

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

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ARSIA

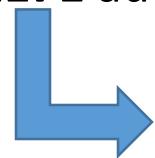
Association Régionale d'Identification et de Santé Animales

Symposium de Santé Animale – Symposium voor Dierengezondheid – Sciensano – 22/03/2023

Pneumonia in cattle in ARSIA : some figures

In 2021 :

2171 autopsied bovine animals (all ages groups)



1416 autopsied calves (0-6 mois)



405 calves (+/- 30%) with respiratory lesions (pneumonia)



Causal agent(s) found in +/- 80% of the cases

On the basis of :

Macroscopic examination

Histology

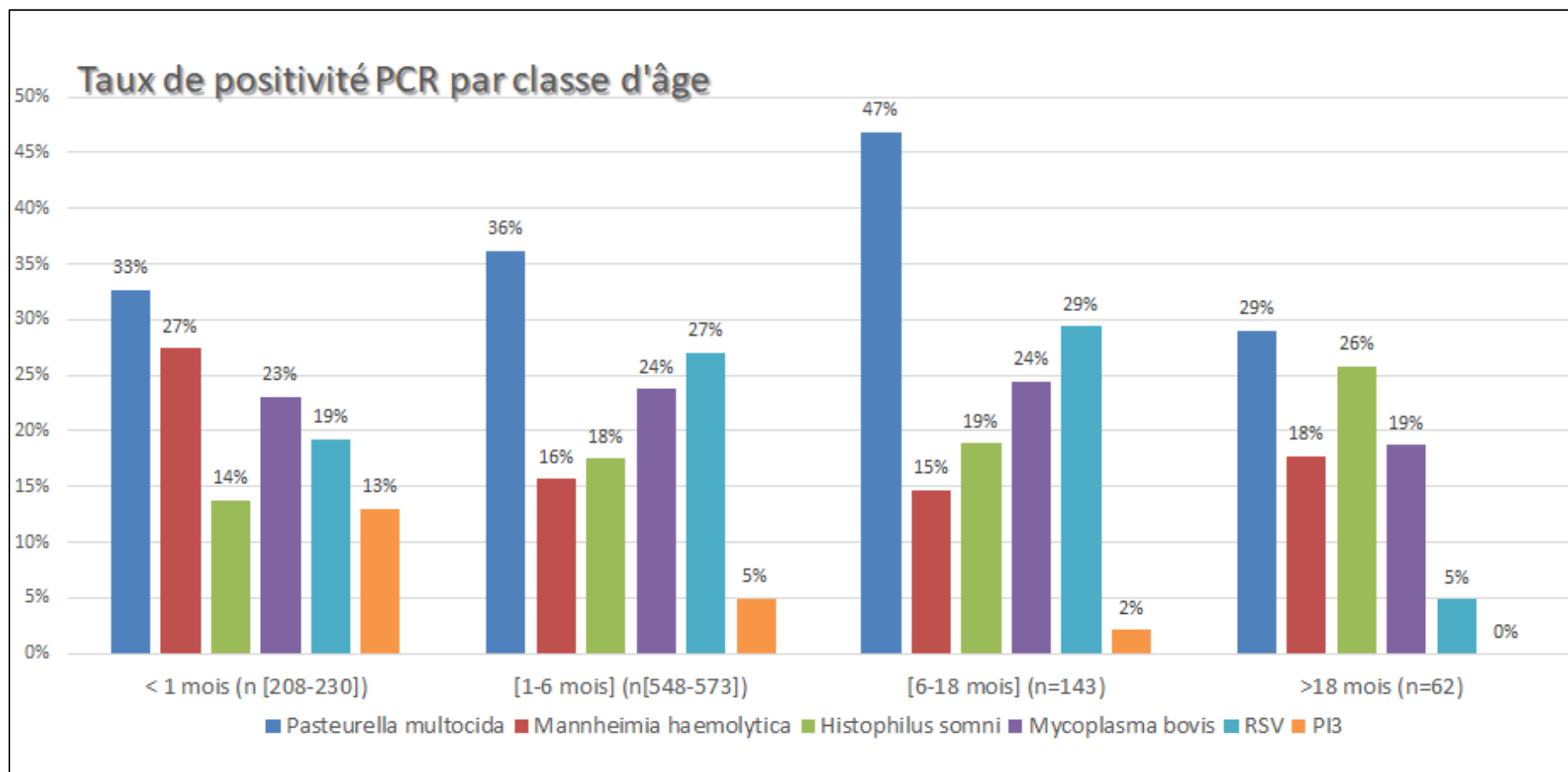
Bacteriological culture (Gassner – blood agar – chocolate agar – CNA – PAM)

