

Laboratory confirmation and attribution of biological threats

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NRL, EURL, RL WOAHA, RC FAO for Foot-and-Mouth Disease

ANSES/LSAn/UMR VIROLOGIE



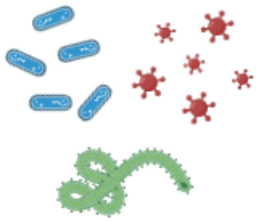
EURL
European Union Reference Laboratory for
FOOT-AND-MOUTH DISEASE

OMSA Laboratoire de référence
pour la Fièvre Aphteuse

Centre de référence  Organisation mondiale
de la santé animale
Fondée en tant qu'OIE



Biological threats



- Living microorganisms (bacteria, virus, fungi...) or toxins from microbes
- Cause disease or death of humans, animals or plants
- Apparently easy to produce and use of infectious microorganisms
- A large number of states or non-state groups wished to own a biological arsenal

Biological threats



- High infectious power, high virulence, not or few treatment or vaccine available (only few biological agents)
- Need to be weaponized to be efficient (in most cases)
- Agroterrorism also (against cultures, against livestock, fishes)
- **Not to forget:** the panic among population (disorganization...)
- The use of biological weapons is very old (since Antic times)
- Used during wars (Unit 731 in Japan, Biopreparat in Soviet Union, COAST project, Irak program...)



[Distribution de couvertures contaminées par la variole en 1764](#)

Biological weapons are considered as weapons of mass destruction



United
Nations

Office for Disarmament
Affairs

UNODA

[Treaties Database Home](#)

Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on Their Destruction

Status of the treaty

[Text of the treaty](#)

Opened for signature at London, Moscow and Washington: 10 April 1972

Entered into force: 26 March 1975

Depositary Governments: Russian Federation, United Kingdom of Great Britain and Northern Ireland and United States of America

Number of Signatory States: 109

Number of States Parties: 184

But these microorganisms cause also natural diseases

The example of Foot-and-Mouth disease



(USDA, 1916)

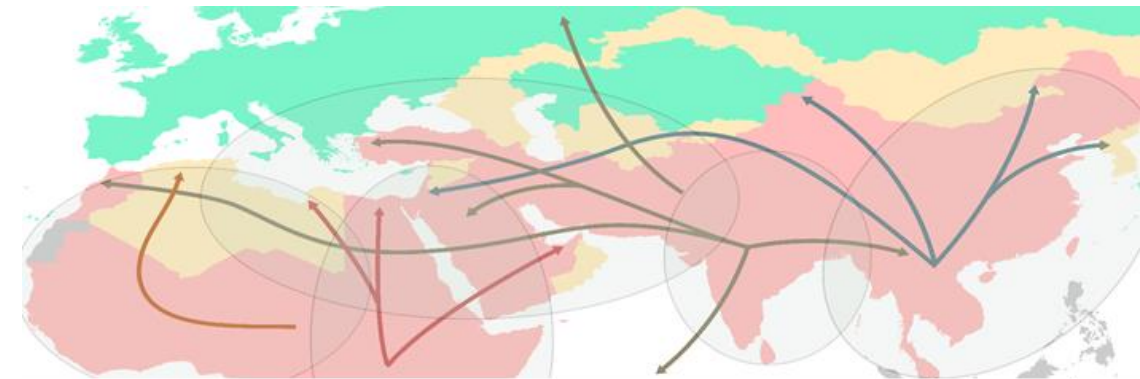


[FAO, 2013. EMPRES-Santé Animale 360, No. 42, Février 2014, Rome](#)

Foot-and-Mouth Disease

An ancient disease but still with us, and a threat for zones free from FMD
➔ socio-economic impact, losses, embargos...

