Bacterial 16S metagenomics by high-throughput sequencing: a tool for the etiological diagnosis of pneumonia in cattle?

➢ Study done by Fabien Gregoire, ARSIA
➢ Reported by Christian Quinet FESASS

In 2020 :

1490 bovine necropsied (all ages)

- ❖ 1177 bovine necropsied (0-6 months)
- ❖ In this age group, 293 animals with respiratory lesions

Approximately 80% of respiratory cases elucidated

Based on:

- ✓ Macroscopic lesions
- ✓ Histology
- ✓ Culture (Gassner - blood agar - chocolate - CNA - PAM)
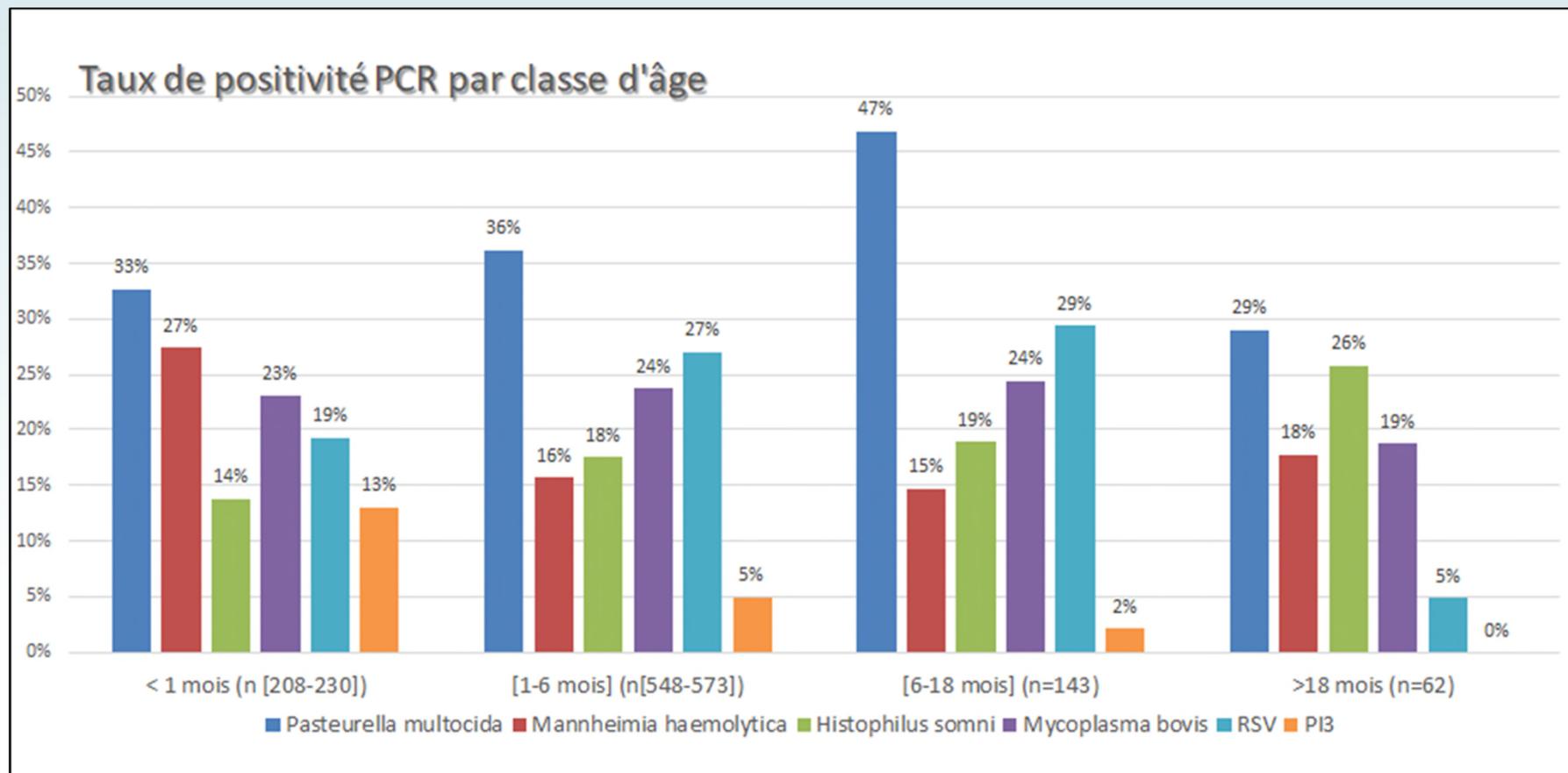- ✓ PCR (6 pathogens)

