PPP Annual Report 2019

PPP projects which are under supervision of the "Topsectoren" must report annually on the scientific content and financial progress. This form is used to report the progress of the content of the project. PPP projects that finish in 2019 should make use of a different form: "PPP-final report."

The annual report will be published on the TKI / topsector website. Therefore, please ensure that there is no confidential information in the annual report.

The PPP-annual report must be sent, at the latest, by the 1st of March 2020 to the "TKI's": info@tkitu.nl or info@tki-agrifood.nl. For Wageningen Research, the report has to be sent to the "Topsector secretary" of your respective institute.

General information	
PPS number	**AF-16160**
Title	**1H4F-SaferFood-BIGdata**
Top section and innovation theme	Smart Agri & Food, Food safety
Public research institutes	WBVR and WU (both from WUR)
Project leader (research)	Dr. Alex Bossers (alex.bossers@wur.nl)
PPS coordinator (private part)	Vion Food NL (penvoerder) - Dr. Bert Urlings - Dr. Martijn Bouwknegt (secretary)
Contact government	Cor Wever
Status (ongoing or finalized)	Ongoing
Official start date	2017 April 1
Official end date	2021 March 31

Approval secretary / consortium The secretary / consortium will review the annual report. The Topconsortia voor Kennis en Innovatie (TKI) (top consortium for knowledge and innovation) would like to be informed about any remarks on the annual report.	
The secretary on behalf of the consortium has (…) the annual report	**X** approved ☐ disapproved
Any remarks on the annual report:	**-**

Planning and progression (please indicate changes with respect to the project plan)	
The PPS is going according to plan?	Yes Project GO/NO-GO per 1st April 2020
Organization / management structure	Representatives of each private partner (Vion, Thermo Fisher (TF), IBM) and the DLO/WUR-partners (WBVR and WU) consult quarterly with each other through teleconferencing. Vion organizes and IBM facilitates the plenary teleconferences. At least once each year a physical meeting takes place, in which results are presented and plans for the coming period are outlined and approved.
Counseling structure (sounding board etc.)	Partner WU (prof Marcel Zwietering) additionally acts as an advisor for the project contents. The consortium as such outlines the project and its tasks, while the 1H4F advisory and steering group reviews the progress and content.
Are there changes in the consortium / project partners?	Yes, The postdoctoral researcher Cynthia Ho has left WBVR. Her duties have been divided over WBVR and WU as well as that some improvements were made to the work plan still leading to the same goals but by a slightly different route (postdoc Jeroen Koomen working at WU group of Marcel Zwietering/Tjakko Abee has taken over (with consortium approval) some of the Salmonella genomics and biofilm characterization studies.
Are there any delays or postponing of completion date?	No

Summary of the project
With big data analysis and machine learning algorithms we aim to identify microbiome-derived biomarkers/signatures for the early detection of potential product contamination in slaughterhouses. Through this early warning system, early interventions can take place to avoid recalls and further contamination of meat products with food pathogens. This monitoring will lead to improved food safety for consumers and reduced contamination related economical losses. To achieve this we aim to identify such microbiome-related biomarkers within a model of pathogenic bacteria (Salmonella) that can potentially be found on the carcasses in the pig slaughter line. The expertise's of the various partners have been used to set-up and perform several experiments to assess microbiome measurement feasibility on carcasses in the pig slaughter line and whether sample pooling strategies can be used to reduce analysis costs without losing analytical power (#1), whether there is an early warning signal in microbiome community structure associated with presence of pathogenic Salmonella (#2), and whether isolated Salmonella's harbor biofilm embracing genetic and biochemical signatures (#3).

Results

Main objectives for 2019;

1) assess microbiome measurements feasibility on the pig slaughter line and whether sample pooling strategies can be used to reduce analysis costs without losing analytical power.
2) whether there is an early warning signal in microbiome community structure associated with the food pathogen Salmonella.
3) whether isolated Salmonella's harbor biofilm embracing genetic / phenotypic signatures.

Point 1 has been presented as preliminary data at the physical meeting in April 2019 in Utrecht, which was hosted by Vion. Point 2 was ongoing for 2019 while work at point 3 was approved in April 2019 and started just after summer 2019 to be completed before summer 2020. As soon as we have a proof of concept to identify biomarkers using described methods, the project will shift focus towards the usability of such markers in to be developed diagnostics and to allow modelling and assessment of intervention strategies.

Achieved results:

#1 initial insights from routine microbiome measurements

Samples for routinely monitoring on pig carcasses were doubled to provide samples for this project. With little adjustment these samples are proven to be useful for assessing the microbiome composition. Over 4,000 samples were collected to span processing over one year and additionally aerobic viable counts, *Enterobacteriaceae* counts and Salmonella status (positive/negative of pooled carcass samples) were determined. The collected samples were subjected to 16S-rRNA gene amplicon sequencing (16S-barcoding) and subsets were analyzed by shotgun metagenome sequencing to determine and relatively quantify the bacterial community composition. All 2,000 samples from the first half year of collection were analyzed for their bacterial community structure and whether there was any signal in there preceding or on the detection of Salmonella positive days.

