

Diagnóstico de referencia de arbovirosis en España

ARBOVIROSIS: COMPARTIENDO EXPERIENCIAS

13 DICIEMBRE 2019 🇪🇸 9:30 HORAS

ESCUELA NACIONAL DE SANIDAD

SEMINARIOS 

María Paz Sánchez-Seco
Centro Nacional de Microbiología
Instituto de Salud Carlos III

ORGANIZA
DEPARTAMENTO DE SALUD INTERNACIONAL
ESCUELA NACIONAL DE SANIDAD
INSTITUTO DE SALUD CARLOS III

LABORATORIO NACIONAL DE REFERENCIA PARA ZONOSIS.

Real Decreto 1940/2004, de 27 de septiembre, sobre la vigilancia de las zoonosis y los agentes zoonóticos.



Funciones

1. Apoyo científico-técnico a la Administración General del Estado, a las Comunidades Autónomas y al Sistema Nacional de Salud en:
 - Prevención, diagnóstico y tratamiento de las enfermedades infecciosas
 - Diagnóstico microbiológico de infecciones humanas emergentes o inusuales
 - Participación en el control de las alertas en enfermedades infecciosas
 - Detección de resistencias a los antimicrobianos
 - Evaluación de vacunas frente a patógenos humanos
 - Formación, docencia y asesoría, relativas a la microbiología humana
2. Desarrollar la investigación biomédica en:
 - Patología infecciosa, focalizada en la caracterización de los procesos moleculares relacionados con estas infecciones (patogenia, dianas terapéuticas, variabilidad patogénica)
 - Innovación tecnológica para mejorar la caracterización de virus, bacterias, hongos y parásitos
 - Inmunología: ontogenia y activación linfocitaria, respuesta inmune, regulación génica, inflamación y patogenia de enfermedades de base inmunológica
3. Fomentar la colaboración científica y técnica con instituciones similares al CNM en:
 - Proyectos de investigación coordinados.
 - Cursos especializados.
 - Tecnologías avanzadas.

- 1. Diagnóstico de referencia
- 2. Fuente de material de referencia
- 3. Consejo científico
- 4. Colaboración e investigación
- 5. Monitorización, alerta y respuesta



EVITAR

Red de investigación en enfermedades víricas
transmitidas por artrópodos y roedores

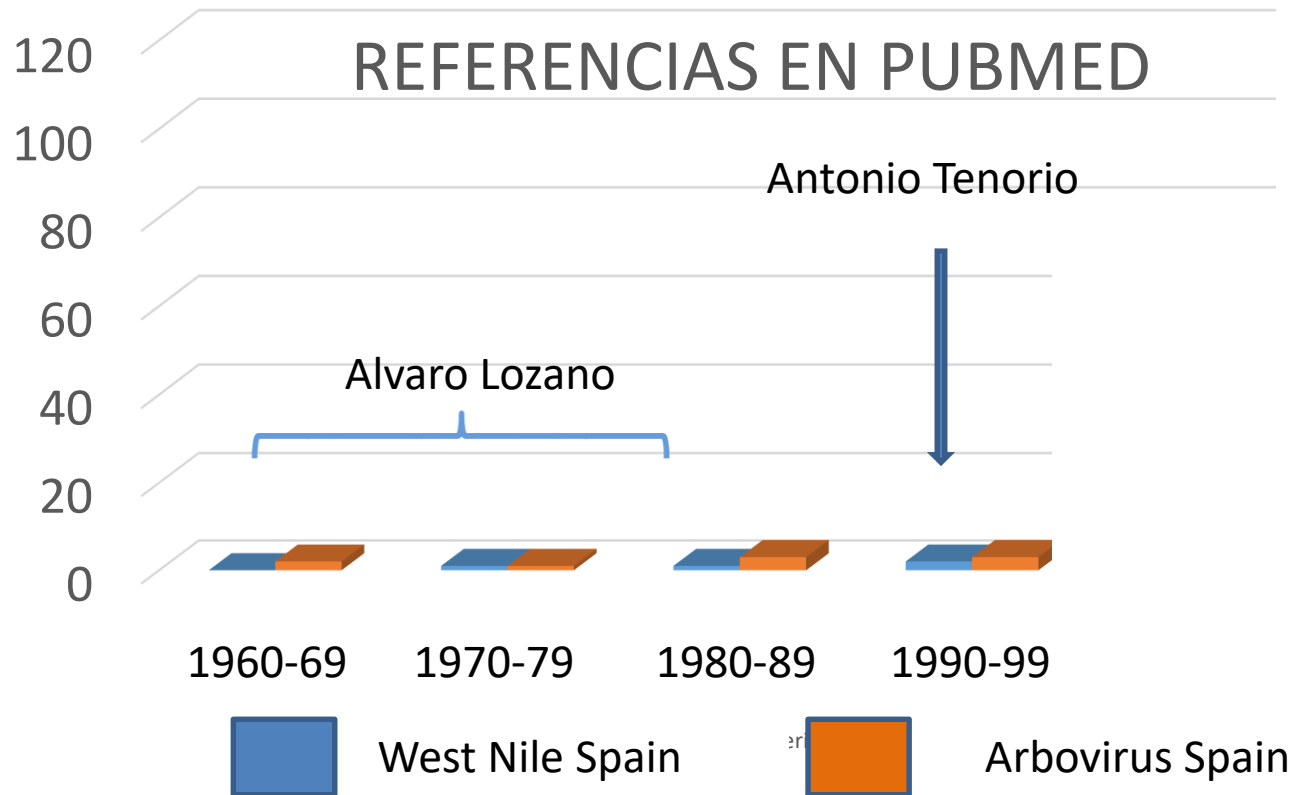
Red Temática de Investigación Cooperativa "Identificación de factores de riesgo y caracterización de arbovirosis y robovirosis en España", 2003-2006

Multidisciplinar: ornitólogos,
entomólogos, veterinarios, clínicos,
epidemiólogos, laboratorio, biólogos
de campo...

