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Philippe Pérot, Béatrice Regnault, Franck Bielle, Jacques Fourgeaud, Éric Troadec, et al.. Necker-Pasteur Clinical Metagenomics: Part 2. Contribution to Pathogen Discovery. 6th International Conference on Clinical Metagenomics, Oct 2021, Geneva, Switzerland. . pasteur-03467165

HAL Id: pasteur-03467165
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Submitted on 6 Dec 2021

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Necker-Pasteur Clinical Metagenomics: Part 2. Contribution to Pathogen Discovery

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BACKGROUND

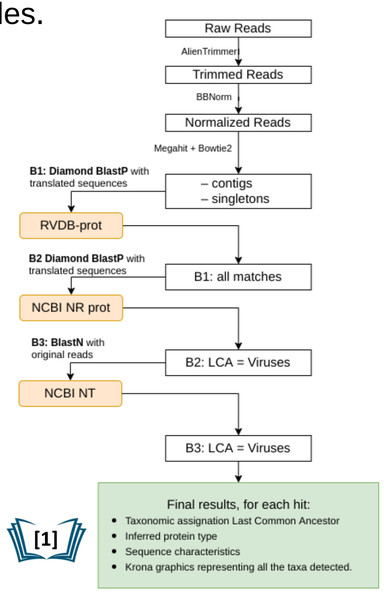
Clinical metagenomics fuels pathogen discovery but a comprehensive research environment is required to address critical questions such as imputability, physiopathology of novel identified infectious agents, and development of new diagnostic tools. The Institut Pasteur, in close collaboration with specialized hospital clinical services and laboratories including (neuro)pathologists, along with other academic labs, seeks to address these topics.

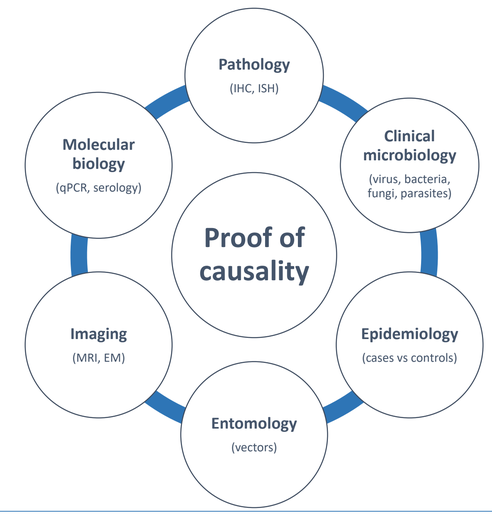
METHODS

We present the research environment, including sequencing and analyzing tools, molecular and cellular biology, virology, histology and entomology resources, that promotes the exploration of novel or unexpected pathogens identified from patient samples.



1. A dedicated sequencing platform, BioIT infrastructure and pipeline





2. A network of trained (neuro)pathologists, virologists, entomologists and molecular biologists

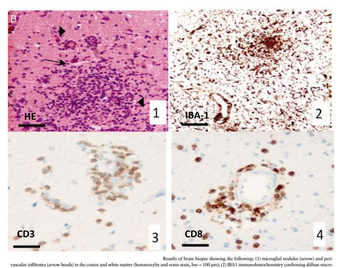
RESULTS

We review some of the major identifications done in the course of our historical partnership with hospitals, by highlighting the place and the contribution of research works following identification of new or unexpected microorganisms. The list includes novel human pathogens with a zoonotic potential (Astrovirus VA1, Umbre orthobunyavirus, European Bat Lyssavirus 1) and known pathogens with unexpected or atypical presentation (Rubella virus, Rabies, Dengue).

