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Molecular characterization of Mycoplasma hyopneumoniae and control strategies

BACKGROUND:

Mycoplasma hyopneumoniae (M. hyopneumoniae) is the cause of mycoplasmas pneumonia of swine, often known as enzootic pneumonia. It is chronic disease and may be endemic, or spread slowly but progressively through a facility over the course of weeks. It is a non-fatal disease of pigs, with morbidity rate up to 70-100%. Although young pigs can develop the disease, it is most important in grower-finisher pigs and play a crucial role in the Porcine Respiratory Disease Complex (PRDC).

Clinical expressions of the disease are coughing, reduced feed conversion and poor weight gain. Economic impact and the key role in PRDC lead to be an eligible disease of eradication/control plan. The connection between laboratory diagnosis and on-field control is the fully knowledge of the pathogen, which means a genotyping characterization. Determining the circulating genotyping can drive a more efficient control plan.

ACTIVITY:

Aim of this month conducted at University of Minnesota (UMN) in the veterinary diagnostic laboratory was to perform a phylogenetic analysis on the genotype data collected in 2 years of work in several Italian fattening pig farms. Furthermore, phylogenetic analysis can help veterinary practitioners to develop control plan of *M. hyopneumoniae.*

M. Hyopneumoniae PCR has been performed in up to 1500 samples come from TBS (Trachea Bronchial Swab) and lung tissue of fattening pigs involved in Mycoplasma outbreaks. Each sample has been tested for *M. hyopneumoniae* through Real Time PCR (amplified P102 gene region). Positive sample has been subsequently subject to genotyping analyses, through MLVA (Multi Locus Variable number tandem repeat Analyses). MLVA is based on the Mycoplasmas genomic feature of repeat region, that can change in the number of repeat (VNTRs) Variable Number Tandem Repeats.

IZSLER (Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emila Romagna) and UMN (University of Minnesota) use both MLVA technique but, based on its experience, the genomic regions investigated are different. IZSLER use Locus1, Locus2, P97/1 and P97/2 regions while UMN use P97 (repeat N 1) and P146 (repeat N 3) with the common characteristic of variability. IZSLER use four tradition PCRs, UMN use multiplex PCR to amplify the two regions, both the amplified was analysed in a capillary electrophoresis tools. Output of capillary electrophoresis can be upload in a dedicate software for both the protocols.

MLVA results has been analysed using BioNumerics, a software platform able to do integrated analyses. Genotypes are arranged in order to farm, type of sample and every other variable can be reason to suspect an association. Genotypes are disposed on a Minimum Spanning Tree (MST), a graphic outcome where genotypes are arranged in a dot plot, each plot is a different genotype, as large as the number of the samples that composed it, distance between the dots gauge the difference in numbers of repeats regions.

The results show the presence of "cluster" of genotypes in many farms, it's a group of genotypes very similar, that can be considered deriving from an only one. In some farms has been detected only one genotype and even the same, that can be "dominant" or more stable than other, relationship with management systems need to be assessed. Furthermore, the "dominant" genotype could be correlated with lung's lesion score at slaughterhouse, a novel result than previous studies.

Genotypes analysis within a pigs in the samples come from lung's lobes show a different pattern in right and left lobes, that means the pathogens follows a definite pathway in the airflow.

My experience at University of Minnesota included also on field work in *M. hyopneumoniae* control plan. On work plan of practitioners vets, there is the detection of *M. hyopneumoniae* and it is performed through airways swabs, oral fluid, blood serology and also environmental samples. Environmental presence of Mycoplasmas also provides information regard the bacterial spread and the air load that is the main vector carrier. Effective methods, but still on research focus are damp environmental swab (cages, walls), environmental dust collection along fixed time and also using air collection tools (cyclone systems). All the samples are re-suspended in a buffer solution ad tested with PCR.

Environmental surveillance can estimate the bacterial pressure before and after an outbreak and evaluate if it is resolved or still active as well as the bacterial air load, during a voluntary exposure in a control/eradication plan.

Control and eradication plan are usually performed in US sows farm when *M. hyopneumoniae* is a considerable problem and cause substantial economic losses, since is compatible with a simultaneous eradication plan of PRRS, it is a possibility to combine the two strategies. Procedure consists in a voluntary exposure of *M. hyopneumoniae* to the sows and gilts; the material used is always lungs homogenate make from scarified pigs with *Mycoplasma-like* lung lesions. Until recently was performed using intra-tracheal inoculation of this homogenate, but this procedure need many time, loss of time and money. A novel technique allows simultaneous exposure of the all animals, it's the spreading in the air of the homogenate through fogging system. Procedure start with the collection of *M. hyopneumoniae* positive lungs homogenate and intra-tracheal inoculation in a *M. hyopneumoniae* free pigs, these pigs are treated with cephalosporin for 1 week to eliminate all other bacteria and scarified. Lung's lobe with *Mycoplasma-like* lesions are tested for the respiratory pathogens that must be negative, if *M. hyopneumoniae* PCR is positive with Ct lower than 25-30, lungs are able to use for homogenate preparation. Homogenate is made with a whisk process (it can be fine the kitchen blender) and stored at -18°C for 1 week.

During the exposure day the homogenate is unfreeze ad resuspended in Friis medium (even in powder form) at the concentration of 60:40 (homogenate:medium) and a final concentration of 1×10^4 CCU/ml. Final exposure is obtained through the application of the resuspended homogenate in an electric "fogger" (same that poultry vaccination) and run for 30 minutes with all windows close and fans off. In this way all the animals receive a *M. hyopneumoniae* aerosol up to the lower respiratory tract, boosted with the deeper breath of the animals due to the higher air temperature generate with fans off.

Few days later significant numbers of animals are tested with TBS with the aim to assess that infection has been occurred in all of the herd. When whole herd is infected we consider day zero.

From day zero the farm should be close for at least 8 months, time necessary because all animals are no longer shedder. Three-four weeks before the end of closure period all animals are treated with medication (macrolide in water and injection for sick pigs). Before the opening of the farm, the animals should be tested with TBS and verify the negative results of PCR, if not there are three alternative: extended closure time, medicate again or test again.

This procedure allows to have a free *M. hyopneumoniae* sows farm and need to have a GDU (gilt development unit) where do same exposure before the gilts transfer from GDU to sows farm or buy free gilts.

FUTURE PROSPECT:

Based on the UMN experience and our datasheet, we can proceed with final data processing regarding *M. Hyopneumoniae* genetic variability with different point of view.

A possible study could be the comparison of the two techniques (IZSLER and UMN) to see different genotype characterization, and asses the most useful to use in Italian diagnostic field. Once obtained, it could be part of IZSLER's diagnostic routine.