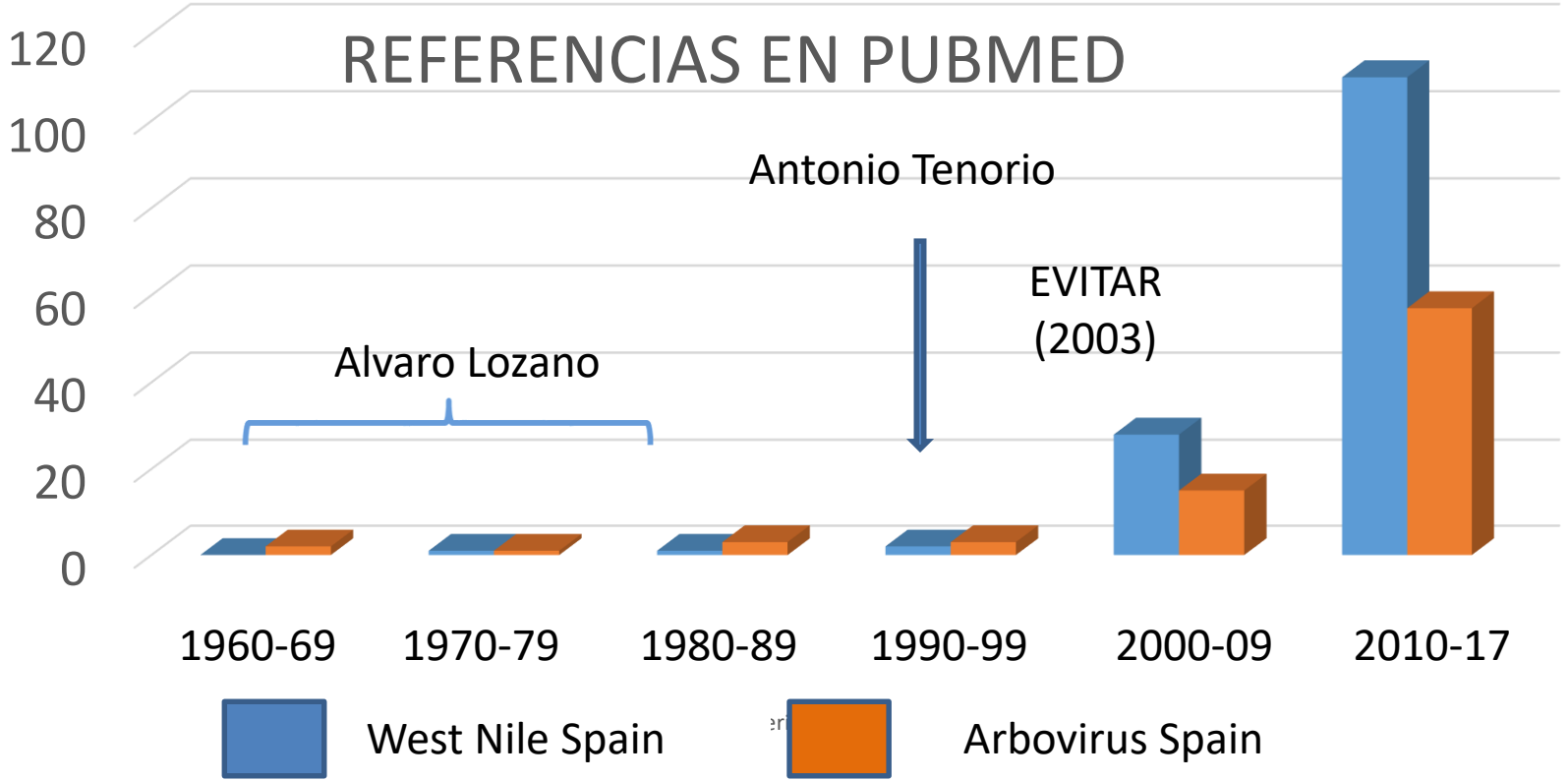
Ambito nacional: Andalucía, Madrid,
Galicia, Cataluña, Baleares

Muestras animales, humanas, vectores





Los arbovirus en España



- INVESTIGACIÓN
 - Nuevas herramientas diagnósticas
 - Epidemiología molecular
 - Estudios de prevalencia de diferentes arbovirosis
 - Importados: Dengue, Chikungunya, Zika
 - Autóctonos: Toscana, West Nile (LCM)
 - Fiebres hemorrágicas: Crimea-Congo
- CARTERA DE SERVICIOS
 - Dengue, Chikungunya, Zika, West Nile, Toscana, Crimea-Congo, West Nile, otros alpha-, flavi-, phlebovirus, Hantavirus, Ebola, Lassa

Chikungunya: Llamando a nuestras puertas

de Salud
Carlos III



Journal of Virological Methods 95 (2001) 153–161



SURVEILLANCE AND OUTBREAK REPORT

Chikungunya virus infections among travellers returning to Spain, 2008 to 2014

MD Fernandez-García^{1,2,3}, M Bangert^{1,3,4,5}, F de Ory¹, A Potente¹, L Hernandez¹, F Lasala¹, L Herrero¹, F Molero¹, A Negrodo¹, A Vázquez¹, T Minguito¹, P Balfagón¹, J de la Fuente¹, S Puente⁶, E Ramírez de Arellano¹, M Lago⁶, M Martínez^{7,8}, J Gascón⁷, F Norman⁹, R Lopez-Velez⁹, E Sulleiro^{10,11}, D Pou¹¹, N Serre¹¹, RF Roblas¹², A Tenorio¹, L Franco^{1,3,13}, MP Sanchez-Seco^{1,3}

A generic nested-RT-PCR followed by sequencing for detection and identification of members of the alphavirus genus

M. Paz Sánchez-Seco^{a,*}, Delfina Rosario^b, Evelia Quiroz^c,
Guadalupe Guzmán^b, Antonio Tenorio^a

- PCR genérica para Alphavirus. Diagnóstico serológico realizado en el Institut Pasteur (2005)



