Case report: Autochthonous Cutaneous Loxoscelism with Oedematous Predominance (CLEP) in Madrid, Spain

Caso clínico: loxoscelismo autóctono cutáneo con predominancia edematosa (CLEP) en Madrid, España

A 69-year-old Spanish woman was admitted to our hospital due to sudden onset facial oedema after an arthropod bite. During the night, while she was asleep, an arthropod walking across her face was noted and she tried to remove it. She noted an acute pain over the upper lip. No flying or terrestrial arthropod was seen when the light was switched on. Nevertheless next day a partially "knocked out" spider was captured between the sheets of her bed. The patient lives in a single-family house in Talamanca del Jarama, a village located in a rural area of the Province of Madrid (3500 inhabitants).

On admission at the emergency room the burning pain was still present, and an asymmetrical left sided facial oedema was noted. The bite occurred at the left side of the upper lip approximately 2 cm from philtrum and a double mark corresponding to the bite of the arthropod (the chelicerae or jaws of a spider bite marks) could be lightly noticed. Surrounding this bite mark a pale plaque of approximately 1.5 cm of maximum diameter could be seen, surrounded by an erythematous halo of approximately 2.5 cm. General symptoms such as general discomfort, fever, dyspnoea or rash were absent. No lymphadenopathies were noticed. Methylprednisolone and amoxiciline–clavulanate were started at the emergency room. Blood parameters were normal. A facial CT-scan was performed, showing mild subcutaneous oedema, with no other bone or soft-tissue lesions.

Antibiotics and steroids were stopped at hospitalisation (48 h after those treatments were started). The patient had a very good self-limiting evolution and was discharged. At ambulatory follow-up, six days after the bite, necrosis signs started at the chelicerae bite marks and expanding approximately 1 cm of diameter (where it was the white plaque). The pain was well relief with habitual analgesics. Drainage and chirurgic debridement were not required.

An entomological study categorised the spider as *Loxosceles rufescens*. Together with the typical clinical manifestations, this gave us a definitive diagnosis of Cutaneous Loxoscelism with an Oedematous Predominance (CLEP) (Fig. 1).

Spider of the genus *Loxosceles* are also called "brown spiders". Their main characteristic is a brown violin-shape pattern located on the prosoma (cephalothorax).[1] They habit in isolated places indoors, rather than outdoors. Bites occur when they feel endangered and have been reported mainly in spring and summer.[2]

L. rufescens is known to live in the Mediterranean Basin.[1] However, not many cases of *Loxosceles* spider bite have been reported in the Iberian Peninsula. According to modified loxoscelism criteria settled by Rader et al.,[3] the case presented should be considered as "documented". Only few other documented cases have been reported in Spain,[4,5] although there are some more presumptive and probable cases[6–8] reported.

Two main subtypes of loxoscelism have been described: Cutaneous and systemic or visceral loxoscelism.[1]

Most common symptoms of cutaneous loxoscelism are oedema and erythema, burning pain and perilesional hyperesthesia. Few hours after the bite a pale-livedoid plaque appears surrounded by an erythematosus area. This pale patch is caused by vasoconstriction and can develop in necrosis in a period of 4–5 days.[2] This is explained by the cytotoxic effect of the venom, which activates complement and induces neutrophil chemotaxis and apoptosis of keratinocytes, producing this dermonecrosis.[1]

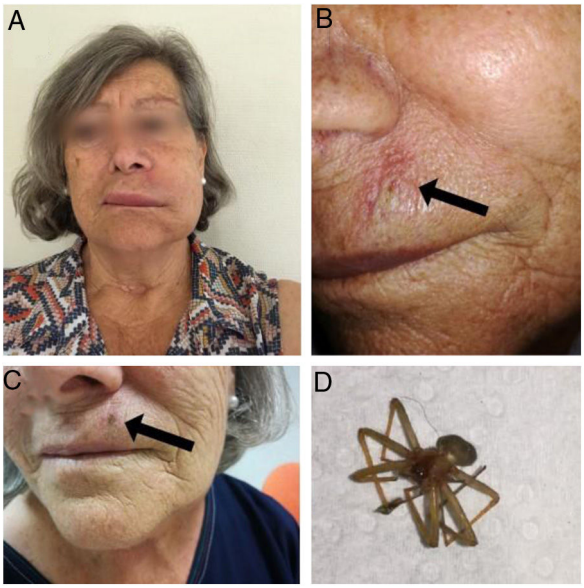

Fig. 1. Clinical features and *Loxosceles rufescens* specimen. (A) Left facial asymmetric oedema 6 h after spider bite. (B) Pale plaque (arrow) 24 h after spider bite. (C) Necrotic eschar (arrow) 7 days after spider bite. (D) *L. rufescens*.

Visceral loxoscelism occurs when venom is injected directly in the blood stream, triggering hemolysis, vasculitis and coagulation alterations. Patients develop fever, haematuria and jaundice in the first 6 h, progressing to decreased level of consciousness and coma with a 25% of mortality.[1]

Oedema is not a common finding of loxoscelism, most of the bites occur at the limbs.[2] A rare type of cutaneous loxoscelism is CLEP, described mainly when the spider bite takes place in the face, usually with a benign prognosis. CLEP is presented frequently with an important oedema, that causes difficulties in diagnosis, and necrotic eschar, is often absent or very small. It is postulated that oedema may abort the necrotic process as it dilutes the venom injected. CLEP occurs in about 4% of loxoscelism cases.[9] To our knowledge, we present the first reported case of CLEP in Spain.

