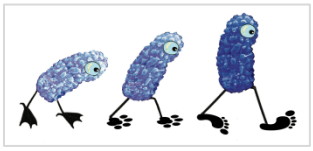

Minutes MedVetKlebs Year 1 Meeting

(10-11 January 2019, Institut Pasteur, Paris)

Day 1. Presentations from the partners and special guests from other consortia (Davide Sassera from SpARK project, Orjan Samuelsen from NOR-KLEB, Yann Reynaud, Damien Thiry, and Francois Gravey.

If all agree, the presentations could be shared by Sylvain thorough our Google Drive folder. ACTION: all

Achievements/Highlights from Year 1:

Methods for the isolation, detection and identification of Kp complex members (detailed information in the presentations)

IP (Sylvain Brisse and Carla Rodrigues, Paris, France)

- MALDI-TOF MS to discriminate within the members of the Kp complex (Kp1 to Kp6, update Kp7 to see if there are specific peaks): *done and published*
- Contact Bruker and BioMerieux in order to show our work and see if they are interested in update their databases. ACTION: Sylvain/Carla
- Other option – disseminate within the partners our BioNumerics MALDI-TOF Kp database spectra. ACTION: partners that would like to develop an identification library in their BioNumerics database are welcome to ask Sylvain/Carla the reference spectra.

IP + INRA (Pascal Piveteau and Elodie Barbier – Dijon, France)

- **zkir qPCR** - detection of the members of the Kp complex (Kp1 – Kp7) directly in the enrichment broth. ACTION: Elodie/Sylvain: draft manuscript
- ACTION by Elodie/Carla: Update the **new primers** and add the **protocol of DNA preparation** from the enrichment in the Google Drive
- Identification of phylogroups – Taqman multiplex: good results were obtained. ACTION Elodie/Sylvain: validate and distribute the protocol with partners

IP + IZSAM (Francesco Pomilio – Teramo, Italy)

- Productivity of 3 different culture media (SCAI, SIGMA and Liofilchem) compared to Nutrient broth (reference method); conclusion: Similar productivity/recovery for the 4 media: SCAI is therefore highly productive; besides being cheaper, easy to prepare. ACTION Francesco/All: Contact commercials in order to explore possibility to introduce SCAI agar plates on the market (is not commercialized yet).
- SIGMA – better selectivity than Liofilchem. Easier to identify Kp in comparison with SCAI. However, supplement (carbenicillin) of the media needs to be bought separately – expensive.

- SCAI at 37°C and 44°C – all groups from the Kp complex are able to grow. No apparent differential selectivity.

NUIG+AGES+IZSAM+SSI

- Optimized collectively the Kp isolation protocol from chicken meat using peptone buffered water and SCAI media (protocol in the Google Drive).

INRA

- Optimization of the Kp isolation protocol from soil using SCAI (in the Google Drive)

Broad Sampling (detailed information in the presentations)

ANSES (Marisa Haenni, Raquel Garcia Fierro. Jean-Yves Madec – Lyon, France) and IP

- 2498 fecal samples from animals – 220 Kp (8.8%)
- AST and WGS of the 220 Kp
- **Ongoing studies** - characterization of the collection, GWAS studies

AGES (Kathrin Hauser, Kornelia Votsch – Vienna and Graz - Austria)

- Ready-to-eat salads, onions, chicken meat – higher % Kp

NUIG (Dearbhaile Morris and Niam Cahill – Galway, Ireland)

SSI (Eva Möller Nielsen and Gosia Ligowska-Marzeta- Copenhagen, Denmark)

INRA (Pascal Piveteau and Elodie Barbier – Dijon, France)

- Soil samples - ~ 10% Kp

Transmission studies (detailed information in the presentations)

NCOH (Rob Willems, Utrecht, The Netherlands)

- Healthy carriers (n=125) and dogs – 15 samples from human carriage were Kp+ (12%) – already in WGS – high diversity
- Until now dogs were all negative for Kp

IP (Philippe Glaser/Thierry Naas – Paris, France)

- Screen different patients at 10 different times in rehabilitation centers; genomic comparisons. Use of SCAI to evaluate diversity ACTION Philippe/Sylvain

Modelling (detailed information in the presentations)

IP (Sylvain & Lulla Opatowski, Paris, France) and DTU (Sara Pires, Copenhagen, Denmark)

Goal - broad Kp ecology/transmission picture. Two approaches in mind: dynamic modelling and source attribution. Discussions highlighted the challenge in implementing these approaches for *K. pneumoniae*. Difficulties with Klebsiella include the fact that defining

'subtypes' is a clearly identified problem for Kp sublineages (either too broad at the level of phylogroups, or too narrow at the level of sublineages).

Budget Discussion

Presentation by Marina Caillet (IP): financial and reporting aspects

Each year, partners of MedVetKlebs will need to obtain from their financial/administrative person, the budget spend on MedVetKlebs project and send it to Pasteur (M Caillet) for information. ACTION Sylvain/Marina

Important point - budget can be moved within a partner among different lines (e.g., publications to consumables) and also between MedVetKlebs partners (hiring personnel, …). This needs to be approved by the EJP One Health board. Action all: identify difficulties with budget (either too much, or not enough).