Enfermedades Infecciosas y
Microbiología Clínica

www.elsevier.es/eimc



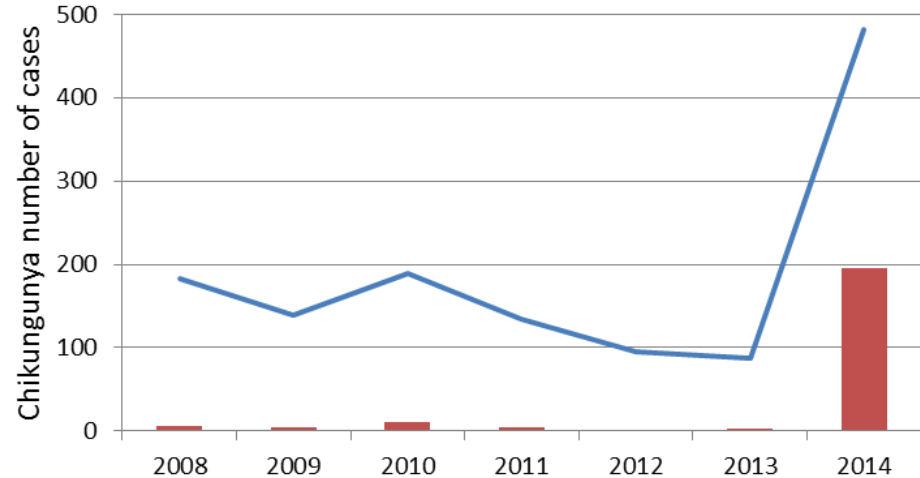
Original

Diagnóstico microbiológico del virus chikungunya importado en España (2006–2007): detección de casos en viajeros

María Paz Sánchez-Seco^{a,*}, Ana Isabel Negrodo^a, Sabino Puente^b, M^a Jesús Pinazo^c, Isabelle Shuffenecker^d, Antonio Tenorio^a, Cesare Giovanni Fedele^e, Cristina Domingo^a, José Miguel Rubio^f y Fernando de Ory^a

- Implementación de métodos para serología y PCRs específicas (2006-

confirmed suspected



Hospitales del SNS capacitados para la detección de Chikungunya (2014)

- Métodos moleculares (comerciales/caseros, Tiempo real, convencional, genéricas/específicas)
- Serológicos (IFI, NT y NT diferencial con ONN, Mayaro). En breve Ross River, Encefalitis equinas

Chikungunya: Llamando a nuestras puertas

Infection with chikungunya virus in Italy: an outbreak in a temperate region

G Rezza*, L Nicoletti*, R Angelini, R Romi, A C Finarelli, M Panning, P Cordioli, C Fortuna, S Boros, F Maaurano, G Silvi, P Anaelini, M Dottori, M G Ciufolini, G C Majori, A Cassone, for the CHIKV study group†
Lancet 2007; 370:1840-46

Chikungunya Virus, Southeastern France

Emerging Infectious
Diseases •
www.cdc.gov/eid • Vol.

17, No. 5, May 14, 2011
Marie Grandadam, Valérie Caro,
Sébastien Plumet, Jean-Michel Thiberge,
Yvan Souarès, Anna-Bella Failloux,
Hugues J. Tolou, Michel Budelot,
Didier Cosserat, Isabelle Leparç-Goffart,
and Philippe Desprès

Eurosurveillance, Volume 20, Issue 17, 30 April 2015

Surveillance and outbreak reports

CHIKUNGUNYA OUTBREAK IN MONTPELLIER, FRANCE, SEPTEMBER TO OCTOBER 2014

E Delisle (delisle.elsa@gmail.com)¹, C Rousseau¹, B Broche², I Leparç-Goffart³, G L'Ambert⁴, A Cochet¹, C Prat³, V Foulongne⁵, J B Ferré⁴, O Catinolis¹, O Flusin³, E Tchernonog⁵, I E Moussion², A Wiegandt², A Septfons⁵, A Mendy², M

RAPID COMMUNICATION

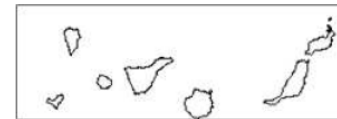
Preliminary report of an autochthonous chikungunya outbreak in France, July to September 2017

Clémentine Calba¹, Mathilde Guerbois-Galla^{2,3}, Florian Franke¹, Charles Jeannin⁴, Michelle Auzet-Caillaud⁵, Gilda Grand^{2,3}, Lucette Pigaglio⁵, Anne Decoppet⁵, Joel Weicherding⁵, Marie-Christine Savail⁶, Manuel Munoz-Riviero⁶, Pascal Chaud¹, Bernard Cadiou⁷, Lauriane Ramalli^{1,8}, Pierre Fournier⁹, Harold Noël¹⁰, Xavier De Lamballerie³, Marie-Claire Paty¹⁰, Isabelle Leparç-Goffart^{2,3}

RAPID COMMUNICATIONS

Detection of a chikungunya outbreak in Central Italy, August to September 2017

Giulietta Venturi^{1,2}, Marco Di Luca^{1,2}, Claudia Fortuna¹, Maria Elena Remoli¹, Flavia Riccardo¹, Francesco Severini¹, Luciano Toma¹, Martina Del Manso¹, Eleonora Benedetti¹, Maria Grazia Caporali¹, Antonello Amendola¹, Cristiano Fiorentini¹, Claudio De Liberato³, Roberto Giammattei⁴, Roberto Romi¹, Patrizio Pezzotti¹, Giovanni Rezza¹, Caterina Rizzo¹



Aedes albopictus

Distribución del vector: diciembre 2015

- Presencia del vector
- Sin datos o no identificado

Distribución Ae. albopictus 2014. Ministerio de Sanidad (CCAES)

Chikungunya: Llamando a nuestras puertas

Instituto
de Salud
Carlos III



COMMUNICABLE DISEASE THREATS REPORT

CDTR

Week 25, 16-22 June 2019

All users

New! Chikungunya - Spain - 2019

Opening date: 17 June 2019

Latest update: 20 June 2019

In June 2019, a cluster of four chikungunya virus disease cases associated with travel to Alicante, Spain was reported by Iceland. Epidemiological, entomological and molecular investigations are ongoing in order to inform the assessment of the risk of transmission. Additional molecular characterisation (RT-PCR and sequencing) is being performed in order to clarify laboratory findings and confirm the diagnosis.