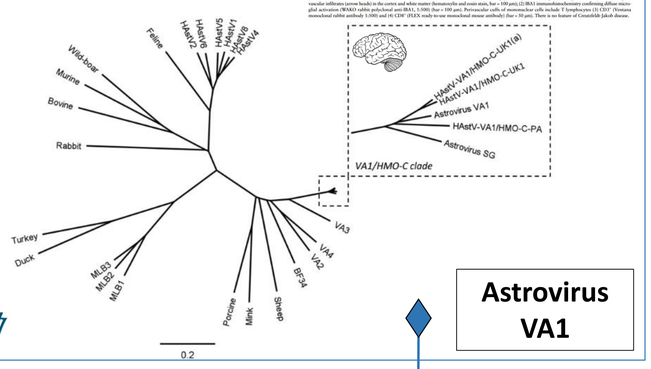
Novel human pathogens with a zoonotic potential

Astrovirus VA1

- 14 year old boy, Bruton agammaglobulinemia
- Cognitive impairment noted over the last 4 years
- Ataxia, erratic myoclonies, and dysarthria
- MRI: diffuse cortical-subcortical brain atrophy and periventricular hypersignals
- CSF: normal.
- Histologic findings consistent with acute pan-encephalitis
- Astrovirus VA1 identified by NGS from brain biopsy (in 3 days from sample to result)
- Actionable treatment: ribavirin + pegylated IFN



Results of brain biopsy showing the following: (1) microglial nodules (arrows) and perivascular infiltrates (arrow heads) in the cortex and white matter (hematoxylin and eosin stain, bar = 100 µm), (2) IBA1 immunohistochemistry confirming diffuse microglial activation (WAKO rabbit polyclonal anti-IBA1, 1:500) (bar = 100 µm). Perivascular cuffs of enormous-size cells include T lymphocytes (3: CD3+ (Tentarra monoclonal rabbit antibody 3.1500) and (4) CD8+ (FLEX ready-to-use monoclonal mouse antibody) (bar = 50 µm). There is no feature of Creutzfeldt-Jakob disease.

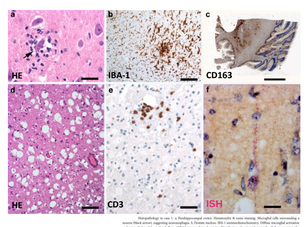


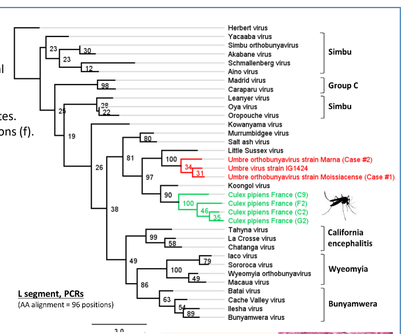
[2-3]

Umbre orthobunyavirus

Case #1

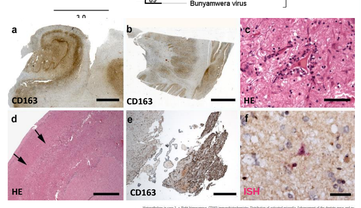
- 21 year old man, Bruton agammaglobulinemia.
- No trip outside France.
- Rapid deterioration of the neurological state, tetrapyramidal syndrome, progression to mutism and grabatisation.
- Post-mortem brain samples: spongiosis (PrP neg), diffuse microglial activation and peri-vascular inflammatory infiltrates.
- Umbre virus detected by NGS and confirmed by ISH in neurons (f).





Case #2

- 58 year old woman, complexe immunologic disorder.
- Trip in South of France (Camargue) a few weeks before symptoms appear.
- Psychomotor slowing down then deterioration of the neurological state over 4 months, leading to mutism then coma and death.
- Post-mortem brain samples: diffuse microglial activation, perivascular lymphocytic infiltrates, severe neuronal loss with complete loss of Purkinje cells.
- Umbre virus detected by NGS and confirmed by ISH in neurons (f).



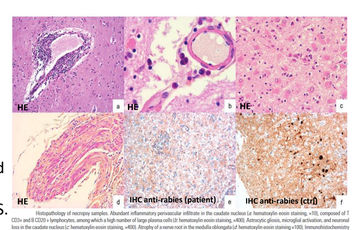
Analysis of Camargue mosquitoes

- 4,000 Culex pipiens mosquitoes captured in 2015, 133 pools analyzed (RT-PCR)
- 4 pools positif fo rKoongol (green) related to Umbre virus (red)

