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Ebola Foresight - 2815FSEBOL

The relevance of livestock, domestic animals and wildlife as a source of Ebola virus infection – a cooperation project between the Friedrich-Loeffler-Institut, the Institut Pasteur in Conakry, Guinea, the Sierra Leone Agricultural Research Institute and the Njala University in Sierra Leone

Country	Sierra Leone, Guinea
Funding Agency	Bundesministerium für Ernährung und Landwirtschaft – BMEL
Project executing Agency	Bundesanstalt für Landwirtschaft und Ernährung – BLE
Coordinator	Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health
Partners	Sierra Leone Agricultural Research Institute (SLARI); Njala University (NU), School of Agriculture in Sierra Leone; Institut Pasteur Guinea (IPGui), Conakry, Guinea
Project Budget	1.680.000 €

Project Duration	01.10.2016 – 30.09.2019
Key Words	Animal Health, Zoonosis, Virology, Filoviruses, Ebolavirus, Diagnostic Assays, Host animal, High containment laboratory (Biosafety Level 4)
Background	<p>The Ebola virus outbreak in West Africa from 2014–2016 has been the largest outbreak known in history. More than 28.000 human cases of Ebola virus disease have been reported, of which more than 11.000 patients died. In the most severely affected countries Guinea, Sierra Leone and Liberia, the epidemic reached unprecedented dimensions with disastrous economic and humanitarian consequences for the local population.</p> <p>Ebolaviruses are zoonotic viruses that can be transmitted from infected animals to humans. Bats have been widely discussed to act as a natural virus reservoir. However, unequivocal proof for this hypothesis is still missing.</p> <p>Furthermore, very little is known about the potential role of livestock and domestic animals in Ebola virus ecology and the potential spillover into the human population.</p> <p>After the initial spillover into the human population however, human-to-human transmission is the predominant route of transmission and responsible for the rapid spread of the virus.</p>
Objectives	<p>Although the zoonotic origin of Ebola virus outbreaks in humans has been known for a long time, there is a lack of knowledge concerning the susceptibility of different animal species, their role as potential intermediate hosts for ebolaviruses in general and the pathogenesis in these hosts.</p> <p>Aim of this project was, in a close collaboration between the FLI, IPGui, SLARI and NU, to study the role of livestock, domestic animals and wildlife as potential reservoir and amplifying hosts in</p>

ebolavirus infections. The project further aimed to develop diagnostic assays for the detection of ebolavirus infections in relevant animal species, which were then supposed to be implemented in the respective African partner laboratories according to the capacity building aspect of this project. An additional aim was the development of detection methods for novel or even unknown viruses related to ebolaviruses.

As part of the capacity building, two PhD students from Sierra Leone and Guinea were trained at FLI over several weeks. Key topics included practical trainings in well-established laboratory methods as well as theoretical lessons in biosafety and virology. Furthermore, the PhD students gained knowledge and experience in the scientific presentation of laboratory results. Following their stay at the FLI, the students were accompanied by FLI staff back to their home countries, in order to transfer the newly gained knowledge to their home institutions. These attempts were supported by teaching local laboratory staff in biosafety and laboratory methods. Besides educating local staff, another focus has been to implement a functional and sustainable laboratory infrastructure in Sierra Leone and Guinea by supporting partners in their efforts to procure relevant equipment.

Results

Apart from laboratory analyses, the safe sampling of animals was trained in multi-day workshops. Here, particular emphasis was put on safe sampling of livestock, domestic animals and bats. After sampling, the PhD students and lab staff were trained in both the analysis of the collected samples as well as the interpretation of the data. To this end a number of serological and molecular assays have been established at FLI to be used for the detection of ebolavirus infections in relevant animal species. Particularly the indirect ELISA as an important first screening assay of sera has been successfully implemented in all partner institutions. Further, protocols for modern third generation sequencing methods were established and published in a scientific journal, and the African partners were trained in this method during a workshop held at the Institut Pasteur in Guinea.

Using the whole panel of newly established serological tests, the analysis of pig serum samples from Sierra Leone and Guinea suggests an exposure of pigs to ebolaviruses or ebolavirus-like viruses. However, it remains to be further investigated whether pigs have been exposed to a known, pathogenic ebolavirus or rather to a novel, antigenically related ebolavirus of unknown pathogenic and/or zoonotic potential. So far, this work has resulted in two original articles published in relevant scientific journals.

Furthermore, the analysis of 300 dog serum samples from Sierra Leone revealed serological evidence for a past ebolavirus exposure. Here, based on our information with regards to the age of these dogs, exposure of them to the highly pathogenic ebolavirus that caused the West African outbreak is likely to have occurred during this outbreak. However, ebolavirus-specific seroconversion was also detected in younger animals, suggesting a more recent virus contact. Nonetheless, a role of dogs as a so-called virus reservoir seems rather unlikely, considering the spatial proximity to humans and the small number of recorded outbreaks. Future studies should investigate whether dogs act as passive virus carriers mechanically spreading the virus after licking and feeding on infected carcasses or fomites, or whether dogs can actually replicate and shed infectious virus. The latter would increase the likelihood of potential transmission to humans.

These serological investigations were complemented by basic research efforts assessing the susceptibility of cells from more than 30 different animal species to ebolaviruses as well as the related marburg- and cuevaviruses. To this end a number of assays were developed that allow these kinds of analyses in absence of infectious virus. This has the advantage that for this work no extensive infrastructure is necessary (work with infectious ebolaviruses has to be performed in maximum containment laboratories with the highest biosafety level 4), so that they can be performed in regular laboratories in a safe manner. In addition, they allow rapid investigations of novel or newly discovered viruses related to ebolavirus. In order to find such viruses, a novel detection system was

developed, which detects the presence of ebolavirus-related viruses in a biological sample not by detecting virus components, as is the usual strategy, but rather visualizes their biological activity.

In summary, the Ebola Foresight project has been the starting point for collaborations between the participating countries and institutions. Moreover, it has initiated the education and training of young African scientists, resulted in the availability of novel detection and analysis methods for ebolaviruses and related viruses, and has contributed to our understanding of the role of pigs and dogs in ebolavirus epidemiology.



Figure 1: Participants of the Kick-off Meeting in Conakry, Guinea, in June 2016.

Photos



Figure 2: Sampling of livestock by a SLARI sampling team.



Figure 3: First hands-on experience of the two PhD students at the FLI Laboratories.



Figure 4: Impressions collected during workshops in Guinea and Sierra Leone. Left: Loading the MinION third generation sequencing device. Middle: Participants of a sequencing workshop in Guinea. Right: In the lab in Sierra Leone.



Figure 5: Closure Meeting of the Ebola Foresight Project with all project partners at the premises of the Institut Pasteur Guinea in Conakry.

Recommendations

In terms of sustainability, we strongly recommend to continue the project activities that have just shown to gain momentum.