PCR (BRSV – PI3 – *Mycoplasma bovis* – *Pasteurella multocida* – *Mannheimia haemolytica* – *Histophilus somni*) Thermo Fisher



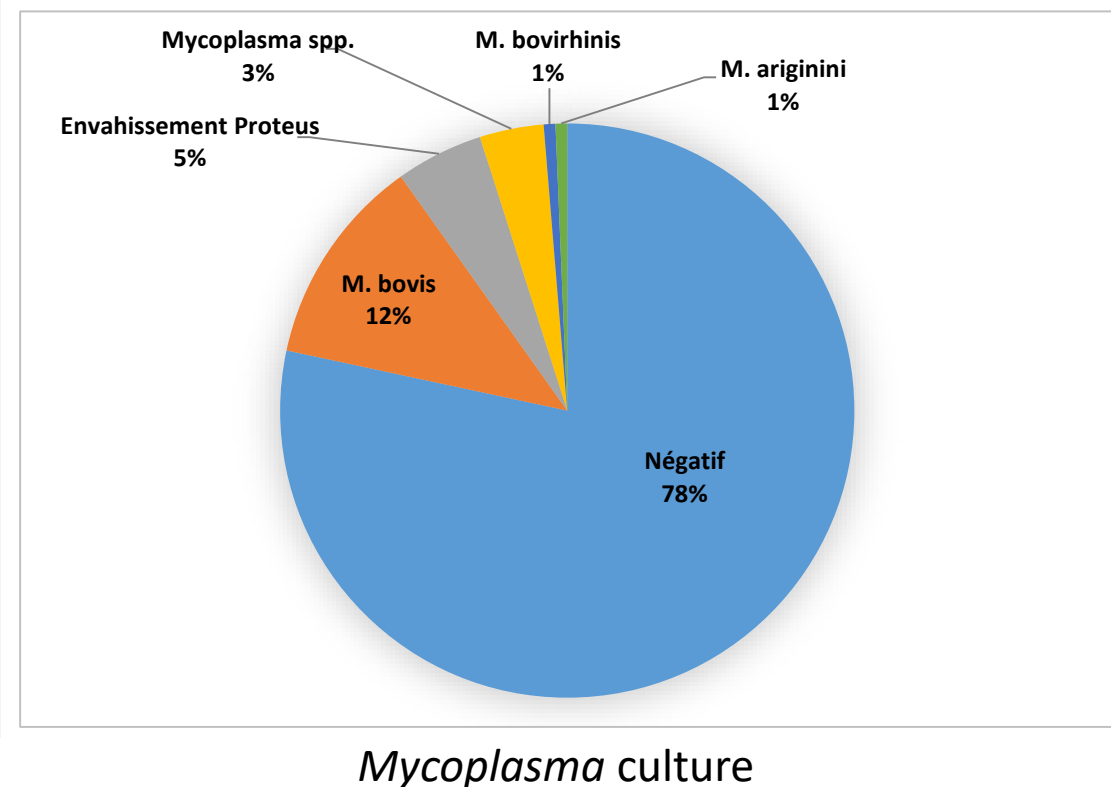
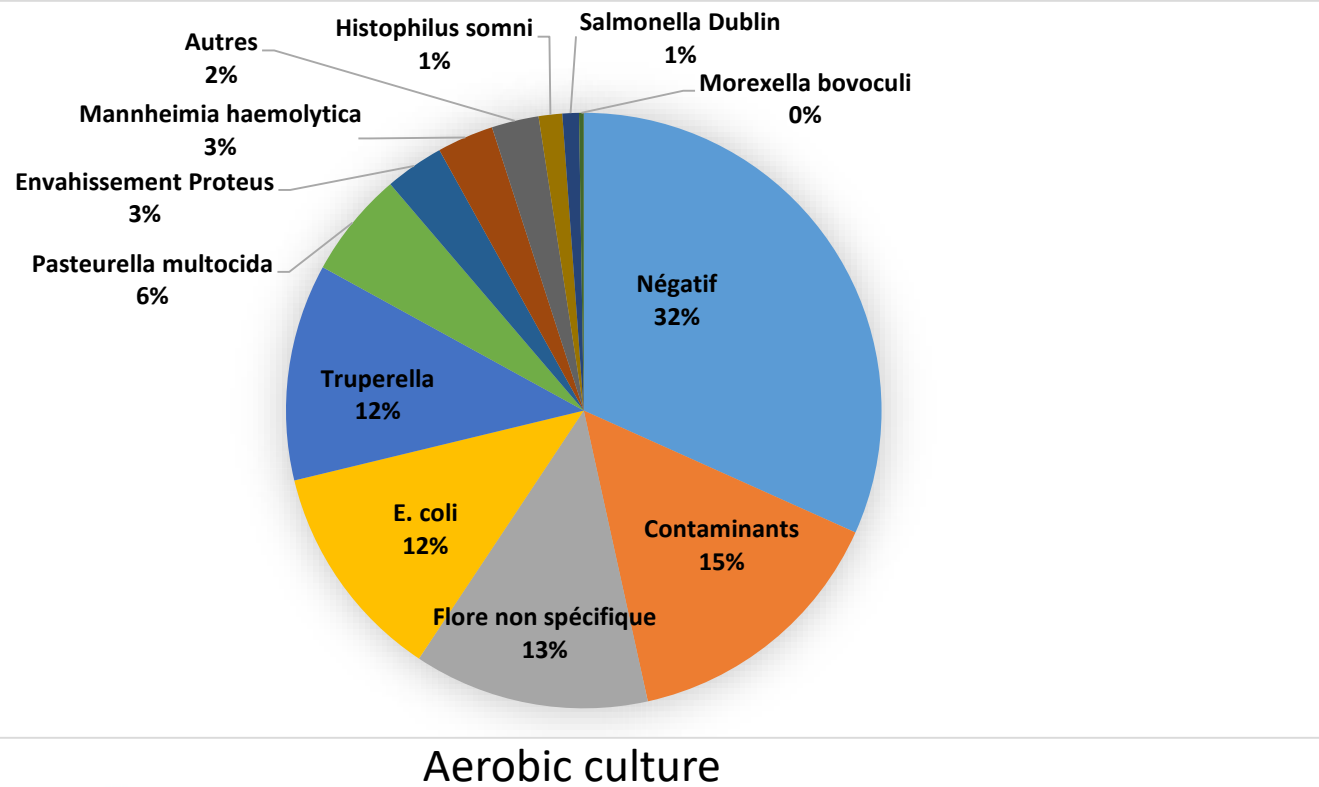
Pneumonia in cattle in ARSIA : some figures

