disease has spread steadily north and east over the past 15 years from Africa through the Middle East and the eastern Mediterranean. There is clear host variation in response to LSD infection since a proportion of the animals develop no clinical signs when a herd is infected. Here, we present the first attempt to detect genome regions that affect resistance of the host to LSD. We collected bio-banked samples from natural LSD outbreaks and experimental infections from participating countries. The samples for DNA included blood samples, tissue samples (e.g. skin), or hair (with the hair roots attached). The study integrated DNA and clinical data from

animals that developed clinical symptoms as well as animals from the same herd that did not develop clinical symptoms. With 238 animals and > 50 000 DNA markers, we could identify five genome regions that appear to differentiate animals that developed clinical symptoms from those that did not. While these findings have to be confirmed with more animals, our study illustrates the importance to collect both data and samples during disease outbreaks. This way, we can develop genetic tools for increased resistance against this disease.

The potential epidemiological role of cattle subclinically infected with lumpy skin disease virus

Lumpy skin disease is characterized by the formation of nodules throughout the bodies of cattle and water buffaloes. In addition to the effect on animal health, the disease also has an important socio-economic impact, as it leads to a sharp drop in milk yield, infertility (temporary or permanent), trade restrictions, reduced market value due to damaged skins, and abortion in pregnant cows. However, only some LSDV infected animals develop these typical LSDV nodules. The role of subclinically infected animals, those without nodules, in LSDV epidemiology is unclear. In order to shed light on this knowledge gap, a number of bulls were infected with LSDV and monitored clinically and by laboratory tests. Stable flies were allowed to feed briefly upon animals which had no nodules but were PCR positive in the blood (=donors). These flies were subsequently transferred to naïve susceptible bulls (=acceptors) for a second blood meal. Two out of the 5 acceptor animals became positive for the virus, clearly demonstrating that LSDV can be transmitted from subclinical infected animals by stable flies. Interestingly, one of the acceptors became subclinical infected while the other developed the typical nodules. Although subclinical infected animals are probably not the engine of a LSDV outbreak as the viral load is relatively low compared to clinical infected animals, they need to be kept in mind by decision makers when designing control policies as they could explain, for example, geographical jumps when transport of seemingly healthy animals is allowed.

Lumpy skin disease virus transmission under low vector abundance conditions

Although lumpy skin disease virus (LSDV) epidemiology is incompletely understood, the transmission by means of vectors has been proven in the last few years. Several haematophagous arthropod species have been put forward but the involvement of Stomoxys calcitrans, also known as the stable fly, has been clearly demonstrated under experimental conditions. This was achieved by transferring multiple times, large numbers of stable flies, fed on viremic animals (=donors) to naïve acceptor animals. This setup is representative for field conditions or stables without vector control. However, it remained unclear if transmission could also occur if only limited numbers of flies are present. This question was addressed by performing an animal experiment whereby only 20 flies were transferred once to acceptor animals after having been allowed a single feeding on viremic donors. LSDV transmission was observed to 5 out of 10 acceptors as evidenced by the development of nodules, viremia and seroconversion. In general, the time between vector feeding/transfer and the nodule formation was between 10 and 19 days (n=4) but went up to 35 days (n=1). The fact that only a few vectors are sufficient to transmit LSDV, albeit under experimental conditions, has a number of implications. Transport, for example, of clinical diseased animals is not without risks and needs to be kept in mind in any crisis management plan. Also vector management needs to be efficient and even then vigilance is warranted. The prolonged incubation period observed in this study shows that implemented quarantine periods need to be sufficiently long.

Ensuring Gender Equality in a Research and Innovation Action.

The Commission has established gender equality as a cross-cutting objective for all policy areas. This includes the promotion of gender equality at all levels in research and innovation teams. Within the DEFEND project, we adopted gender equality as a central pillar of the Action right from the proposal design stage. Our aim has been to promote the concept of gender equality amongst partner and stakeholders, for example by having as close as possible to 50:50 men and women in teams and among the leading roles. We have achieved this through strategies such as developing and implementing a DEFEND gender equality plan, using gender neutral language in all documents, actively considering gender balance in all teams including the Multi-Actor Panel, Ethics Advisory Board, Enabling Impact Team, promoting gender equality via our Defend communication channels – our website, newsletter, at annual conferences and on our social media pages, providing a childcare support grant to enable caregivers to attend annual meetings, and seeking out best practice by attending conferences on gender equality. Specific examples include advertising Women and Girls in Science Day on our twitter feed, and the participation of our consortium lead, Professor Pip Beard, in the conference 'Research and Innovation Excellence through gender equality: New pathways and challenges' in Helsinki in 2019. We recommend these actions to other research consortia.

The role of conflicts, human mixed migration on animal infectious diseases spread.

The effectiveness of surveillance and control measures for transboundary animal diseases (TAD) can be increased when socio-economic factors and a deep knowledge and understanding of local dynamics are understood and incorporated. This becomes even more critical in cases of conflict, insecurity and poverty. Activities were carried out in six countries (three EU countries, two in the Balkans and one in the Middle East region), focusing on the effects of ongoing conflicts in Ukraine and Syria and on human mixed migration along the Western Balkan route on the spread of TADs. The main focus was on the TADs African swine fever and lumpy skin disease although the findings could apply to other high consequence diseases of animals. The short- and long-term consequences of conflicts on animal health were examined. Short-term effects include, for example, immediate challenges such as worsening food security and essential infrastructure degradation, direct damage to the country's agrifood and zootechnical sector, weakness in animal health services and diseases surveillance while the long-term consequences concern protracted issues such as reduced livestock capacity, reduction of farmers' capacity in proper animal management and biosecurity, and post-conflict challenges related to borders and ethnic minorities and related movements. Regarding mixed migration along the Western Balkan route, the study found that long-distance migration does not significantly impact the spread of transboundary animal diseases (TAD) due to the rare presence of animals and animal products brought by migrants. However, the recent crisis in Ukraine raises concerns about the role of pets in migration. There is a need to coordinate efforts to address animal welfare during emergencies, including implementing internationally harmonized actions and promoting data collection on the presence of animals in refugee camps and their needs. As for livestock, their importance as animals in long-term refugee camps has emerged. Even if their presence is not reported in refugee camps in the EU and the Balkans, livestock still represent one of the primary food sources worldwide, often supplementing the food aid received. It is, therefore, essential to consider them in the planning and management of camps and to support local veterinary services to guarantee both animal and human health and food safety.

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