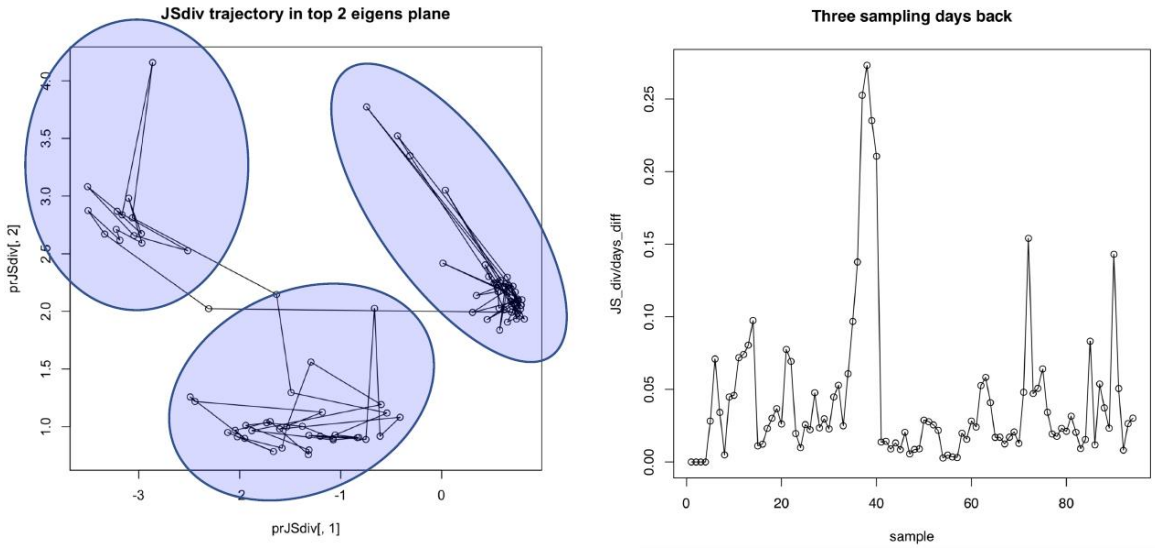


Figure 1. Microbiome divergence over time. A) Principle Coordinate Analysis (PCoA) ordination of Jensen-Shannon divergence between all microbiome samples. Individual days in the time line can be followed by the connector lines. B) JS divergence over 3 day sliding window clearly shows the transition points between the three major clusters. Thus far these clusters have not been correlated with Salmonella positivity yet.

There is substantial evidence of systemic bacterial presence as can be seen from the community composition analysis (figure 1). The Jensen-Shannon divergence seems to be temporally grouped with clear transition points. To investigate this further we first need to rule out any systematic batch effects from the analysis (meta-analysis). In addition to this, we are investigating whether the measured community structure changes (compositional data) are due to additional taxa added to a basic community structure or whether the community structure has changed without the detected increase of bacterial load on the carcass. To measure these bacterial load the 16S qPCR will be used on all previously and currently analyzed samples.

To increase the number of analyzed samples and to decrease overall cost it was decided to test whether there will be a significant data loss by pooling of samples. A pilot study was initiated in which various pools of 5 and 10 samples which were analyzed for microbiome composition by 16S barcoding and the results were compared to that of the individually sequenced samples. Samples were selected such that it would potentially include the preliminary signals picked up from the data by IBM. Overall the analyses showed that pooling 10 samples per day did not result in any data loss and actually improved the signal to noise ratio. It could also be concluded that different carcasses sampled in the chilling room on the same day have strong similarity in the microbiome. Based on these results we additionally analyzed the microbiome composition of the 1,000 samples from the second half of the sampling year. Microbiome measurements have been completed and are currently being analyzed.