PCR results
results on autopsy lungs (2018-2019-2020):

Taux de positivité PCR par classe d'âge

Legend: Pasteurella multocida, Mannheimia haemolytica, Histophilus somni, Mycoplasma bovis, RSV, PI3

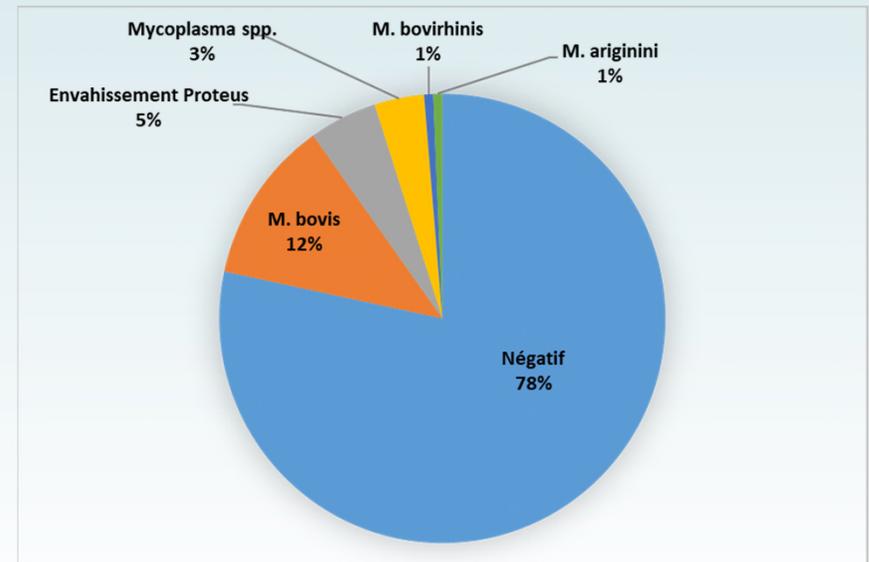Age classes: < 1 mois (n [208-230]), [1-6 mois] (n[548-573]), [6-18 mois] (n=143), >18 mois (n=62)

Results of autopsy lung cultures (2020):
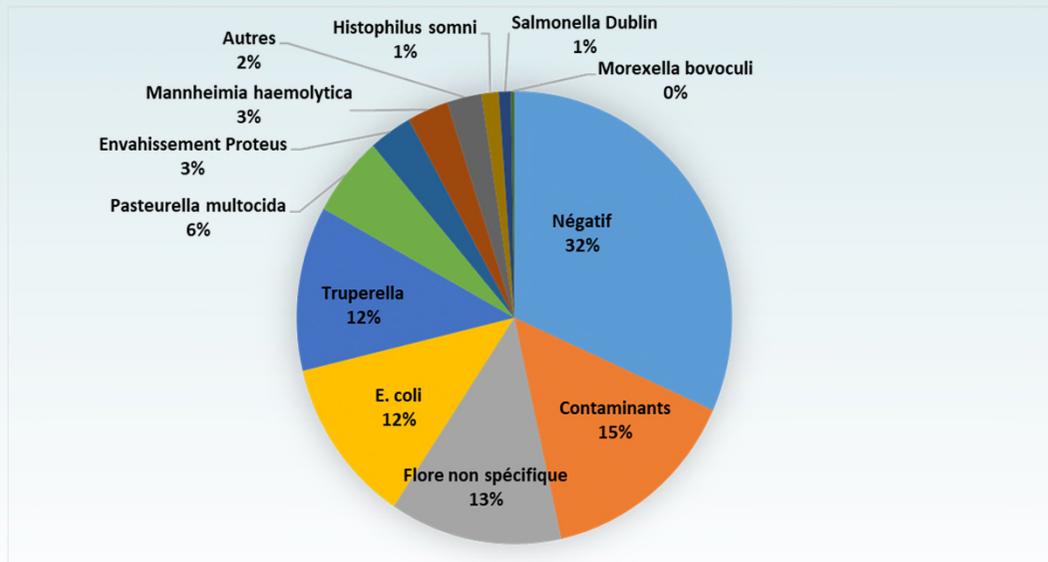
Aerobic culture

