Monkeypox in Spain

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24 h/7 days per week: Rapid Response Unit
Detection and identification of orthopoxviruses using a generic nested PCR followed by sequencing

ABSTRACT

Some orthopoxviruses are considered to be potential agents. After the smallpox eradication campaign, vaccination was stopped around 1980, so a significant portion of the world's population is no longer protected against infection. Infection with related orthopoxviruses and non-smallpox infections can be used to diagnose and confirm cases.

Use of Internally Controlled Real-Time Genome Amplification for Detection of Variola Virus and Other Orthopoxviruses Infecting Humans

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Smallpox, once a devastating disease, was eradicated in 1980. However, in recent years, particularly following the 2001 anthrax attacks in the United States, there has been considerable interest in the development of rapid diagnostic assays for orthopoxviruses. The assay described here uses internally controlled real-time PCR technology to detect orthopoxviruses. The assay is rapid, sensitive, and specific, allowing for the identification of variola virus and related orthopoxviruses.

Graph showing Delta Ct vs Cycle with 10^6 pfu and 1 pfu.
External Quality assays

• 2002 European Network for Diagnostic of Imported Viral Diseases (ENIVD)
• 2004 ENIVD
• 2018 Emerge (Robert Koch)
• 2019 Emerging Virus Diseases Laboratory Network (EVDLabNet, ECDC)
• 2019 Refbio (Robert Koch)
May 17th: first communication; 7 possible cases
May 18th: arrival of samples
    **Vesicular fluids**, serum, urine, nasopharyngeal exudate
May 18th: results: all positive
May 18th: first draft for national surveillance protocol
May 19th: Sequences (400 bp) available: MPXV Western African clade
May 25th: complete genome sequences: analysis ongoing.
Clinical criteria:
A person with a clinical picture highly suggestive of monkeypox infection (MPX)* in which they have been ruled out or the differential diagnosis indicates that there is very low suspicion of other pathologies.

*Vesicular or pustular rash (especially if it is umbilicated) in any part of the body with one more of the following: fever (>38.5°C), severe headache, myalgia, arthralgia, back pain, lymphadenopathy.

Epidemiological criteria:
If in the 21 days before the onset of symptoms you meet one of the following:
— Have had close contact with a confirmed or probable case of MPX
— Has had sex in risky sexual contexts
— Has a history of travel to endemic areas of West or Central Africa where circulation of the virus has been identified.

Laboratory criteria:
Detection of MPX virus genome (MPXV) by specific or generic PCR for Orthopoxvirus in clinical sample.
Isolation in Vero E6 cells

Negative staining of a clinical sample (swabs obtained from the lesion in viral transport media)

Western Africa Clade

Congo Clade

Cowpox

Monkeypox
RESULTS

June, 1st.
125 positive cases (36 with sequencing)
148 negative cases
No clinical information (Mild illness: Most of them umbilicated skin lesions, fever, astenia, mialgia, inguinal adenopaties)

No epidemiological information (many of them MHM, Canary Islands party, sauna)

Methodolofy transfer to hospitals and laboratories of the National Health System
Implementing MPXV specific PCRs
Trying to obtain inmunofluorescence assays for serology
WP1: Clinical Research
- Patients evolution
- Risk groups
- Contacts and pets

WP2: Virological research
- Virus in fluids
- Immunity
- Sequencing

WP3: Standardization of proceedings for control and detection
- Differential diagnosis
- Methodological standardization

Coordination
Monkeypox in Spain

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