The **OLIVE PRESS**



COSTA BLANCA OTHER NEWS HEALTH LEAD

MISTAKE: 'LABORATORY ERROR' LED TO FALSE DIAGNOSIS OF CHIKUNGUNYA BY ICELANDIC HOSPITAL AS SPAIN REMAINS

CRIMEA CONGO



- I-África Occidental
- II-RDC
- III-Sudáfrica y África Occidental
- IV- Asia y Oriente Medio
- V-Europa y Turquía
- VI-Grecia.

• Western Africa cluster

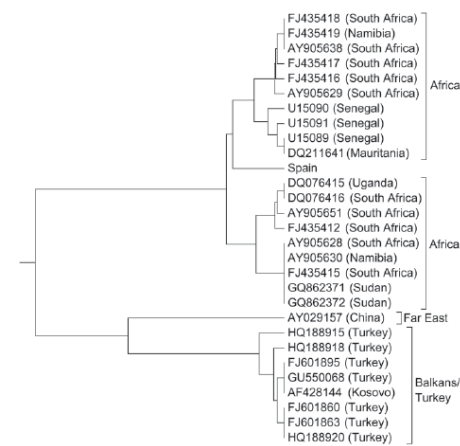
Crimean-Congo Hemorrhagic Fever Virus in Ticks, Southwestern Europe, 2010

To the Editor: Crimean-Congo hemorrhagic fever virus (CCHFV; family *Bunyaviridae*, genus *Nairovirus*) causes outbreaks of severe hemorrhagic fever in humans with case-fatality rates $\leq 30\%$. The disease was initially recognized by Russian scientists in the 1940s and the virus was first isolated in the Democratic Republic of Congo 40 years later (4). CCHFV is present throughout broad regions of Europe, the Middle East, and Africa. Reports linking transmission of the virus with an infected vector involved ticks of the genus *Hyalomma* spp. (5). It appears that maintenance of the virus is dependent on *Hyalomma* spp. within periods of silent activity in vertebrates are involved in the transmission cycle (6). Transmission of CCHFV to humans occurs through tick bites, direct contact with blood or tissues of infected animals, person-to-person spread, or by nosocomial infection (1).

In southeastern Europe, the Balkans are the known western limit for CCHFV (7). This finding is of special interest because *Hyalomma marginatum*, the main tick vector in the western Palearctic (an ecozone that includes temperate and cold areas of Eurasia and North Africa and several archipelagos and islands in the Atlantic and Pacific Oceans), is common throughout the Mediterranean Basin (7), where clinical cases of the disease or the virus have not been reported. Unsupported claims of the effects of climate on virus distribution have been reported but never empirically demonstrated (8).

We report the detection of CCHFV in ticks collected in southwestern

Europe. A total of 117 semi-engorged adult *H. lusitanicum* ticks were collected from 28 adult red deer (*Cervus elaphus*) in November 2010, at a site (39.63°N, 7.33°W) in Cáceres, Spain. Live ticks were transported to the special pathogens laboratory at Hospital San Pedro-CIBIR in Logroño (northern Spain), classified, and frozen at -80°C . For RNA extraction, specimens were washed in 70% ethanol and then in 100% ethanol.



of CCHFV as described (9). Negative controls (with template DNA but without primers and with primers and containing water instead of template DNA) were included in all assays.

For the second round of PCRs, 2 of 12 pools showed amplicons of the expected size (211 bp). Only 1 amplicon could be sequenced. MEGA5 (www.megasoftware.net) was used to compare the sequence with representative small segment sequences of CCHFV available in GenBank (Figure). (Aligned sequences are available from the authors.) Pools of cDNA were submitted to the Spanish National Center of Microbiology (Madrid), where results were confirmed. The CCHFV sequence we report showed 98% genetic similarity (204/209 bp) with sequences recorded for CCHFV

in Mauritania and Senegal, on the western coast of Africa.

This finding suggests the circulation of CCHFV in southwestern Europe. The close affinity of the strain from Spain with strains circulating in western Africa and the lack of similarity with isolates from eastern Europe suggest the introduction of this virus from nearby countries of northern Africa. Migratory movements of birds of the virus cause birds nature *H. reportedly through along the . Because similarities movements ates from report our*

this virus or if other a because western not been *suslanicum* isolated trip from

Sicily to Portugal. The Mediterranean rabbit and ungulates, the main hosts for immature and adult *H. lusitanicum* ticks, respectively, are residents of the collection area; however, the movement of these animals through trade has not occurred for several years. Thus, *H. lusitanicum* ticks could not serve as a spreading vector in the western Mediterranean region. The CCHFV strain from southwestern Europe has been found in ticks restricted to hosts that cannot spread long distances. Therefore, although it would be unlikely, given the strain's similarity with CCHFV isolates from Senegal and Mauritania, we should not exclude the possibility of an ancient existence for this strain. Additional data collected in the Mediterranean Basin are necessary to establish the actual range of CCHFV.