- Highly contagious transboundary animal disease
- Infecting cloven-hoofed animals (cattle and wildlife, more than 70 species are sensitive)
- Unpredictable spread of lineages between pools and over long-distance
- Identification and characterization of circulating strains help to understand the transmission pathways and to identify future lineages that may pose a threat
- Two incursions of the virus in EU in 2025 (Germany, then Hungary and Slovakia)
- Two incursions in 2026 (Cyprus, Greece)



Long-distance "trans-pool" FMD virus movements



FMD in UK in 2001



Reflections on the Foot-and-Mouth Disease Epidemic of 2001: a United Kingdom Perspective
WOAH Bulletin

Rev. Sci. Tech. Off. Int. Epiz., 2016, 35 (1), 175-189

Genomics and outbreaks: foot and mouth disease

G.L. Freimanis, A. Di Nardo, K. Bankowska, D.J. King, J. Wadsworth,
N.J. Knowles & D.P. King*

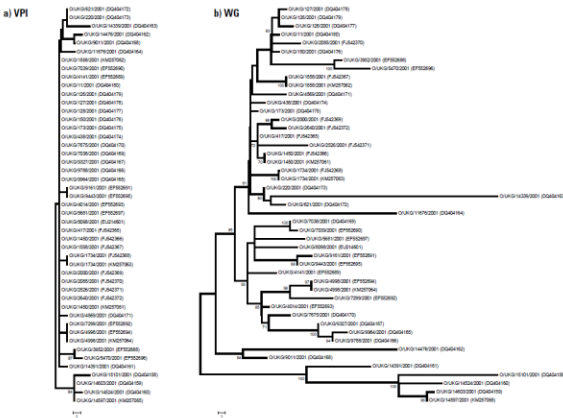


Fig. 4
A comparison between phylogenetic analysis of data generated from the same foot and mouth disease virus isolates collected during the 2001 foot and mouth disease epidemic in the United Kingdom using VPI only or WG (whole genome)
Maximum likelihood trees were produced using MEGA v. 6.06 (70)

- 6,45 millions of animals culled
- Estimated cost of more than 8 billions £
- Virus with a very high mutation rate
- Genomic investigations using sequencing
- The index case was not identified

Transmission Pathways of Foot-and-Mouth Disease Virus in the United Kingdom in 2007

Eleanor M. Cottam^{1,2}, Jemma Wadsworth¹, Andrew E. Shaw¹, Rebecca J. Rowlands¹, Lynnette Goatley¹, Sushila Maan¹, Narender S. Maan¹, Peter P. C. Mertens¹, Katja Ebert¹, Yanmin Li¹, Eoin D. Ryan¹, Nicholas Juleff¹, Nigel P. Ferris¹, John W. Wilesmith³, Daniel T. Haydon², Donald P. King¹, David J. Paton¹, Nick J. Knowles^{1*}