Mycoplasma culture

16 S Sequencing Technology

16 S Sequencing Technology
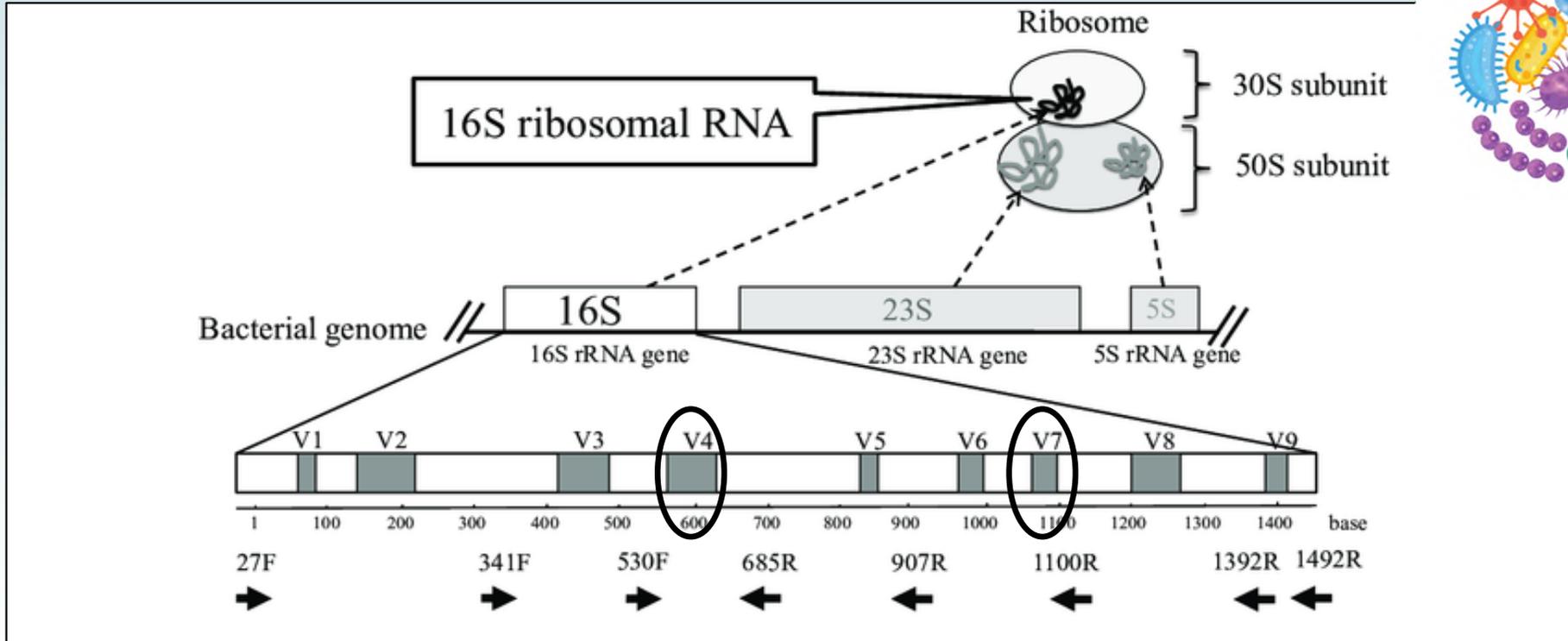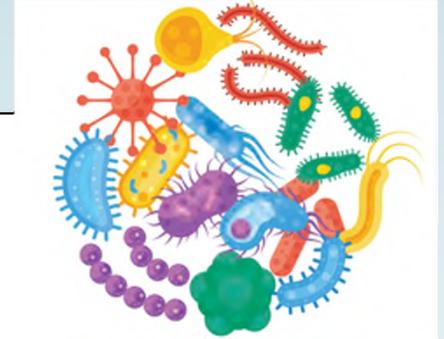
Lung sample
Storage -20°C
Ethanol disinfection (min 24h)
PBS rinsing and heart sampling

DNA extraction
DNA quantification (QuantusTM Promega)

PCR 16S regions V4 and V7 (ThermoFisher primers)