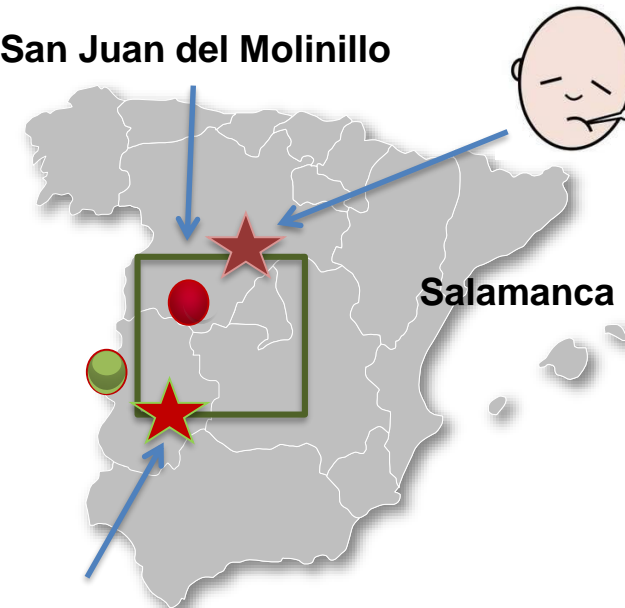
2016 y 2018. Casos humanos

Autochthonous Crimean–Congo Hemorrhagic Fever in Spain

A. Negrodo, F. de la Calle-Prieto, E. Palencia-Herrejón, M. Mora-Rillo, J. Astray-Mochales, M. P. Sánchez-Seco, E. Bermejo Lopez, J. Menárguez, A. Fernández-Cruz, B. Sánchez-Artola, E. Keough-Delgado, E. Ramírez de Arellano, F. Lasala, J. Milla, J.L. Fraile, M. Ordoñas Gavín, A. Martínez de la Gándara, L. López Perez, D. Díaz-Díaz, M.A. López-García, P. Delgado-Jimenez, A. Martín-Quirós, E. Trigo, J.C. Figueira, J. Manzanares, E. Rodríguez-Baena, L. García-Comas, O. Rodríguez-Fraga, N. García-Arenzana, M.V. Fernández-Díaz, V.M. Cornejo, P. Emmerich, J. Schmidt-Chanasit, and J.R. Arribas, for the Crimean Congo Hemorrhagic Fever@Madrid Working Group*