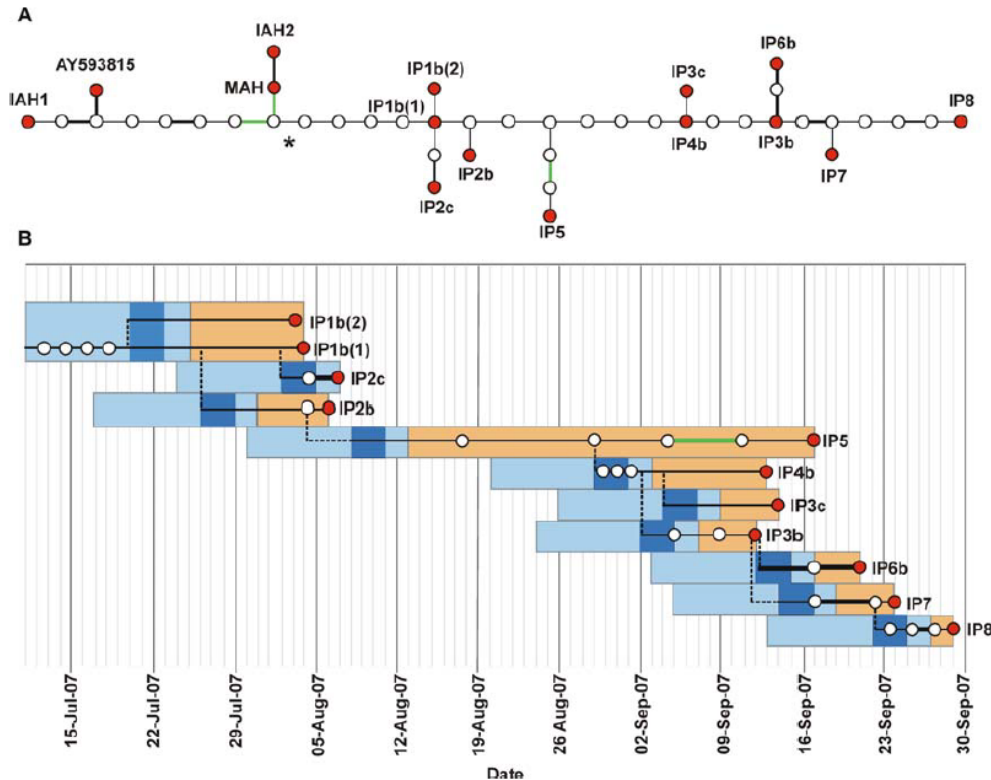


Figure 2. Analysis of sequence data. **A)** Statistical parsimony analysis by TCS [19] of complete genome sequences of 14 FMDVs; connecting lines represent a nucleotide substitution, thicker lines represent non-synonymous substitutions, with substitutions indicative of adaptation to cell culture coloured green. Sequenced haplotypes (red circles), and putative ancestral virus haplotypes (white circles) are shown. AY593815 is a previously published sequence [21] of FMDV O₁ BFS 1860. The asterisk indicates the start of the tree in 2B). **B)** Lesion age derived infection profiles of holdings overlaid with the outbreak virus genealogy. The orange shading estimates the time when animals with lesions were present from the oldest lesion age at post-mortem [22]. For IP2c, there were no clinical signs of disease. The light blue shading represents incubation periods for each holding, estimated to begin no more than 14 days prior to appearance of lesions [23]. The dark blue shading is the infection date based on the most likely incubation time for this strain of 2–5 days [24]. Each UK 2007 outbreak virus haplotype is plotted according to the time the sample was taken from the affected animal (x axis). The dashed lines link the TCS tree together but do not denote any genetic change.
doi:10.1371/journal.ppat.1000050.g002

- Complete genome sequence analysis of FMD viruses conducted in real-time
- Identification of the initial and intermediate sources of these outbreaks
- Demonstrate the value of such techniques in providing information useful to contemporary disease control programmes.

- The origin of the strain was known
- Originated from a lab

But...



No match...

FMDV positive sample



FGS obtained



**No recent sequence, or
not public/shared**

The main problem is linked to the databases and/or shared data...