Need to follow-up Rob's UMCU/RIVM budget Action Sylvain/Rob

Hired personnel:

IP (genomics): **Carla Rodrigues**

Administrative support (IP): **Marina Caillet**

SSI: **Gosia Ligowska-Marzeta**

INRA: **Elodie Barbier**

ANSES: **Raquel Garcia Fierro**

ISZAM: **technician**

IP (modelling – 5 months): to be defined. Action Sylvain/Lulla/Sara

Day 2. Discussion of the next steps

HOW? WHERE? WHAT?

HOW?

1. **Culture media**

Validation of SCAI medium (Citrate Simmons agar from Bio-Rad (Ref 64834), *myo*-inositol from Sigma Aldrich (Ref I5125))

Freezing effect - evaluate the impact of freeze/unfreeze samples in the recovery rate of Kp. Might be a small project to be executed by one the partners. ACTION: who volunteers? Please tell Sylvain

Lauryl Sulfate Tryptose Broth – use to detect of coliforms in water, wastewater, dairy products and other food samples. Damien Thiry used in the isolation of Kp from pig feces – enrichment step, more selective than LB. Less contaminants than with LB+Ampicillin. Question by Philippe about colistin R isolates? According to Damien they grow well in this medium (tested after the meeting already).

2. **qPCR**

Detection:

Validation of zikr qPCR by the other partners - multicentric validations. The idea is to perform a PCR directly from the enrichment to detect Kp; and plate in SCAI only the positive samples. Send the positive control DNAs to all partners (*K. pneumoniae* ATCC700603) ACTION: Dearbailhe and ACTION all volunteers: evaluate qPCR

Identification:

Identification of phylogroups – Taqman multiplex

ACTION Carla: Send reference strains for phylogroups (reference strains of Kp1 to Kp7) and also define a negative control (*E. coli* ATCC25922 ok for all?) ACTION all volunteers: evaluate qPCR

Quantification – was discussed – seem to be too time consuming for most partners at the moment, we will see if arises later

WHERE? WHAT?

Broad sampling can continue but if we are to valorize data together we need to use identical protocols. ACTION Carla/all: make list of protocols according to sample types and validate it with partners.

Sampling campaign: ACTION Carla: Distribution of the template to be filled with the type of samples to be tested by the different partners; ACTION all: please fill in the template based on what you can/will/all willing to sample.

Need of **harmonized protocols** for **water** samples – sewage, seawater, freshwater (partners interested – NUIG, IZSAM, AGES): ACTION Dearbhaile & others for validation

How many colonies should we take from the plate?

- At this step only 1 colony per plate/sample should be enough, as multiple colonies are likely to be identical
- To access variability within samples, it was decided to keep mixed colonies (take all the content of the petri dish using a swab and freeze at -80°C);
- ACTION Carla: Define best bulk microbial community -80°C storage from plates (BHI + 20% glycerol??) and diffuse the info.
- ACTION all: keep swab of plate from all samples once storage medium is defined.
- ACTION Sylvain: Ask Orjan Samuelsen/Ed Feil about mixed colony sampling sequencing
- Role of insects in the transmission of Kp – ACTION all: check for groups working with insects nearby; or sample insects!

Sequencing Plan

It will be important to know if all partners have a sequencer in house or if they need to send the isolates/samples to the other partners; and corresponding budget. Action Carla: make census of available sequencers at Partners institutions

ACTION Pasteur: sequence samples from INRA/soils: one colony, Kp1 only, per soil sample.

NEXT STEP – combined qPCR, PREVALENCE and GWAS STUDY DESIGN

Study for qPCR validation (NUIG, AGES, ISZAM, INRA, SSI, ANSES)

- **Ready-to-eat salads** + **chicken meat** – 25-30 samples of each
- Enrichment in BPW – follow Elodie DNA preparation
- **Detection**: Plate on SCAI and simultaneously perform the zkir PCR
- **Identification**: ID by MALDI-TOF MS vs qPCR ID phylogroups
- **Need to design chicken meat + salad + bacteraemia study for qPCR & genomics studies – GWAS ACTION Elodie/Sylvain**

Ontology

Check EFSA SSD2 (Standard Sample Description) format and FoodOn consultation.

ORION project (EJP) ACTION Carla/Sylvain/Eva

Create a template for sample description ACTION Carla

Interaction with other consortia

Possible cross-validations of hypotheses in different datasets: SpARK (Pavia) vs. MedVetKlebs (Europe); pool isolate collections from some sources together to increase statistical power for prevalence or GWAS studies.

Communication

- Sharing of documents through a Google Drive folder
- Make a short video for YouTube presenting the MedVetKlebs project: maybe ACTION Sylvain
- Small article for EJP Newsletter ACTION Carla
- Use the OneHealth EJP site - https://onehealthejp.eu/. Log in and request membership to MedVetKlebs group. ACTION Sylvain/Carla: try it
- If "validated": Share strain coding system, reference strain lists & genomes via website
- Social Networks – Twitter – use the hashtag **#MedVetKlebs** and link to @OneHealthEJP and @sylvainbrisse
- A group photo was taken on Day 2, early afternoon – it is available on the Google Drive.
- **Congresses** – One Health EJP ASM 2019 (poster with the presentation of the overall project ACTION Carla; animal Klebsiella ACTION Carla; qPCR ACTION Elodie),ECCMID (not so many participants from MedVetKlebs apparently), IMMEM 2019 (possibly qPCR, population structure studies).

Date of next plenary meeting:

Mid November 2019 – at Institut Pasteur, Paris. ACTION Sylvain: Make a Doodle to decide the final date!