Illumina Miseq sequencing

Analysis of results with Geneious software
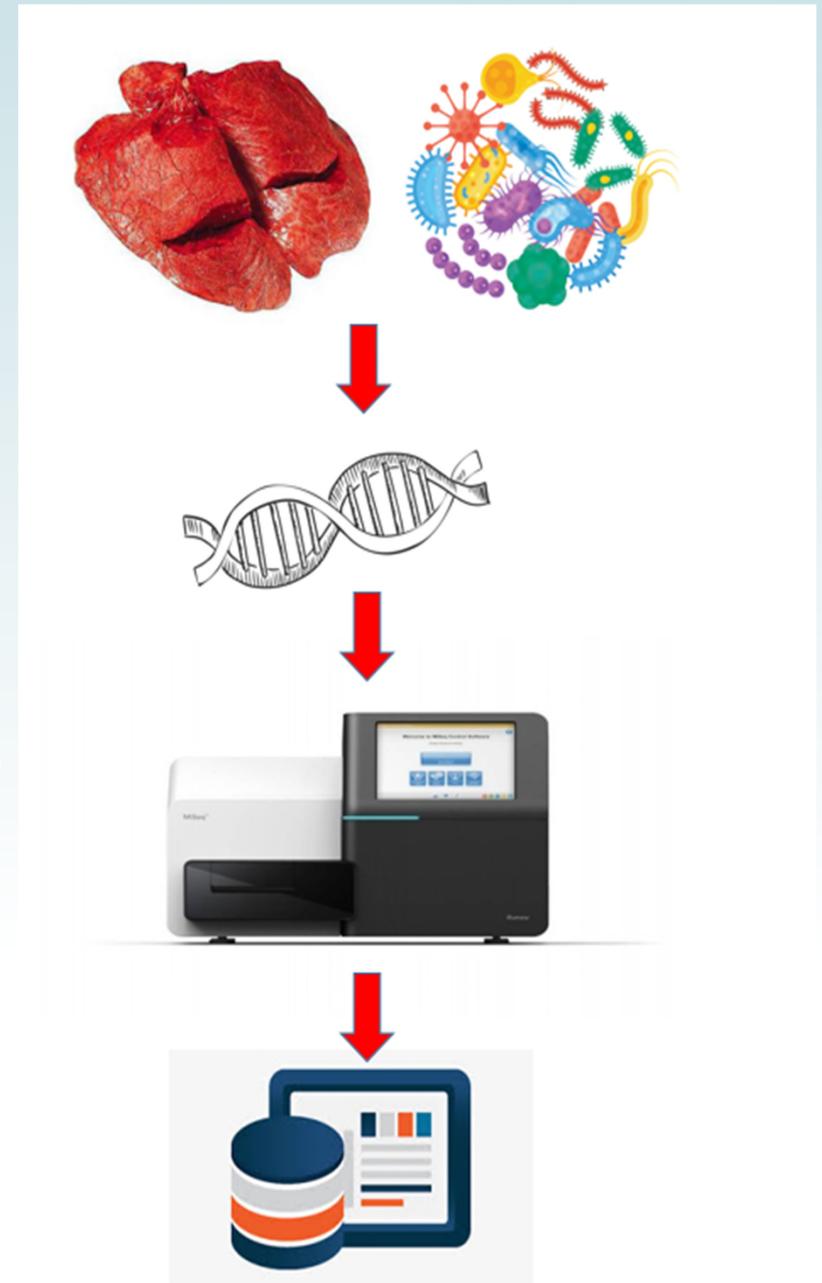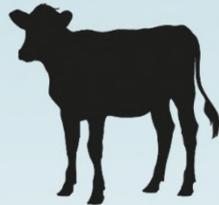(reference base: NCBI)
Processing of results

Case/control study

Calves 1-6 month
**From necropsy room**

With pneumonia lesions
N = 174

58 « resolved »
116 « not resolved »

Healthy lungs
N = 24

Trueperella pyogenes strain ATCC 19411 16S ribosomal RNA 30321 18.73%

Fusobacterium necrophorum strain JCM 3718 16S ribosomal RNA 29902 18.47%
Bacteroides heparinolyticus strain HEP 16S ribosomal RNA 22274 13.76%

Porphyromonas endodontalis strain JCM 8526 16S ribosomal RNA 18678 11.53%
Mycoplasma bovirhinis strain NBRC 14857 16S ribosomal RNA 12178 7.52%
Unclassified (conflicting taxonomy) 8538 5.27%
Porphyromonas somerae DSM 23386 strain JCM 13867 16S ribosomal RNA 7945 4.91%
Porphyromonas crevioricanis strain NUM 402 16S ribosomal RNA 6889 4.25%
Helcococcus ovis strain s840-96-2 16S ribosomal RNA 5002 3.09%
Parvimonas micra strain JCM 12970 16S ribosomal RNA 4696 2.90%
Peptoniphilus indolicus ATCC 29427 16S ribosomal RNA 3407 2.10%
Mycoplasma dispar strain 462/2 16S ribosomal RNA 3005 1.86%
Filifactor alocis strain ATCC 35896 16S ribosomal RNA 2509 1.55%
Unclassified (no match) 1603 0.99%
Bacteroides pyogenes strain DSM 20611 16S ribosomal RNA 1245 0.77%
….
….
….