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



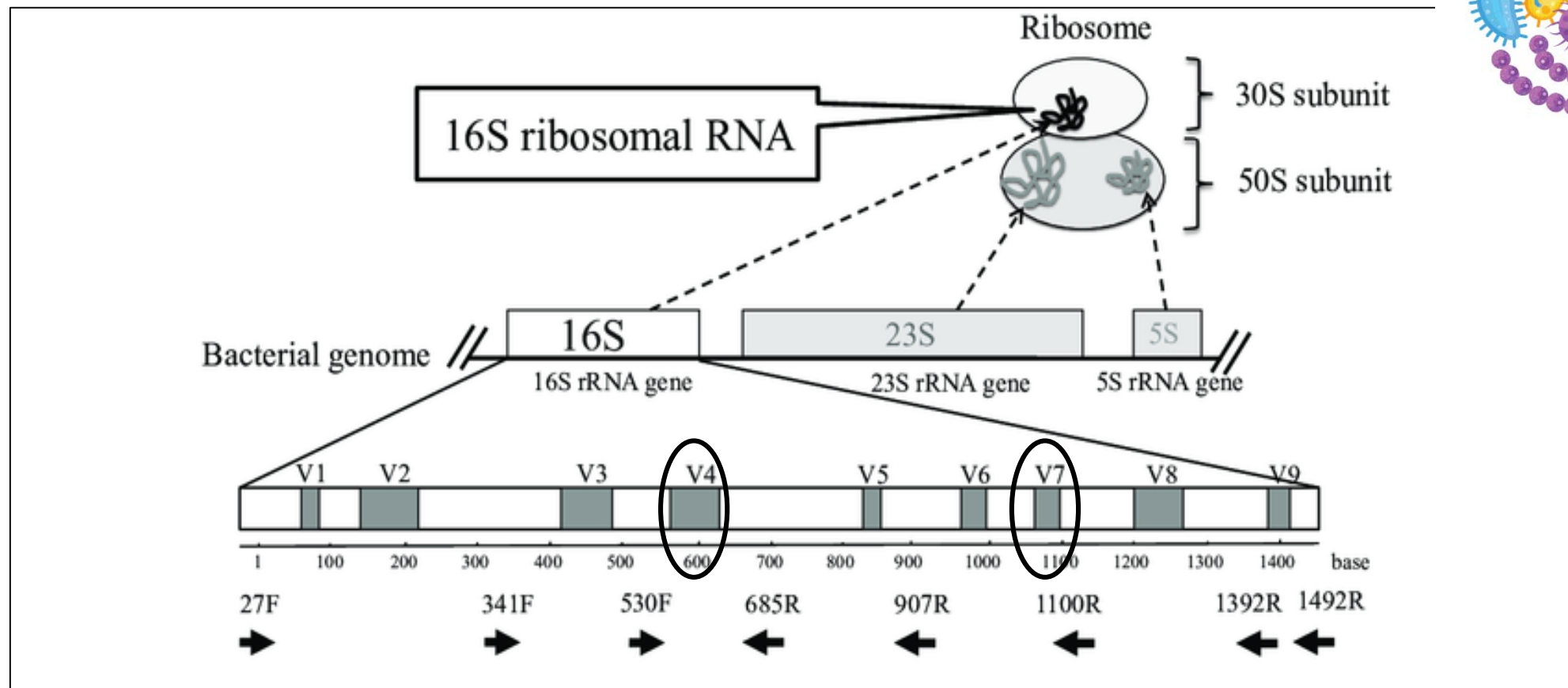
Pneumonia in cattle in ARSIA : some figures

Results of bacterial cultures on lungs from autopsy (2020):



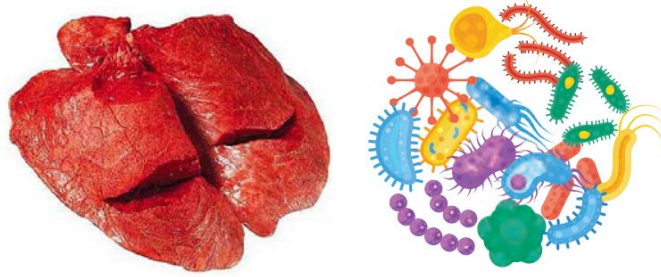
Exploring the 20% unknown gap with 16S rRNA metagenomics

Project financed by the Belgian Public Health ministry



Kazumasa Fukuda, 2016. DOI:[10.7888/juoeh.38.223](https://doi.org/10.7888/juoeh.38.223)





Lung Sampling during necropsy
Storage at -20°C
Disinfection with ethanol (min 24h)
Rinsing with PBS et sampling in the center of the piece



DNA extraction
DNA quantification (Quantus™ Promega)

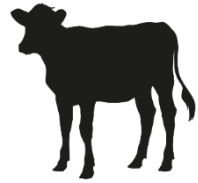


PCR 16S regions V4 et V7 (Primers ThermoFisher)
Index PCR (Nextera XT Index kit - Illumina)
Librairies preparation
Sequencing Illumina Miseq



Data analysis with software logiciel Geneious
(reference database: NCBI)
Results processing





