that possible outbreaks can be identified before they can have major consequences. Epidemiological and entomological surveillance is necessary to detect and control further outbreaks and to minimize the risk of local transmission in vector-colonized areas.

Fundings

This work was supported by the Network of Tropical Diseases Research RICET (RD16/0027/0002) and FEDER.

Competing interests

None.

Bibliografía

1. Wu X, Lu Y, Zhou Sen, Chen L, Xu B. Impact of climate change on human infectious diseases: empirical evidence and human adaptation. Environ Int. 2016 Jan;86:14–23, http://dx.doi.org/10.1016/j.envint.2015.09.007.
2. Tomasello D, Schlagenhauf P. Chikungunya and dengue autochthonous cases in Europe, 2007–2012. Travel Med Infect Dis. 2013;11:274–84, http://dx.doi.org/10.1016/j.tmaid.2013.07.006.
3. Delisle E, Rousseau C, Broche B, Leparc-Goffart I, L'Ambert G, Cochet A, et al. Chikungunya outbreak in Montpellier, France September to October 2014. Euro Surveill. 2015;20, pii:21108.
4. Venturi G, Di Luca M, Fortuna C, Remoli ME, Riccardo F, Severini F, et al. Detection of a chikungunya outbreak in Central Italy August to September 2017. Euro Surveill. 2017;22:13, http://dx.doi.org/10.2807/1560-7917.ES.39.17-00646201722.
5. Calba C, Guerbois-Galla M, Franke F, Jeannin C, Auzet-Caillaud M, Grard G, et al. Preliminary report of an autochthonous chikungunya outbreak in France July to September 2017. Euro Surveill. 2017;22:514, http://dx.doi.org/10.2807/1560-7917.ES.39.17-00647201722.
6. Succo T, Leparc-Goffart I, Ferré J-B, Roiz D, Broche B, Maquart M, et al. Autochthonous dengue outbreak in Nîmes, South of France July to September 2015. Euro Surveill. 2016;21:3, http://dx.doi.org/10.2807/1560-7917.
7. Marchand E, Prat C, Jeannin C, Lafont E, Bergmann T, Flusin O, et al. Autochthonous case of dengue in France October 2013. Euro Surveill. 2013;18:20661.
8. Panthier R, Hannoun C, Beytout D, Mouchet J. Epidemiologie du virus West Nile: (etude d'un foyer en Camargue, 3—Les maladies humaines. Ann Institut Pasteur. 1968;115:435–45.
9. ECDC. Historical data by year – West Nile fever seasonal surveillance. https://ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/historical [accessed 02.11.18].
10. ECDC. Communicable Disease Threats Report (14–20 October, week 42). https://www.ecdc.europa.eu/sites/portal/files/documents/communicable-disease-threats-report-20-oct-2018_0.pdf [accessed Nov 02.11.18].

Marta Díaz-Menéndez *, Clara Crespillo-Andújar

Infectious Diseases Unit, Department of Internal Medicine, University Hospital La Paz-Carlos III, Madrid, Spain

* Corresponding author.
E-mail address: marta.diazmenendez@gmail.com
(M. Díaz-Menéndez).

https://doi.org/10.1016/j.eimc.2018.12.002
0213-005X/
© 2018 Elsevier España, S.L.U. and Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica. All rights reserved.

Tularemia: diagnosis of an unexpected oculoglandular case in a non-endemic area by universal PCR

Tularemia: diagnóstico de un caso oculoglandular inesperado en un área no endémica mediante PCR universal

Dear Editor,

Tularemia is a zoonotic disease caused by *Francisella tularensis*, a Gram-negative facultative intracellular coccobacillus[1] with four recognized subspecies[2]: *tularensis* (type A), *holarctica* (type B), *mediasiatica* and *novicida*. Type A is found in North America, while type B is located, but not exclusively, in the northern hemisphere.[3] In Spain, it was an uncommon disease until 1997, when the first tularemia outbreak occurred in Castilla y León.[4] Until now, all cases reported in Spain were caused by *F. tularensis* subsp. *holarctica.* Clinical manifestations of tularemia fall into two main forms: ulceroglandular (>90% of cases in Europe)[2] and typhoidal. However, there are three more clinical forms: oculoglandular, oropharyngeal/gastrointestinal and pneumonic.

