1. **Analysis of metagenomics results in a surveillance context (*By Steven Sarrazin, Gent University; Steven.Sarrazin@UGent.be and Roosmarijn Luiken, Utrecht University; R.E.C.Luiken@uu.nl)***

*The objectives of this section are to explore data and data transformations and to produce and interpret a forest plot of a meta-analysis.*

Along with sample collection, at the time of each farm visit, the authority collected information on the total antimicrobial use (AMU) (group treatments) of the sampled animals throughout their entire rearing period. Additionally, the farms were scored for several parameters of internal, external and total biosecurity (measures designed to protect the farm from the entry and spread of infectious diseases). For each category, the higher the score, the higher the biosecurity level of the farm.

Some additional farm-level explanatory data were also collected during the visits.

We provide you with a table that contains data on the above mentioned explanatory variables (EFFORT\_MG\_sampledata\_final.txt), and a R script (Exercise V meta-analysis V4.Rmd) to run statistical analysis on those data using Rstudio within the Virtual Box. Download the .zip file section V material.zip and extract both files into the shared folder Vboxshare.

**NB: The table EFFORT\_MG\_sampledata\_final.txt is the same that was used in section IV exercise – you do not need to save two copies in Vboxshare!**

Launch Rstudio **from the terminal in the virtual machine with administrative rights ("sudo rstudio")**, open the R script Exercise V meta-analysis V4.Rmd, run it step by step, and study the outputs you get. Note that it might not work if you launch Rstudio from the desktop icon!

**NB. You can save your replies to the questions on the script itself, by writing your reply outside of areas of code chunks.**

The analysis is organized as follows:

First, you will focus on the pig data and second on the poultry data. Note that the first half of the script regards pigs and the second half poultry.

You will start with exploring the data, and data transformations. Then you will look at the association between total resistance (the sum of the relative abudance of all resistance genes in a sample) and two explanatory variables – antimicrobial use (AMU) and farm biosecurity - by using linear regression and meta-analysis.

QUESTIONS:

*Exploration of the AMU data – pigs and poultry*

1. What variation do you notice between farms in a country?
2. What variation do you notice between countries?
3. Distribution of the AMU data. To be used as a predictor for AMR in models, the use of very skewed data is not advised. Is the distribution of the non-transformed AMU approximately normally distributed? Does the log10+1 transformation help?

*Exploration of the biosecurity data – pigs and poultry*

1. What variation do you notice between farms?
2. What variation do you notice between countries?
3. What is the relation between external and internal biosecurity at the country level?

*Meta-analysis AMR – AMU pigs and poultry*

1. Interpret the forest plot (TOTAL\_RESISTANCE\_VS\_AMU\_PIG.png picture saved in the folder Vboxshare). What is the general relationship between total AMR and total AMU?Is the relationship positive or negative? What does this mean?

*Meta-analysis AMR – Biosecurity pigs and poultry*

1. Interpret the forest plot (TOTAL\_RESISTANCE\_VS\_EXTERNAL\_BIOSECURITY\_PIG.png picture saved in the folder Vboxshare). What is the general relationship between total AMR and external farm biosecurity in broilers? Is the relationship positive or negative? What does this mean? Is there variation between countries?
2. Can you conclude from this epidemiological analysis on probable associations that may justify the observed differences in AMR between farms? (pigs and broilers)

git remote add origin https://github.com/Ibisanmi1/Metagenomics-applied-to-surveillance-of-pathogens-and-antimicrobial-resistance.git

git branch -M main

git push -u origin main