Not all laboratories/countries declared FMD cases

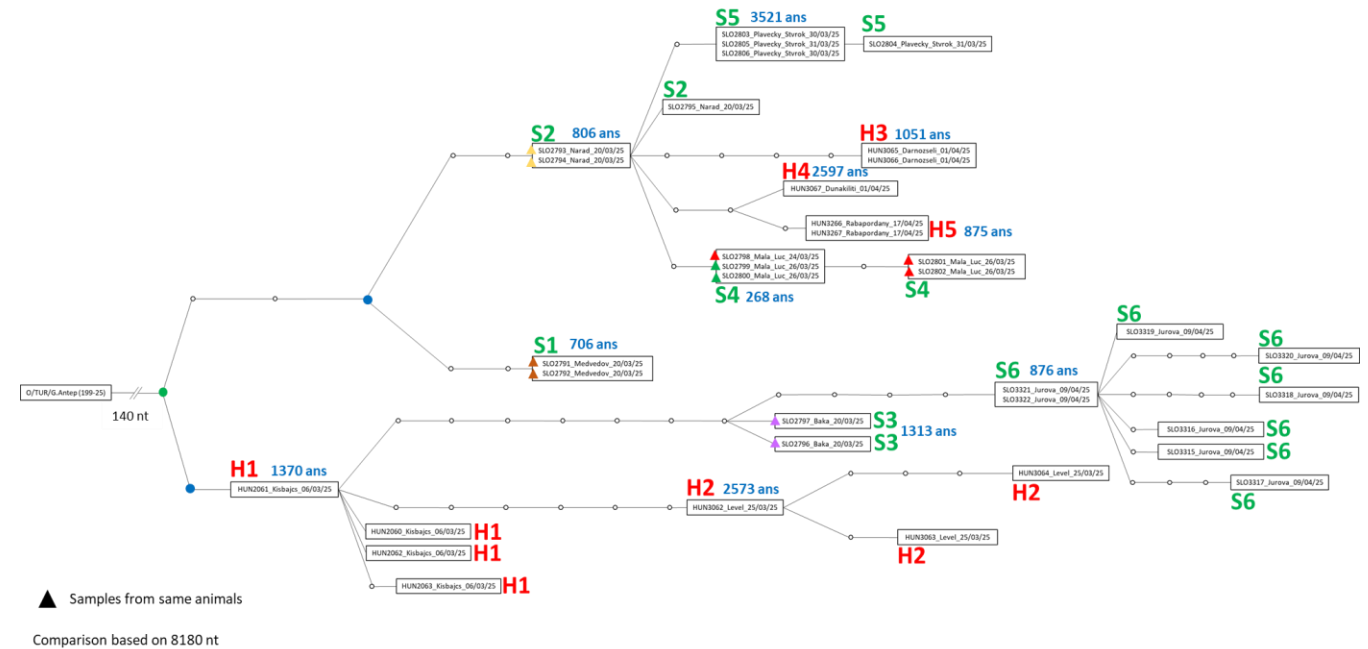
Not all laboratories perform FGS



Critical gap of intelligence

FMD in EU in 2025: Hungary and Slovakia

- 11 outbreaks in two months
- Single introduction of the virus
- “Real” ancestor of the outbreak not sampled (36 sequences in the TCS tree)
- More information is required on strains circulating in West Asia





The example of Anthrax

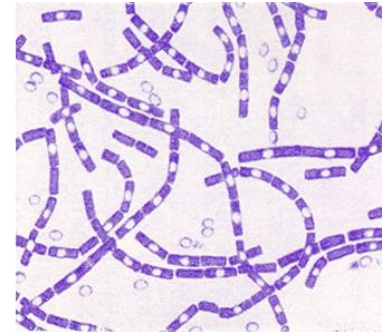


DEATH IN THE WOOLPACK.

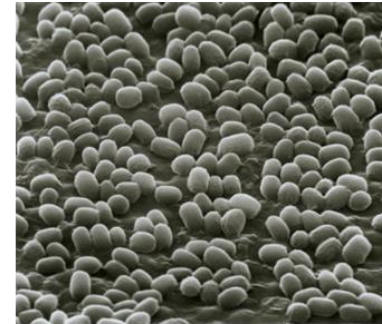
[and the Woolsorter: Bradford doctors against anthrax](#) | 100 Objects

Anthrax

- Zoonotic disease caused by a Gram positive bacteria (*Bacillus anthracis*)
- Not very contagious
- Spores (persistence form in soil for decades)
- 3 forms of the disease in humans: cutaneous (and also injectional), gastro-intestinal and inhalational



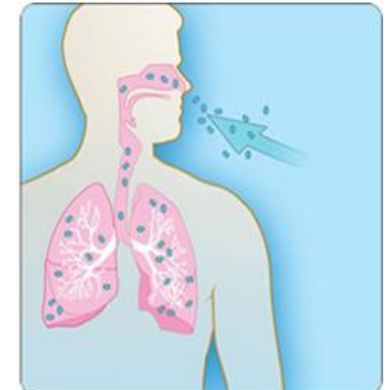
[Protéines à la loupe - "Les toques de l'anthrax - un trio diabolique" par Sylvie Rothblut](#)



<http://fineartamerica.com/featured/spores-of-bacillus-anthraxis-bacteria-nibsc.html>



[A case of human cutaneous anthrax | The Medical Journal of Australia](#)





Anthrax as a bioweapon

In 1942 on Gruinard Island (Scotland): military experiments on the island (bomb with anthrax spores), with sheep herd

- Sheep died quickly, persistence of spores into the environment
- Island put in quarantine for 48 years... decontamination with formaldehyde solution...



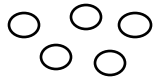
(<http://cs.stanford.edu/people/eroberts/courses/ww2/projects/chemical-biological-warfare/uk.htm>)