**Calves 1-6 months
Autopsy**



**Lungs with pneumonia
N = 174
58 « solved »
116 « unsolved »**

**Healthy lungs
N = 24**

Routine panel
of PCR and cultures

+



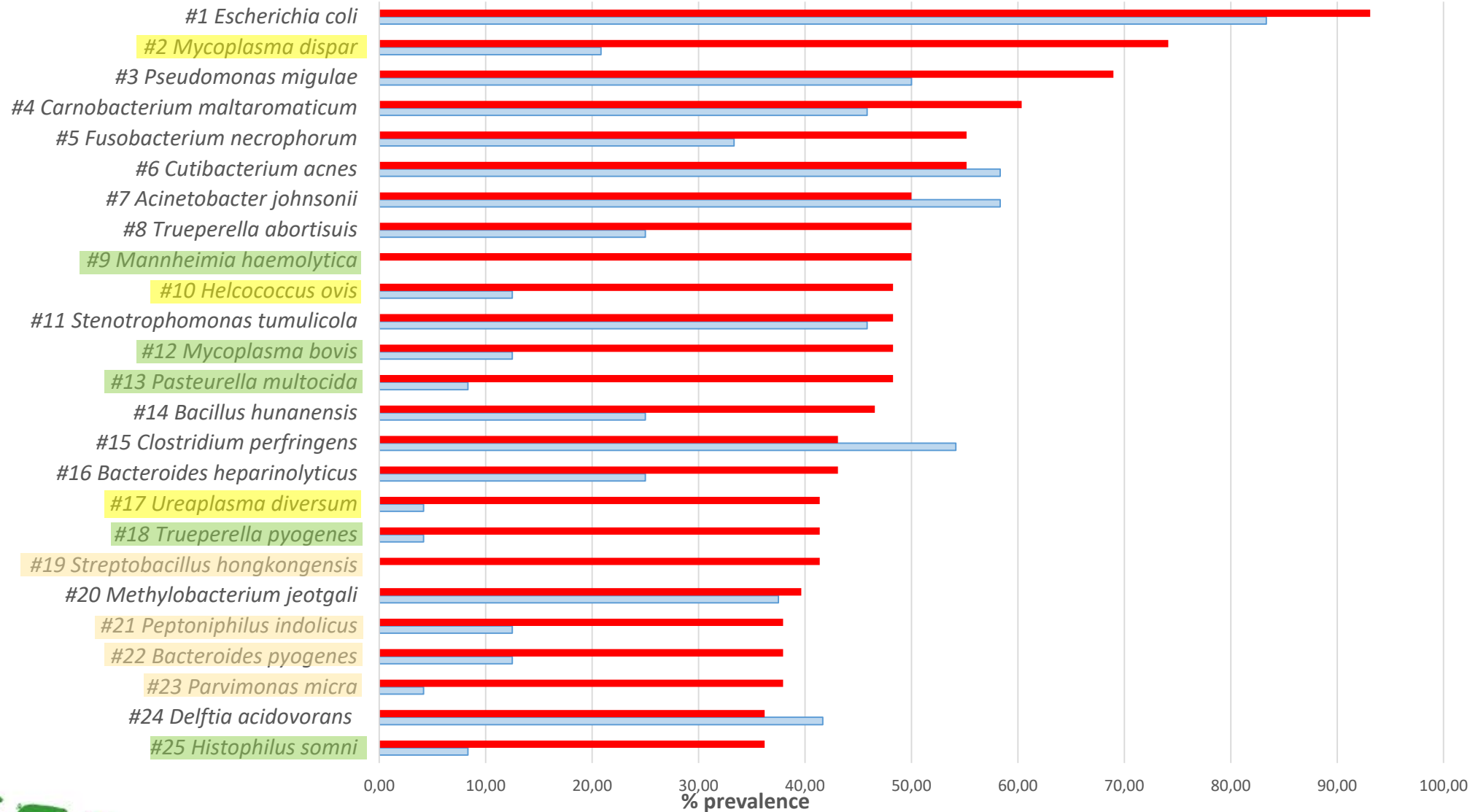
Trueperella pyogenes strain ATCC 19411	16S ribosomal RNA	30321	18.73%
Fusobacterium necrophorum strain JCM 3718	16S ribosomal RNA	29902	18.47%
Bacteroides heparinolyticus strain HEP	16S ribosomal RNA	22274	13.76%
Porphyromonas endodontalis strain JCM 8526	16S ribosomal RNA	18678	11.53%
Mycoplasma bovirhinis 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%
....			
....			
....			