San Juan del Molinillo



Salamanca

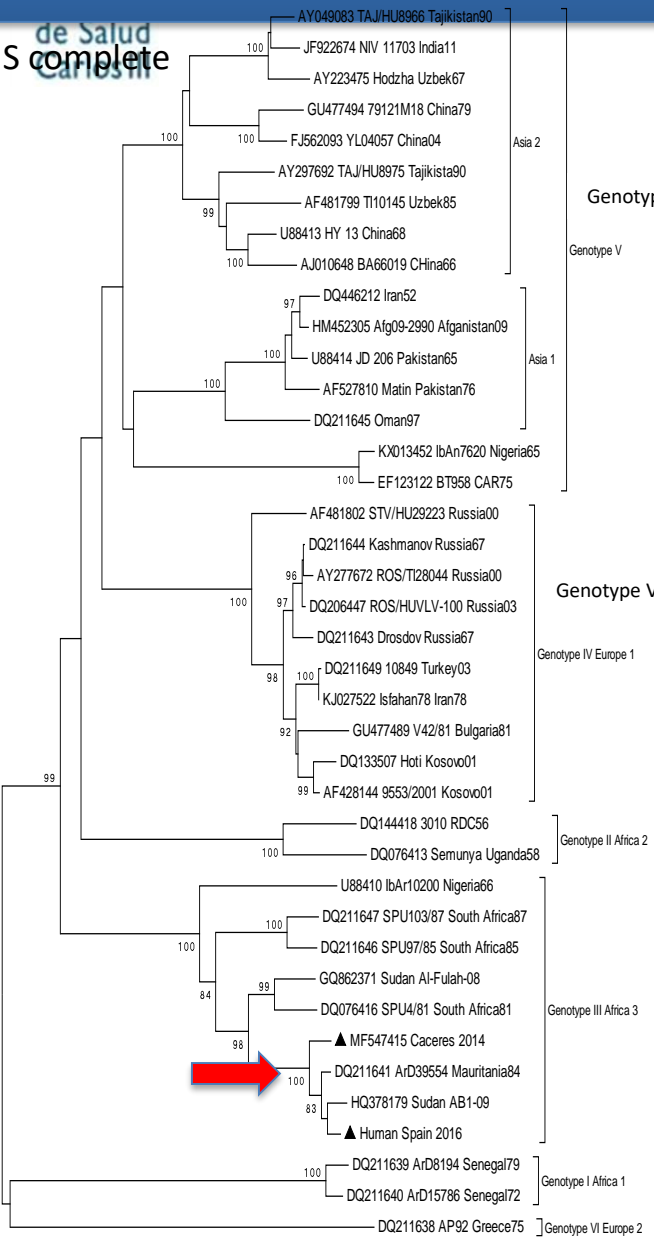


Helechosa de los Montes

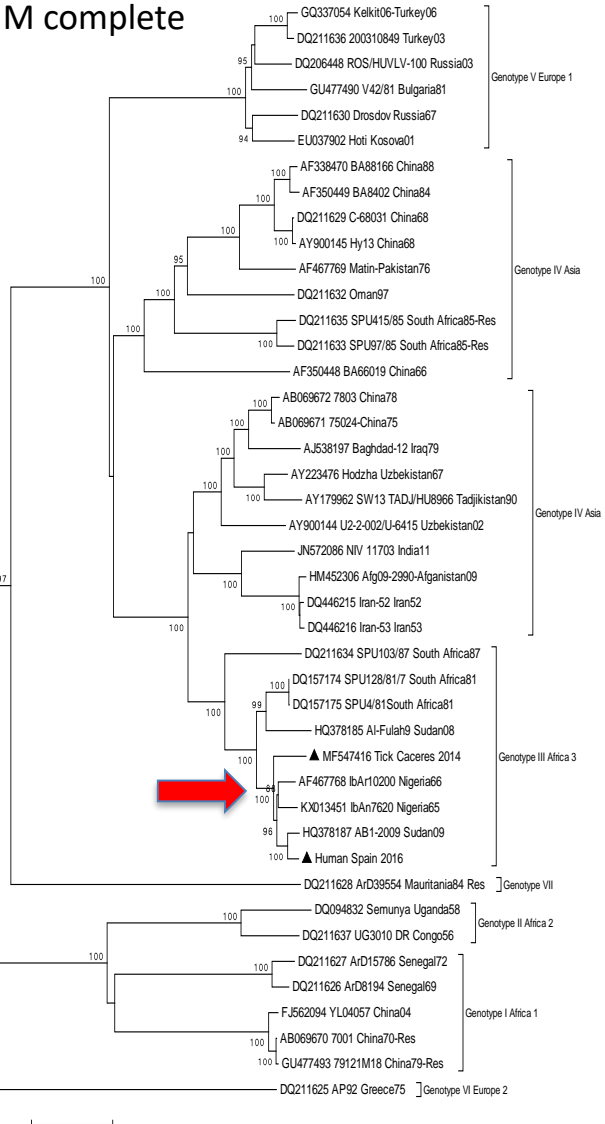
Filogenia (genoma completo): Africa 3

Ramírez de Arellano et al, EID, 2017: 23
 Cajimat et al, VBZD, 2017: 17,

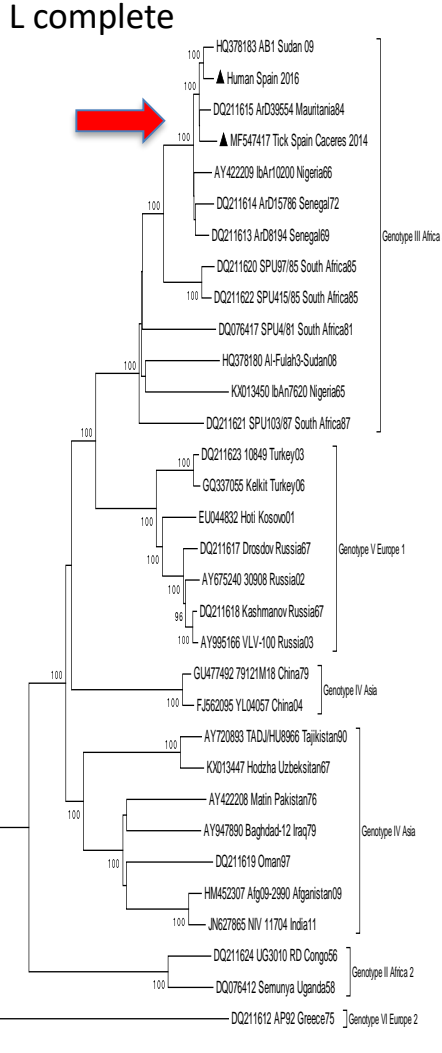
de Salud