We have previously published the first case of ulceroglandular tularemia in a non-endemic area (Asturias, Spain).[5] Here, we present the first reported case of oculoglandular tularemia occurred in the same region which worried us.

An 88-year-old male presented to the emergency department of our hospital in April 2017 for diagnosis and management of pain in his right eye and the presence of conjunctival discharge. He did not have other symptoms, history of trauma, drug intake, or any recently local or systemic infection. His laboratory workup only showed a high value of C-reactive protein (5.5 mg/dL) and his medical and surgical histories were noncontributory. A diagnostic of viral conjunctivitis was done and he was treated with lubricant and anti-inflammatory drops.

Two months later, he was brought to the Department of Oral and Maxillofacial Surgery with a painful cervical mass (Fig. 1), weight loss and discomfort. He did not declared a recent travel, contact to ill people or animals although he lives in a rural area. Clinical examination revealed the presence of a right neck mass measuring 30 mm × 20 mm, with gummy consistency, painful on palpation and without other appreciable alterations.

A CT scan was performed and three neck lymph nodes together with an intraparotid lymph node were seen as pathological, showing necrotic areas.

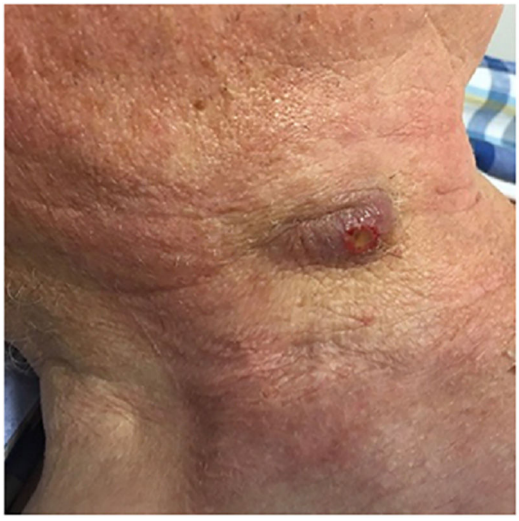

Fig. 1. Cervical mass observed in physical examination one month after conjunctivitis.

A universal PCR was performed in fine-needle aspiration biopsy (FNAB), based on amplification of the gene coding for 16S rRNA and subsequent sequencing (Bigdye® Terminator, Thermo Fisher Scientific). This evidenced the presence of *F. tularensis* subsp. *holarctica*. A second sample of FNAB was sent 10 days later resulting also positive.

In addition, serum samples were sent to the Spanish National Center for Microbiology (Madrid, Spain) in order to study the presence of antibodies against *F. tularensis* by microagglutination resulting positive with a titer of 1/1024.

Ziehl-Neelsen stain, culture and PCR for mycobacteria were negative. Lymphadenopathy-causing viruses (CMV, EB, HHV-6, HHV-7, HHV-8, Adenovirus, Picornavirus, Enterovirus, Mumps, LCMV) and *Toxoplasma gondii* were undetectable by PCR. Serological tests (ELISA) against *Coxiella burnetii* (IgG), *Rickettsia conorii* (IgG) and *Borrelia burgdorferi* (IgG/IgM) were negative.

Once the diagnosis was confirmed, the patient was treated with a 14-day course of intravenous streptomycin at a dose of 10 mg/kg/12 h, with favorable evolution. No surgical excision of the neck mass was needed.

Oculoglandular form of tularemia is very infrequent in our environment. In Spain some studies show an incidence of this form around 4%[4] but this microorganism should be considered in a patient with Parinaud's syndrome even in non-endemic areas.