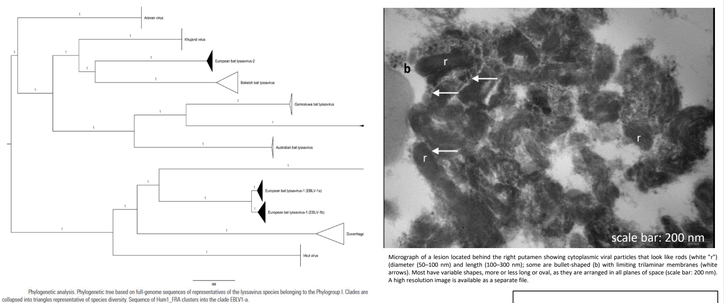
[4]

European Bat Lyssavirus 1

- 59 year old patient without past medical history.
- No travel outside France in the previous 2 years.
- Bats colony lived in an outbuilding of his house.
- Phono- and photophobia, vomiting, dysarthria, and swallowing disorders, progression to a nonreactive coma and clinical seizures.
- abundant inflammatory perivascular in-filtrates, astrocytic gliosis, and microglial activation, limited necrosis. Loss of Purkinje cells.
- Identification of European Bat Lyssavirus 1 by NGS.



Histopathology of necropsy samples. Abundant inflammatory perivascular infiltrate in the caudate nucleus (a: hematoxylin-eosin staining, ×10), composed of T CD3+ and B CD20+ lymphocytes, among which a high number of large plasma cells (b: hematoxylin-eosin staining, ×400). Astrocytic gliosis, microglial activation, and neuronal loss in the caudate nucleus (c: hematoxylin-eosin staining, ×400). Atrophy of a nerve cell in the medulla oblongata (d: hematoxylin-eosin staining ×100). Immunohistochemistry against rabies was negative (e: caudate nucleus, ×200), whereas the cerebral cortex of a rabid patient was positive (f: cerebral cortex ×200).



Phylogenetic analysis. Phylogenetic tree based on full-genome sequences of representatives of the lyssavirus species belonging to the Phylogroup 1. Clades are collapsed into triangles representative of species diversity. Sequence of Hum/_FRA clusters into the clade EBLV1-a.

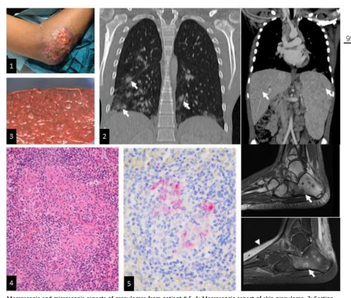
Micrograph of a lesion located behind the right putamen showing cytoplasmic viral particles that look like rods (white "r") (diameter: 150–100 nm) and length (100–300 nm); some are bullet-shaped (b) with limiting trilaminar membranes (white arrows). Most have variable shapes, more or less long or oval, as they are arranged in all planes of space (scale bar: 200 nm). A high resolution image is available as a separate file.

[5]

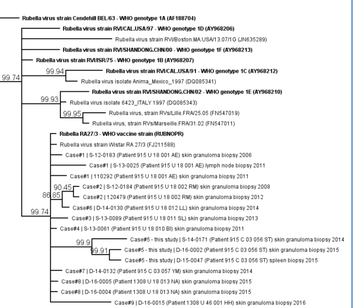
Known human pathogens with unexpected presentation

Rubella virus, vaccine strain (cutaneous and visceral granulomas)

- Persistence of rubella live vaccine in cutaneous and visceral granulomas associated with a Primary Immunodeficiency (PID).
- 9 PID pediatric cases described by our group in the period 2013-2019: Ataxia-telangiectasia (x5), RAG1 deficiency (x1), RAG2 deficiency (x1), Activated phosphoinositide 3-kinase δ syndrome (x1) and Undefined combined Immunodeficiency (x1).