Case/control study

POUMONS LESES POUMONS SAINS

Case/control study

POUMONS LESES POUMONS SAINS
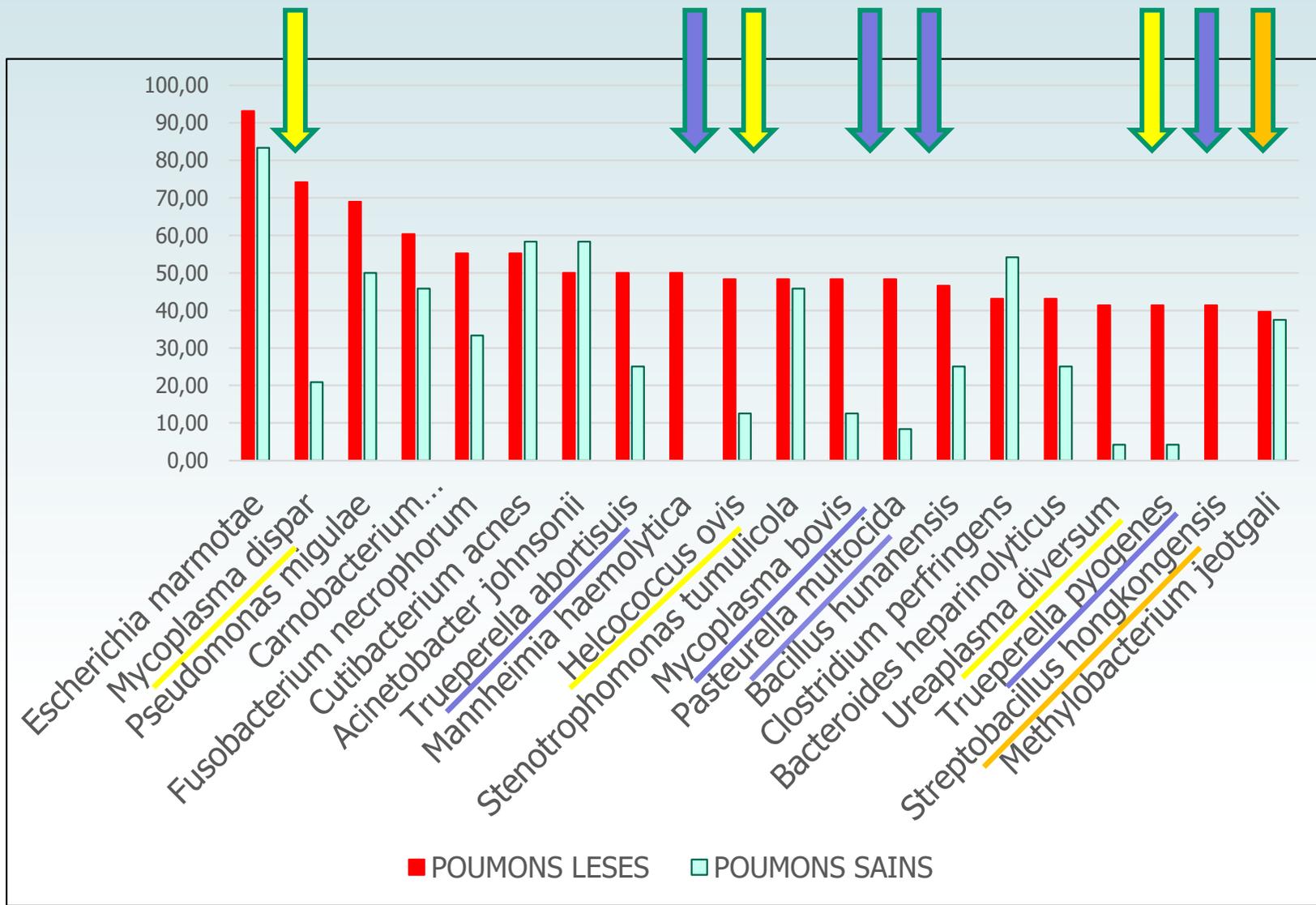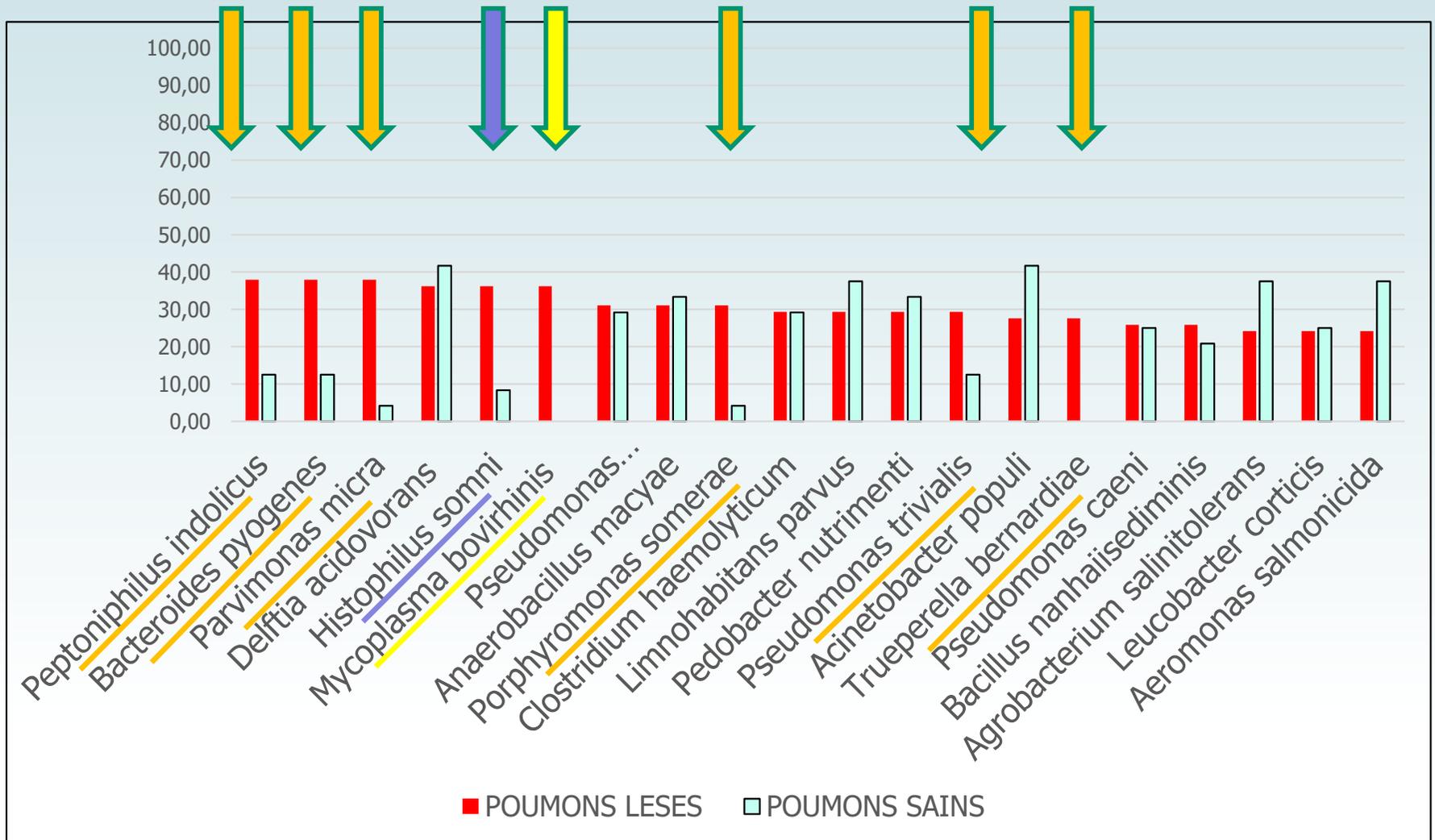
Case/control study

Pathogenic bacteria routinely tested :

Mannheimia haemolytica

Mycoplasma bovis

Pasteurella multocida

Trueperella pyogenes

Histophilus somni

Pathogenic bacteria not routinely tested :

*Mycoplasma dispar**

*Helcococcus ovis**

*Ureaplasma diversum**

*Mycoplasma bovirhinis**

Pathogenicity/identification not confirmed :

Streptobacillus hongkongensis

*Peptinophilus indolicus**

*Bacteriodes pyogenes**

Parvimonas micra

Porphyromonas somerae

Pseudomonas trivialis

Trueperella bernadiae (= Trueperella pyogenes?)