The gold standard for the diagnosis of tularemia is the isolation of the causative agent by culture, however, this is difficult (it requires a medium with cysteine) and hazardous for the laboratory staff (Biosafety Level 2 precautions). Therefore diagnosis is based mainly on serology and results became positive between 10 and 14 days after onset of the disease.[6,7]

Genome amplification by polymerase chain reaction (PCR) is more sensitive than culture and provides rapid, sensitive and specific diagnosis of tularemia.[8–10] There are specific targets of *F. tularensis* genes (e.g. *fopA*, *tul4*, *ISFtu2* or RD1 protein-encoding gen) but when there is no suspicion of a specific etiological agent, it is useful to perform a universal PCR. In this case, if we had not performed the 16S rRNA PCR, the patient wouldn't have been correctly diagnosed.

The present case shows the importance of molecular techniques that amplify panbacterial genes, especially useful for diagnosis of rare infections with great difficulty of isolation of the etiological agent like *Bartonella henselae* (also causing Parinaud's syndrome), *Tropheryma whipplei*, *Borrelia* spp. or *Ehrlichia* spp. Also in those cases without bacterial growth due to antibiotic treatment.

Financial disclosure and conflict of interests

The authors declare that they have not received funding to carry out this study and have no conflict of interests.

Bibliografía

1. Ellis J, Oyston PC, Green M, Titball RW. Tularemia. Clin Microbiol Rev. 2002;15:631–46.
2. Sjöstedt A. Tularemia: history, epidemiology, pathogen physiology, and clinical manifestations. Ann N Y Acad Sci. 2007;1105:1–29.
3. Jakson J, McGregor A, Cooley L, Ng L, Brown M, Ong CW, et al. *Francisella tularensis Subspecies holarctica*, *Tasmania Australia*, 2011. Emerg Infect Dis. 2012;18:1484–6.
4. Pérez-Castrillón JL, Bachiller-Luque P, Martín-Luquero M, Mena-Martín FJ, Herreros V. Tularemia epidemic in northwestern Spain: clinical description and therapeutic response. Clin Infect Dis. 2001;33:573–6.
5. Gallego L, Junquera L, Palacios JJ, de Vicente JC. Cervical tularaemia in a non-endemic area. Med Oral Patol Oral Cir Bucal. 2009;14:E180–2.
6. Sato T, Fujita H, Ohara Y, Homma M. Microagglutination test for early and specific serodiagnosis of tularemia. J Clin Microbiol. 1990;28:2372–4.
7. World Health Organization: Epidemic and Pandemic Alert and Response. WHO guidelines on tularaemia, vol. 73. Geneva, Switzerland: World Health Organization; 2007 http://www.who.int/csr/resources/publications/WHO_CDS_EPR_2007_7.pdf
8. Maurin M, Gyuranecz M. Tularemia: clinical aspects in Europe. Lancet Infect Dis. 2016;16:113–24.
9. Kantardjiev T, Padeshki P, Ivanov IN. Diagnostic approaches for oculoglandular tularemia: advantages of PCR. Br J Ophthalmol. 2007;91:1206–8.
10. Antunes NT, Frase H, Toth M, Vakulenko SB. The class A β-lactamase FTU-1 is native to *Francisella tularensis*. Antimicrob Agents Chemother. 2012;56:666–71.

Paula Donate-Pérez-Molino[a], Cristian Castelló-Abietar[b,f], Jonathan Fernández-Suárez[b,f], Juan C. de Vicente[c,d,e,*]

[a] *Department of Oral and Maxillofacial Surgery, Hospital Universitario Central de Asturias, Oviedo, Spain*
[b] *Department of Microbiology, Hospital Universitario Central de Asturias, Oviedo, Spain*
[c] *Department Head Department of Oral and Maxillofacial Surgery, Hospital Universitario Central de Asturias, Oviedo, Spain*
[d] *School of Medicine and Health Sciences, University of Oviedo, Asturias, Spain*
[e] *Instituto Universitario de Oncología del Principado de Asturias (IUOPA), Spain*
[f] *Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo, Spain*

* Corresponding author.
E-mail address: jvicente@uniovi.es (J.C. de Vicente).

https://doi.org/10.1016/j.eimc.2018.12.003
0213-005X/
© 2018 Elsevier España, S.L.U. and Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica. All rights reserved.