 complete



M complete



L complete



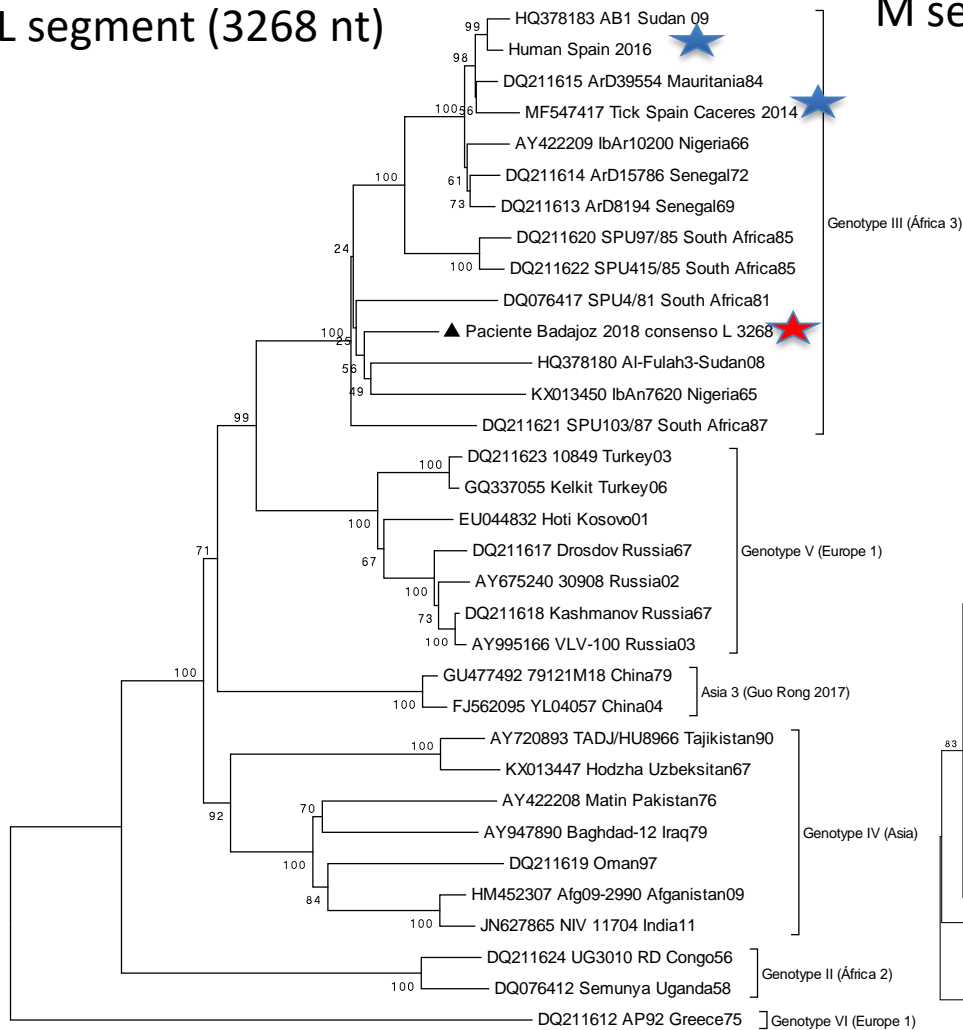
0.02

0.05

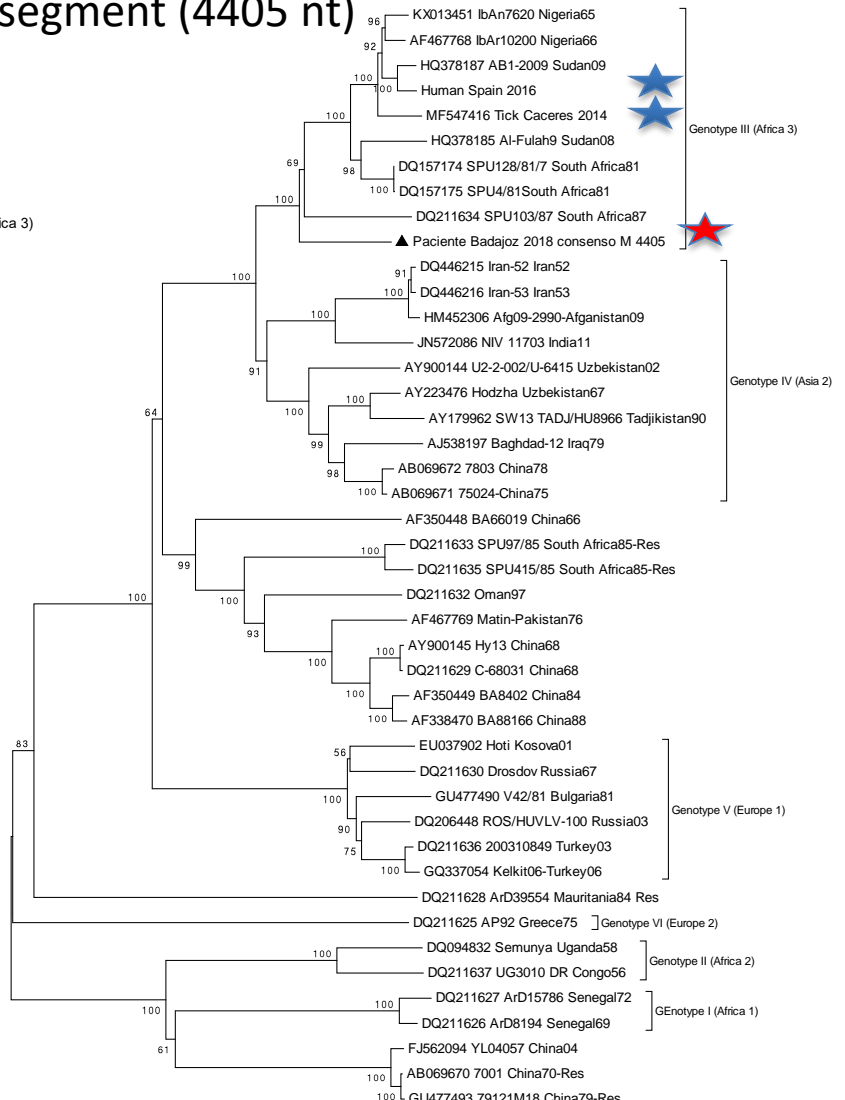
0.02

Filogenia (genoma completo): Reordenamiento Africa 3/ Asia (IV)

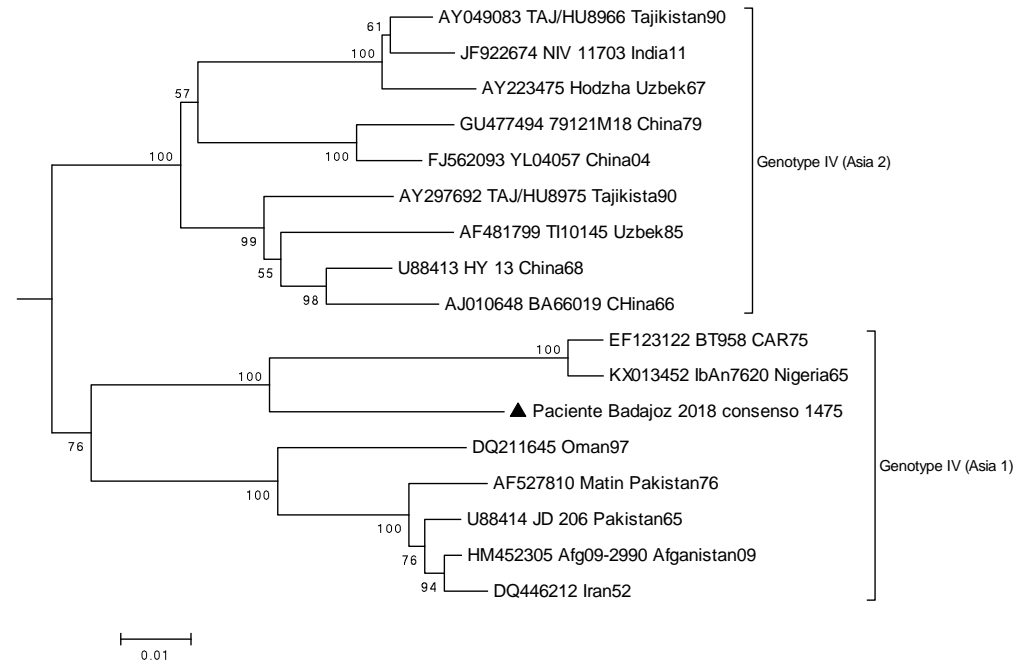
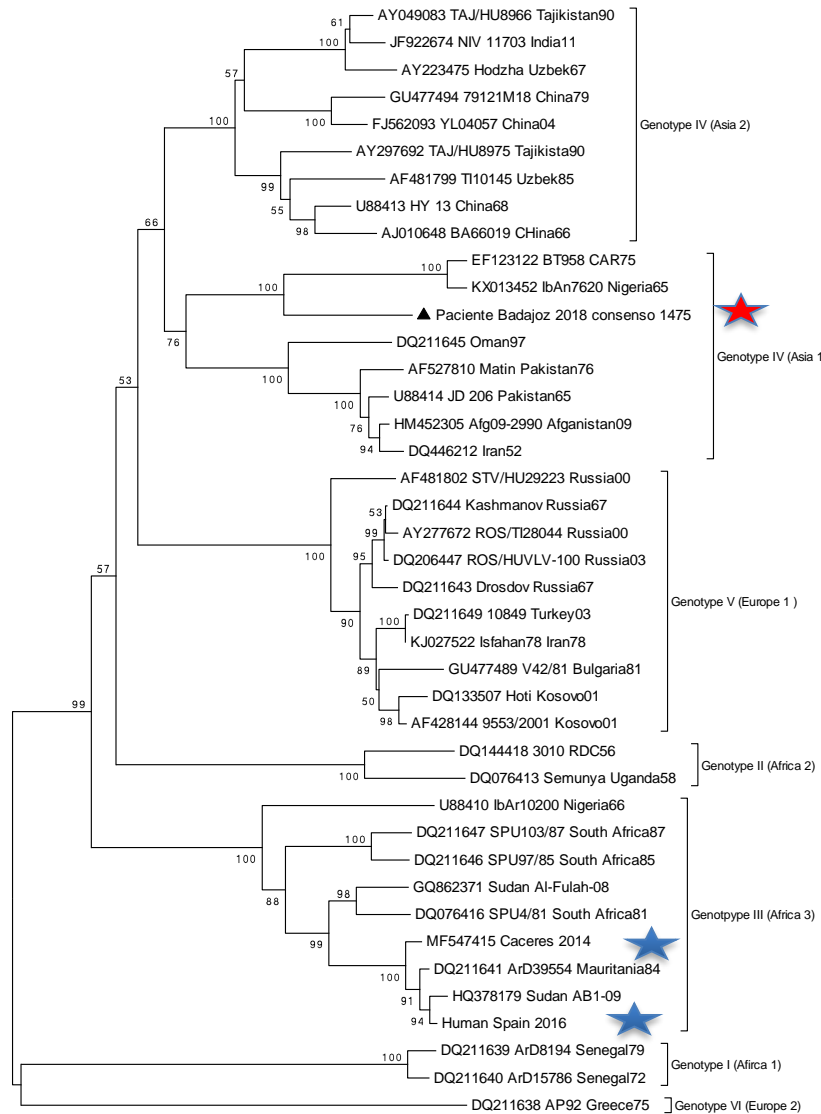
L segment (3268 nt)