Anthrax as a bioweapon



Ex Soviet Union



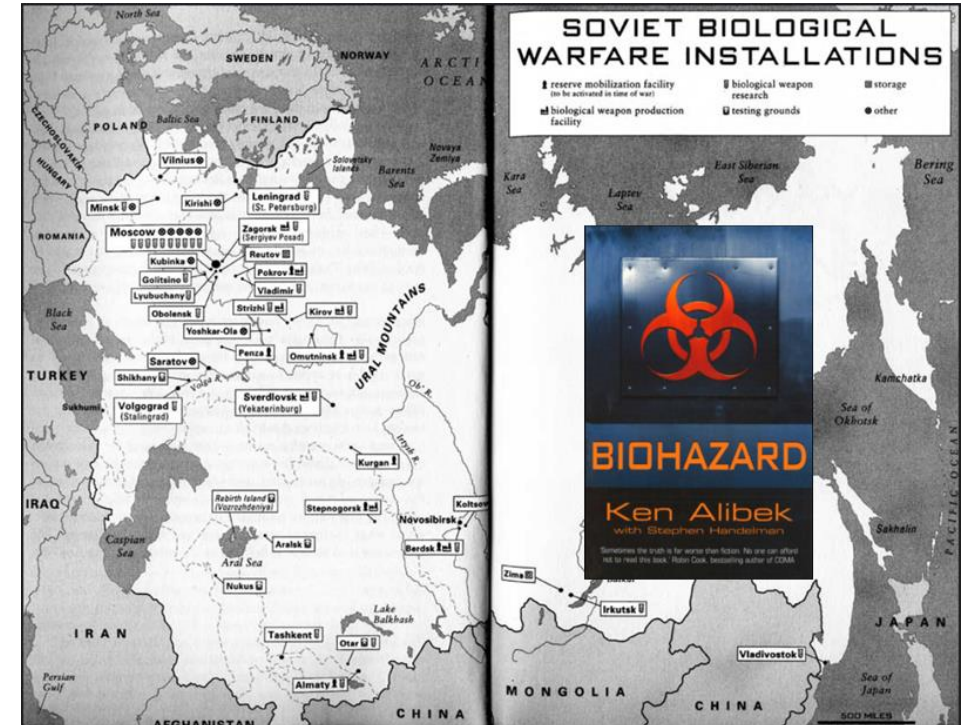
less than 1g of dried spores, accidentally released



68 official deaths

The Sverdlovsk Anthrax Outbreak of 1979

Matthew Meselson,* Jeanne Guillemin, Martin Hugh-Jones, Alexander Langmuir,† Ilona Popova, Alexis Shelokov, Olga Yampolskaya



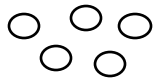


Anthrax as a bioweapon

2001 anthrax attacks : Amerithrax



USA



Contaminated mails



5 human deaths (22 cases)





- **18 September 2001:** Letters sent to media contained brown material (mostly caused cutaneous anthrax)
- **09 October 2001:** Letters sent to the two US senators contained a fine powder (mostly caused inhalational anthrax, almost only anthrax spores)
- All of the material derived from the Ames strain (common strain isolated from a cow in Texas in 1981)
- Genome sequencing was performed (one month) = mutations identified in the anthrax cultures grown from powder material