Macroscopic and microscopic aspects of granulomas from patient # 5. 1: Macroscopic aspect of skin granuloma. 2: Section of the spleen showing macroscopic aspects of granuloma: diffuse multimicrovacuolar pattern linked to the presence of numerous granulomas. 3A: Thoracic coronal CT image showing diffuse nodular and micronodular lesions (arrows). 3B: Coronal reconstruction of thoraco-abdominal enhanced CT showing mediastinal homogeneous adenomegalies (head arrow) and heterogeneous hepatosplenomegaly (arrows). 3C and D: Sagittal T1 and fat suppressed T2 weighted MR of the right ankle reveal multiple and diffuse intraosseous lesions without periosteal reaction, with adjacent soft tissues infiltration (head arrows). 4: Numerous epithelioid granuloma organization of the spleen. 5: Immunohistochemistry with anti-rubella capsid antibody of the spleen, Fast Red: strong cytoplasmic signal within numerous histiocytes and epithelioid cells.

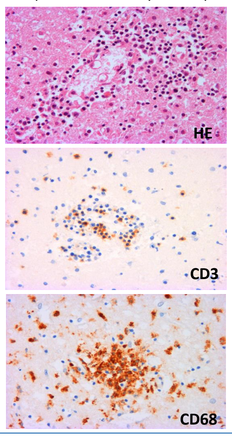


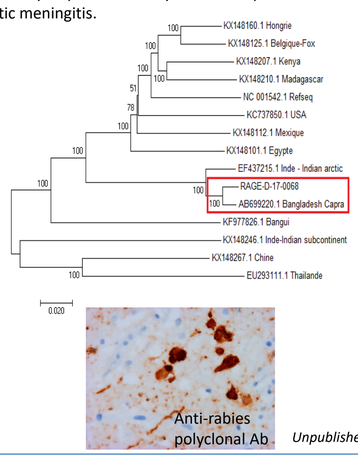
The alignment of the RV sequences identified in the 9 clinical cases (see table S5), along with a selection of references RV strains. The alignment of the RV sequences identified in the 9 clinical cases (see table S5), along with a selection of references RV strains. WHO reference strains for RV genotyping and RV vaccine are indicated in bold. Sequences accession numbers are indicated in parentheses.

[6-8]

Rabies (16 months post bite)

- Czech patient in France for tourism. No notable antecedent, sporty.
- Bitten by a dog during a trip to Bangladesh 16 months ago.
- 5 June 2016: Spontaneous onset of pain in the lower right limb. June 18: febrile context, headache, aphasia, confusion, visual hallucinations and hydrophobia. 13 July 2016: encephalic death.
- Repeated CSF analysis: aseptic lymphocytic meningitis.

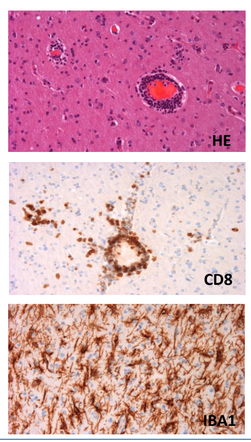


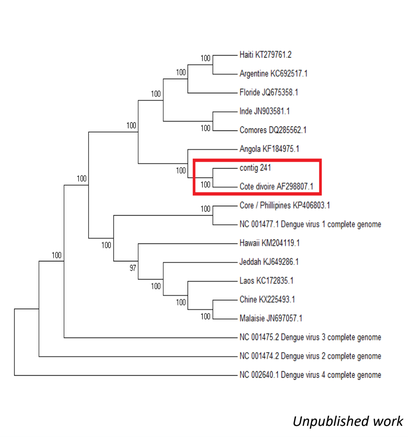


Unpublished work

Dengue (neurologic form)

- Patient from Togo. No recent trip. HIV+ (in therapeutic rupture)
- Neurological worsening in 15 days: headache, ataxia then coma
- Tetrapyramidal syndrome, tetraparesis, pseudo-choreic movements of the 4 limbs
- Normal autoimmune balance and normal tumor markers.





Unpublished work

2013 2014 2015 2016 2017 2018 2019 2020 2021

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CONCLUSION

Pathogen discovery includes the identification and characterization of new or unexpected infectious agents and the demonstration of their causality, with the objectives of understanding their biology and fostering the development of novel diagnostic or therapeutic tools. The goal of the Pasteur-Necker initiative in the coming years is to become a national center for clinical metagenomics combining patient care and fundamental research with potential for medical applications, for the benefit of science and patients.

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