There is no consensus about the best treatment. As it is, in most cases, a self-limiting process, main treatment is supportive.[2]

Conflict of interest

The authors declare not to have any conflict of interest.

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https://doi.org/10.1016/j.eimc.2019.10.005

Stability of viral RNA in clinical specimens for viral diagnosis☆

Estabilidad del ARN viral en muestras clínicas para el diagnóstico viral

In molecular biology, the conservation of genetic material from clinical samples is key when performing experiments with RNA. Therefore, it is important to take the maximum precautions to avoid its degradation, mainly due to RNases that can cause fragmentation of genomic material. These are robust proteins with enzymatic activity that participate in various physiological processes and their main function is to hydrolyse and degrade RNA, and they can also be introduced exogenously into experiments.[1,2]

When clinical samples are kept in the laboratory for long periods of time, their RNA is especially vulnerable to degradation,[3,4] which could influence viral diagnosis by decreasing the sensitivity of amplification techniques.

Various studies have shown that ribonuclease inhibitors exhibit a wide spectrum of inhibitory activities against RNases, effectively protecting RNA during laboratory procedures, allowing great flexibility in experimental design. Their use can be a solution to minimize these degradation problems.[5–7]

The objective of this study was to evaluate the integrity of viral RNA from clinical samples after storage at 4 °C, after a standard working time period (10 days), which is considered sufficient to carry out all the routine processes involved in the viral diagnosis of a sample, and to analyze the efficacy and performance of an RNase inhibitor to prevent its degradation.

Of the samples processed in the Virology Unit of the Hospital Central de Asturias [Central Hospital of Asturias] for two months, 67 exudates (42 nasopharyngeal and 25 pharyngeal) collected in a viral transport medium were studied. Extraction and purification of the genomic material was carried out with the MagNA Pure LC Total Nucleic Acid Isolation Kit Reagent, in the MagNA Pure LC 2.0 automated system (Roche Diagnostics, Switzerland). 100 μl was obtained for a subsequent amplification of fragments of the viral genome and laboratory syndromic diagnostic protocols were applied. In all of them, the quantity and quality of the sample was evaluated, and with the amplification of the human β-globin gene, the normalized viral load (by number of cells) was reported. From the total nucleic extract, two aliquots of 10 μl were separated and in one 1 μl RNase® Inhibitor (Applied Biosystems, USA) was added. Both were stored at 4 °C.

The 67 samples included underwent the usual laboratory diagnostic process for enterovirus (*n*: 53), parainfluenza virus (*n*: 11), and influenza A virus (*n*: 3). After 10 days, the two stored aliquots underwent the same PCR and the amplification cycles (Ct) were compared with and without inhibitor. They were also divided into three groups according to their Ct (<20, 20–25 and 25–35 cycles) and type of virus.

All statistical calculations were performed with the computer program, GraphPad InStat v2.04a (GraphPad Software, USA) according to the specific requirements.

In Table 1, we observe that the presence of viral RNA is evident in all the samples studied, and no statistically significant differences were found in any group after 10 days regardless of whether or not an inhibitor was added. Furthermore, the mean Ct does not vary significantly in any virus.

The samples repeated the amplification range in 59 (88.1%) of the occasions after 10 days, and in 58 (86.6%) of the cases, when RNasin® was added to the extracted sample.

This study shows that, although the use of RNasin® is usually recommended in RNA virus management protocols, it does not seem essential, at least in the viruses studied. In some cases, the performance in the samples with and without an inhibitor decreased slightly, and it is important to note that no sample was undetectable after 10 days.

Thus, we maintain that for viral studies that are conducted in a period of 10 days stored at 4 °C it does not decrease the diagnostic capacity and it would not be necessary to add preservative reagents, which would decrease the handling of the samples and also would not imply an increase in the cost of the diagnostic tests. It would be useful to conduct studies over longer periods of time to establish from what time possible degradation of RNA could be minimized by using an enzyme inhibitor.

Table 1
Ct results in each of the protocols and their mean according to the type of virus.

	Ct	With (without) inhibitor			*p* Value
		<20	20–25	25–35	
Ct	<20 *n* = 18	16 (16)	2 (2)	0 (0)	Ns
	20–25 *n* = 31	4 (1)	20 (24)	7 (6)	Ns
	25–35 *n* = 18	0 (0)	3 (3)	15 (15)	Ns
	Total	Standard	With inhibitor	Without inhibitor	
		24.78 ± 3.44 (21–28)	25.07 ± 2.37 (22–27)	25.28 ± 2.44 (22–27)	
Virus	IA *n* = 3	28.67 ± 4.16 (24–32)	28.34 ± 3.78 (24–31)	28.67 ± 5.13 (23–33)	Ns
	PIV *n* = 11	23.54 ± 5.80 (15–30)	24.09 ± 5.75 (17–31)	24.18 ± 5.87 (17–32)	Ns
	ETV *n* = 53	22.13 ± 4.12 (12–29)	22.79 ± 5.53 (11–36)	23.00 ± 5.25 (12–34)	Ns

Ct: amplification cycles; ETV: enterovirus; IA: Influenza A virus; Ns: not significant; PIV: Parainfluenza virus.

DOI of original article: https://doi.org/10.1016/j.eimc.2019.09.010.
☆ Please cite this article as: García Fernández X, Álvarez-Argüelles ME, Rojo S, de-Oña M. Estabilidad del ARN viral en muestras clínicas para el diagnóstico viral. Enferm Infecc Microbiol Clin. 2019. https://doi.org/10.1016/j.eimc.2019.09.010