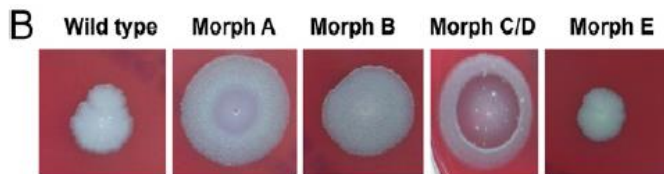
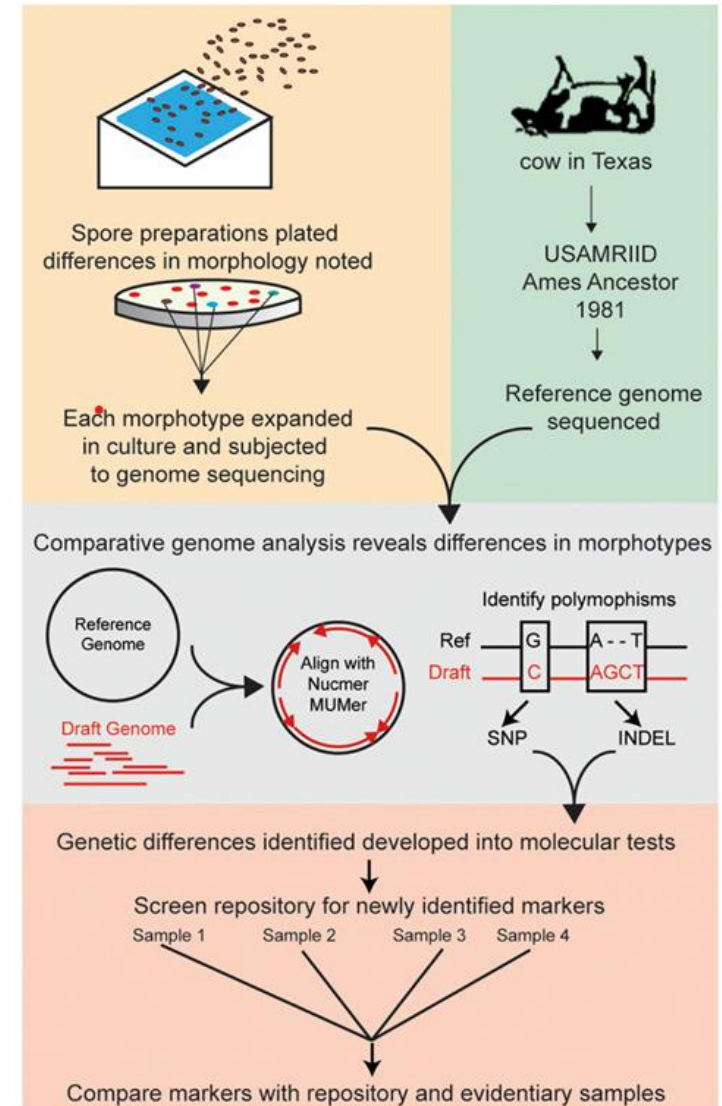
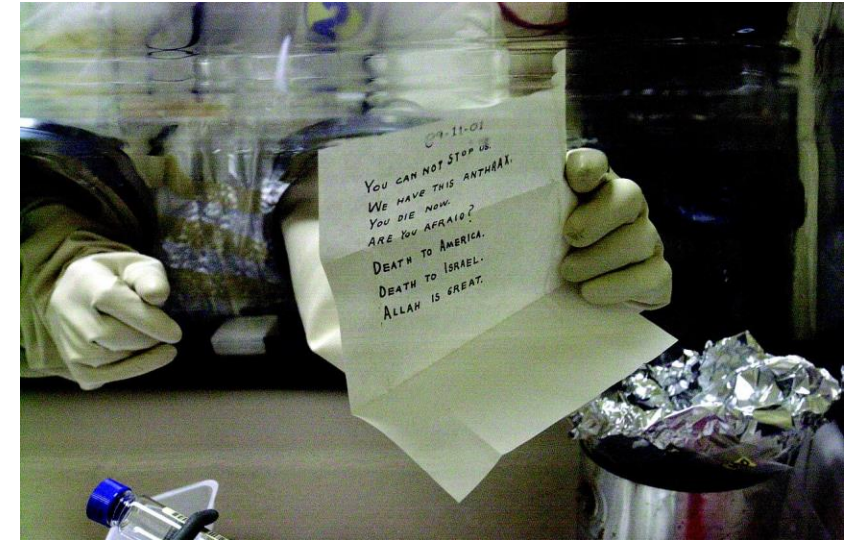


Fig. 1. Microbiological identification of morphological variants of *B. anthracis* Ames. (A) Flowchart used to process the evidentiary material for the identification of the morphological variants. In all cases, the morphotypes are altered in the sporulation phenotype and colony morphology. (B) Image of a representative colony of each of the morphotypes grown on SBA.





- First suspicions were that it was terrorists from Middle East (just after World Trade Center attack + letter)
- Finally, the investigation led to a military lab in USA (Fort Detrick, USAMRIID)
- 2010: FBI concludes that a scientist from this lab was responsible



(FBI)

From a lab perspective: integration of whole-genome sequencing for forensic applications = microbiological forensic (*B. anthracis* is perfect for that, very homogeneous, few mutations; could be more complicated for other microorganisms)

Take-home messages



Virus and bacteria can be used as biothreats



Usually, they are responsible of natural diseases. But these diseases, like FMD and anthrax can be responsible of huge losses (even if non-zoonotic, like FMD) or panic/disorganization (like anthrax)



Laboratory analyses can be helpful to identify the source or the most-likely origin during an outbreak, using Full Genome Sequencing for example.



However, it is mostly related to databases, and critical gaps of intelligence are often observed



Sharing data, sharing intelligence, global cooperation are essential

Thank you for your attention