...

HELCOCOCCUS OVIS ISOLATED FROM SUPPURATIVE ARTHRITIS IN A LAMB

Lucienne Downs, District Veterinarian, Central Tablelands Local Land Services

Posted Flock & Herd August 2020

Biological Characteristics and Pathogenicity of *Helcococcus ovis* Isolated From Clinical Bovine Mastitis in a Chinese Dairy Herd

Kai Liu[1], Zhaoju Deng[2], Limei Zhang[1], Xiaolong Gu[1], Gang Liu[1], Yang Liu[2], Peng Chen[2], Jian Gao[2], Bo Han[2] and Weijie Qu[1]

Association of *Ureaplasma diversum* with reproductive disease in cattle

* Taxons confirmed by PCR (ULiège) or bacteriology

Resolving these 'unresolved' cases by 16S?

Case	Description	1	2	3	4	5	6	7	8	9	10	
CI-19-164888	CI-19-164888-001 AT Avis du pathologiste sur ce dossier Commentaire: CI-19-164888-001 AT Résultat de l'autopsie OK CI-19-164888-001 BA Culture Aérobie Flore non spécifique	Helcococcus ovis strain s840-96-2 16S ribosomal RNA 11090 28.53%	Unclassified (no match) 9166 23.58%	Trueperella bernardiae strain LCDC 89/0504 16S ribosomal RNA 7852 20.20%	Ignatzschineria indica strain FFA1 16S ribosomal RNA 3087 7.94%	Streptobacillus hongkongensis strain HKU33 16S ribosomal RNA 3017 7.76%	Pseudomonas silesiensis strain A3 16S ribosomal RNA 842 2.17%	Streptobacillus felis strain 131000547 16S ribosomal RNA 676 1.74%	Ureaplasma diversum strain A417 16S ribosomal RNA 548 1.41%	Unclassified (conflicting taxonomy) 461 1.19%	Moraxella cuniculi strain CCUG 2154 16S ribosomal RNA 454 1.17%	...
CI-19-167815	CI-19-167815-001 AT Avis du pathologiste sur ce dossier Commentaire: CI-19-167815-001 AT Résultat de l'autopsie OK CI-19-167815-001 BA Culture Aérobie Flore non spécifique	Ureaplasma diversum strain A417 16S ribosomal RNA 60782 71.88%	Pseudomonas chlororaphis subsp. aurantiaca strain NCIB 10068 16S ribosomal RNA 9178 10.85%	Aeromonas salmonicida subsp. masoucida strain NBRC 13784 16S ribosomal RNA 4642 5.49%	Unclassified (no match) 3560 4.21%	Unclassified (conflicting taxonomy) 2962 3.50%	Pseudomonas tolaasii strain ATCC 33618 16S ribosomal RNA 863 1.02%	Erwinia persicina strain NBRC 102418 16S ribosomal RNA 510 0.60%	Pseudomonas stutzeri ATCC 17588 = LMG 11199 16S ribosomal RNA 492 0.58%	Mycoplasma dispar strain 462/2 16S ribosomal RNA 386 0.46%	Serratia liquefaciens strain ATCC 27592 16S ribosomal RNA 161 0.19%	...
CI-19-231163	CI-19-216040-003 BO Biothèque oui CI-19-231163-001 AT Avis du pathologiste sur ce dossier Commentaire: CI-19-231163-001 AT Résultat de l'autopsie OK CI-19-231163-001 BA Culture Aérobie Envahissement par Proteus sp	Pseudomonas mendocina strain ATCC 25411 16S 15433 29.17%	Mycoplasma bovirhinis strain NBRC 14857 16S 9950 18.80%	Pseudomonas aeruginosa strain DSM 50071 16S 7416 14.01%	Unclassified (conflicting taxonomy) 7272 13.74%	Bacteroides pyogenes strain DSM 20611 16S 5992 11.32%	Xenorhabdus hominickii strain KE01 16S 1557 2.94%	Proteus mirabilis strain ATCC 29906 16S 1276 2.41%	Proteus alimentorum strain 08MAS0041 16S 1090 2.06%	Mycoplasma dispar strain 462/2 16S 846 1.60%	Trueperella pyogenes strain ATCC 19411 16S 593 1.12%	...
CI-20-056519	CI-20-056519-001 AT Avis du pathologiste sur ce dossier Commentaire: CI-20-056519-001 AT Résultat de l'autopsie OK CI-20-056519-001 BA Culture Aérobie Contaminants CI-20-056519-001 BA Culture Aérobie Flore non spécifique	Mannheimia haemolytica strain NCTC 9380 16S ribosomal RNA 18136 52.93%	Unclassified (no match) 4692 13.69%	Pseudomonas silesiensis strain A3 16S ribosomal RNA 3545 10.35%	Pseudomonas tolaasii strain ATCC 33618 16S ribosomal RNA 1322 3.86%	Serratia liquefaciens strain ATCC 27592 16S ribosomal RNA 996 2.91%	Gardnerella vaginalis ATCC 14018 = JCM 11026 16S ribosomal RNA 677 1.98%	Unclassified (identity too low) 516 1.51%	Escherichia marmotae strain HT073016 16S ribosomal RNA 490 1.43%	Unclassified (conflicting taxonomy) 384 1.12%	Corynebacterium coyleae strain DSM 44184 16S ribosomal RNA 230 0.67%	...
CI-21-012910	CI-21-012910-001 AT Avis du pathologiste sur ce dossier Commentaire: CI-21-012910-001 AT Résultat de l'autopsie OK	Unclassified (conflicting taxonomy) 16845 39.27%	Unclassified (no match) 6093 14.21%	Pseudomonas tolaasii strain ATCC 33618 16S ribosomal RNA 4910 11.45%	Staphylococcus capitis strain JCM 2420 16S ribosomal RNA 4093 9.54%	Ureaplasma diversum strain A417 16S ribosomal RNA 2981 6.95%	Cutibacterium acnes strain ATCC 6919 16S ribosomal RNA 1196 2.79%	Staphylococcus aureus strain ATCC 12600 16S ribosomal RNA 935 2.18%	Staphylococcus pasteuri strain ATCC 51129 16S ribosomal RNA 686 1.60%	Corynebacterium tuberculostearicum strain Medalle X 16S ribosomal RNA 502 1.17%	Polygonibacillus indicireducens strain In2-9 16S ribosomal RNA 457 1.07%	...