#2 early warning signals in the microbiome

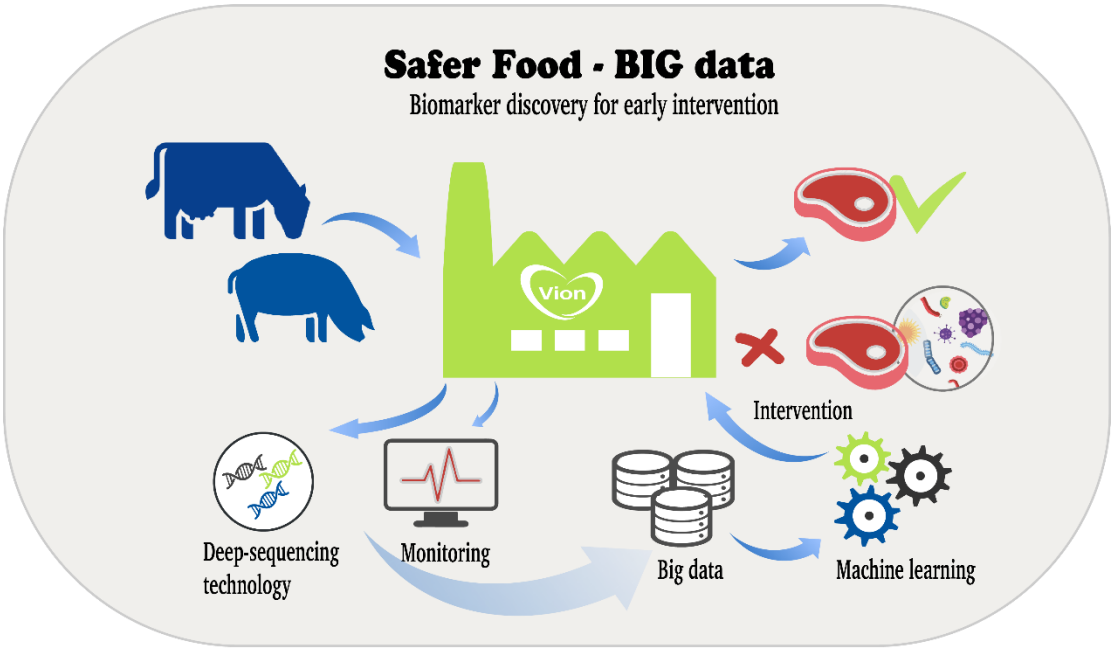
As indicated, the microbiome compositions for different carcass samples show a temporal coherence, meaning that samples on the same or adjacent days show more similarity than samples collected far apart in time. Due to the limited Salmonella positive days in the thus far analyzed samples, it is too early to develop predictive models, for this the microbiomes from many more carcasses need to be sequenced and analyzed. Currently the Salmonella positive cultures seem to appear stochastic (exponential model with a constant rate of random events). This can be potentially improved by adding more samples over time (the 2nd half year of sampling) and by adding additional quantitative qPCR data for Salmonella (*ongoing work*).

#3 Salmonella clonality and biofilm potential

To determine whether Salmonella is derived from external sources or from biofilms present in the slaughterhouses, we set up a study to compare salmonella isolates (genetically for clonality and biochemically for biofilm embracing capacity). Little variation in the genetic sequences would indicate an internal source of contamination. In total approximately 100 Salmonella isolates sourced from slaughter lines were subjected to whole genome sequencing. Salmonella WGS proved to be a good alternative to serotyping methods providing more detail (at single nucleotide level) if strains are clonal or not (high resolution). From the sequence identity analysis, samples cluster according to date of sampling and to a lesser extent on sampling location. Further analysis of genomes is ongoing as well as the analysis of the biochemical potential of these isolates to form/participate in biofilm formation. Clues from the phenotypic/genetic analysis helps to optimize/develop intervention strategies that keep the sanitary up-to-date. Clonality analysis will also be taken a step further using for instance MASH (k-mer profile) distances by putting the genomes in the context of several thousand Salmonella genomes in IBM's genomic database (*ongoing work*).

Social application: give a brief description of the social application / payoff

There are strict food safety regulations for the production of pork and beef to guarantee consumers safety. Despite these regulations contamination with food pathogens such as *Salmonella*, *Listeria monocytogenes* and STEC during the production chain cannot be fully eliminated. Contaminated products can lead to food poisoning, wasting of food products, substantial economic losses and brand damaging in case of recalls. This project aims to further reduce the small percentage of potential contamination using early detection signals (biomarkers) developed with innovative methods. These biomarkers derived from the existing microbiome in the production chain will act as early predictors when contamination occurs with food pathogens. We will use BIG-data and machine learning algorithms to identify such biomarkers which subsequently can be used to develop low-cost rapid routine diagnostics (figure below).



Number of delivered products in 2019 (specify in the appendix the titles and/or descriptions of the products or a link to the products on public websites)					
Scientific articles (*)	Reports	Articles in trade journals	workshops/ invited lectures	Patent applications /first filings	Spin-offs (**)
Not yet (1 article planned)	2018 and 2019 bi-Ann reports	none	20181108 A Bossers, 1H4F najaarscongres <u>Consortium meetings:</u> 20180405 Zurich, Switserland 20190408 Utrecht, NL 20200309 San Jose, USA	none	none

(*) A reminder: in case of financial support from the TKI allowance, the TKIs should be acknowledged in the publications. In case of publications in high impact journals the TKIs would like to be informed in advance.

(**) This indicates: financial support from other parties resulting from this project, additional subsidies and spin-offs activities.

Do you expect patent applications this year?	NO

Planned publications:

<u>Publications in scientific journals</u>

- Genome + biofilm phenotyping paper (Salmonella genome analysis for clonality in meat processing plants and biofilm phenotype characterization). (Planned).

<u>Publication in trade journals/websites:</u>

- 1H4F website: project description