Most prevalent bacterial taxa found in lungs with pneumonia (Calves 0-6 months)

In red : prevalence in lungs with macroscopic lesions

In blue : prevalence in lungs with no lesions





Helcococcus ovis – *Ureaplasma diversum* – *Mycoplasma dispar*

What's next?

Review > *Isr J Med Sci.* 1987 Jun;23(6):621-4.
Comparative pathogenicity of *Mycoplasma bovis* and *Mycoplasma dispar* for the respiratory tract of calves
 C J Howard¹, L H Thomas, K R Parsons
 Affiliations + expand
 PMID: 3312099
 Vol. 46, No. 10

frontiers
in Veterinary Science

published: 18 February 2021
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A Review of *Ureaplasma diversum*: A Representative of the Mollicute Class Associated With Reproductive and Respiratory Disorders in Cattle

Manoel Neres Santos Junior^{1,2}, Nayara Silva de Macêdo Neres¹,
 Guilherme Barreto Campos¹, Bruno Lopes Bastos¹, Jorge Timenetsky³ and
 Lucas Miranda Marques^{1,2,3*}

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JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2008, p. 3291–3295
 0095-1137/08/\$08.00+0 doi:10.1128/JCM.00867-08
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***Helcococcus ovis*, an Emerging Pathogen in Bovine Valvular Endocarditis[∇]**
 Peter Kutzer,^{1*} Christoph Schulze,¹ Andreas Engelhardt,¹ Lothar H. Wieler,² and Marcel Nordhoff²
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Received 6 May 2008/Returned for modification 6 July 2008/Accepted 7 August 2008

Helcococcus ovis – *Ureaplasma diversum* – *Mycoplasma dispar* What's next?

1) Development of a real-time PCR test and testing of routine samples

Ureaplasma diversum (kit developed and commercialized by Genetic PCR solutions™)

Lungs with pneumonia	N = 100	29 %
« Healthy » lungs	N = 20	5 % (1 pos, Ct > 35)

Helcococcus ovis (kit developed in partnership by ULiège)

Lungs with pneumonia	N = 100	26 %
« Healthy » lungs	N = 20	0 %

Mycoplasma dispar (kit developed in partnership by ULiège)

Lungs with pneumonia	N = 100	70 %
« Healthy » lungs	N = 20	5 % (1 pos, Ct > 35)



Helcococcus ovis – *Ureaplasma diversum* – *Mycoplasma dispar*

What's next?

- 1) Development of a real-time PCR test and testing of routine lung samples **In progress**
- 2) Evaluation of histopathologic lesions (ULiège) **Results upcoming**
- 3) PCR tests and specific culture on *In vivo* samples (bronchoalveolar lavage and nasal swabs) **In progress**



Is this 16S metagenomic method applicable for routine diagnostic?

- Good concordance between 16S and targeted real-time PCR
- Currently time-consuming and laborious (technique AND data process)
- Real interest for the non elucidated cases

In our study, about 50% of the cases of the unsolved cases found an etiology with this method

! Only screening the bacteriological sphere !

Wonderful tool for opening new exploration paths and highlighting new pathogens



Ongoing reflexions and upcoming challenges

- Deeper analysis of the results :
 - co-infections
 - « Quantitative » aspect of the presence of bacteria
- Confirmation of taxa of interest
- Epidemiological and pathological aspect of *M. dispar*, *H. ovis* and *U. diversum* (PCR. Serology?)
- Exploration of other systems/pathologies (abortions, metritis, ...)
- Exploration of the viral sphere





A team work!

ARSIA

Molecular biology: Isabelle, Pascale, Julie and the team

Pathology/bacteriology : Thierry, Frédéric, Marc and the team

GPS Projects : Julien Evrard and Marie Léonard

Christian Quinet, Director of the laboratory

ULiège, Faculté de Médecine Vétérinaire

Prof. MM Garigliani

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Thank you for your attention