✓ **Approximately 50% of "unsolved" cases solved with 16S**

Resolving these 'unresolved' cases by 16S?

Limitations of the 16S method:

- Identification of taxa
- Lot of different bacteria
- Considering as pathogenic or not pathogenic
- Quality of samples and "invasion" by contaminating bacteria
- What about the viral sphere?

Conclusions et perspectives

Some (nice) surprises

Some (nice) challenges

Conclusions et perspectives

Some nice surprises!

✓ Confirmation of pathogens researched in routine
✓ Identification of "new" pathogens of interest
✓ Opening new areas for exploration
✓ Usable in routine?
    - Currently complex and time-consuming at the technical and data processing levels --> bioinformatics flow to be consolidated
      - Only targets the bacterial sphere
      - Interesting for "difficult" cases

Most interest is the exploration of underestimated or unknow pathogens => Identification of new/not yet knowed risks for respiratory diseases in bovine

Slide 14

CQ1   Christian Quinet, 15/04/2022

Some nice challenge!

➢ Fu

➢ C

➢ E
interest

> New case/control study for Helcococcus ovis,
> Mycoplasma dispar et Ureaplasma diversum :
>   - Prevalence (with homemade PCR)
>   - Epidemiological study
>   - Standardised histology

Slide 15

CQ1   Christian Quinet, 15/04/2022

Some nice challenge!

➢ Further analysis of the results :
- Quantitative aspect
- Co-infections
- Comparison of reference databases

➢ Confirmation of taxa of interest

➢ Epidemiologic and pathological study on confirmed taxas of interest

➢ Investigation of other systems/pathologies (abortions, metritis, ...)

➢ Investigation of the viral sphere

Slide 16

A team work !

ARSIA

    Molecular Biology Department : Isabelle, Pascale et Julie

    Pathology Department: Thierry, Frédéric and staff

    Julien Evrard, coordinator of all Arsia projects

    Fabien Gregoire, manager of this project

ULiège, Veterinary University of liège

    Prof. MM Garigliani

Thanks for attention !