M segment (4405 nt)

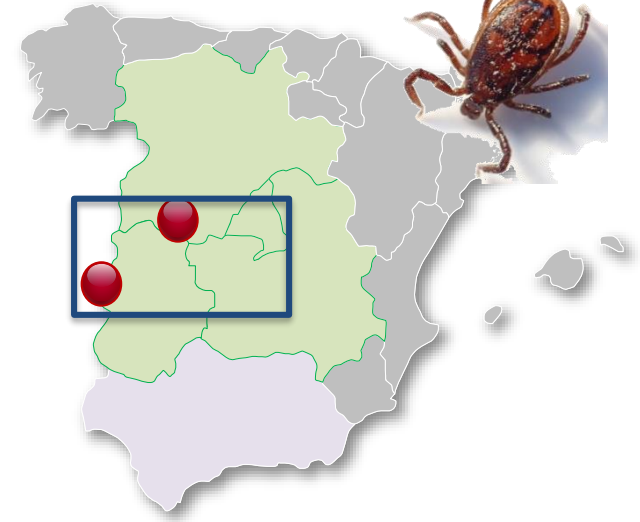


Filogenia (genoma completo): Reordenamiento Africa 3/ Asia (IV)



S segment (1475 nt)

Presencia de VFHCC en garrapatas en regiones cercanas a las garrapatas infectadas y al caso humano



- Región: Castilla León (Avila), Castilla La Mancha (Oropesa y Talavera), Madrid (San Martín de Valdeiglesias) and Extremadura (Cáceres, Coria, Navalmoral y Plasencia), Andalucía



Virus emergentes: preparación

Implementación/Desarrollo metodología
 Controles positivos
 Entrenamiento
 Controles de Calidad

OPEN ACCESS Freely available online



First International External Quality Assessment of Molecular Detection of Crimean-Congo Hemorrhagic Fever Virus

optimal
 Carlile Escabífa^{1,2}, Stephan Ölschläger³, Tatjana Avšič-Zupanc⁴, Anna Papa⁵,
 Jessica Vanhomwegen^{2,6}, Roman Wölfel⁷, Ali Mirazimi⁸, Anette Teichmann¹, Oliver Donoso-Mantke¹,
 Matthias Niedrig¹

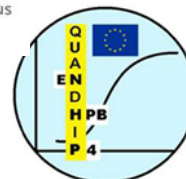


Lab/Sample	OCHF1	OCHF2	OCHF3	OCHF4
INMI	nd	28	36	nd
SMI	nd	25	32	nd
INSERM	nd	26	33	nd
HPA PD Untyped	nd	33;30;pos	nd;37;pos	nd
IMBBw	nd	pos;30 Baghdad	pos;35 Baghdad	nd
BNI	nd	27	34	nd
NICE Hungary	nd	24	31	nd
SPIEZ	nd	26;28	33;38	nd
INSA Port.	nd	30	35	nd
ICEB4 Vert le Petit	nd	25	40	nd
RKI	nd	26;25	33;30	nd
Spain	nd	pos;30 Afg09-2900	pos; 38 Afg09-2900	nd
PUM	nd	27	32	nd

New World Arena
 Hendra/ Nipah

 not detected
 number

NWA
 HN
 correct
 because
 wrong
 nd
 ct value



- Preparación
 - Algoritmos diagnósticos claros
 - Batería de técnicas con sensibilidad/especificidad conocida
 - Reactivos listos para su uso
 - Personal entrenado
 - Flujos de comunicación pre-establecidos
- Identificación agente
- Seguimiento caso

LABORATORIO NACIONAL DE REFERENCIA PARA ZONOSIS

Real Decreto 1940/2004, de 27 de septiembre, sobre la vigilancia de las zoonosis y los agentes zoonóticos.

LABORATORIO DE REFERENCIA E INVESTIGACIÓN EN ARBOVIRUS Y SEROLOGÍA



Laboratorio de Arbovirus y Enfermedades Víricas Importadas

M^a Paz Sánchez Seco
Ana Vázquez
Anabel Negrodo

Laura Herrero
Lourdes Hernández
Paqui Molero

Laboratorio de Serología

Fernando de Ory
Mayte Pérez Olmeda

Jesús de la Fuente
Pilar Balfagón
M^a Angeles Murillo
Desiré Dafouz
Juan Camacho