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RESEARCH ARTICLE

Detection of Mediterranean Black Widow Spider, *Latrodectus tredecimguttatus* (Rossi, 1790) for the First Time in Jordan by DNA Barcoding and a Case of Envenomation Treated with *Ferula assa-foetida* L. (Apiaceae)

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Abstract

Objective: The Mediterranean black widow spider; *Latrodectus tredecimguttatus* (Rossi, 1790) could reasonably be regarded by many health practitioners as living solely in Southern Europe. However, its range extends well beyond the Mediterranean, into central Asia, and reaches parts of China. In this article, we detected this species in Jordan for the first time and confirmed the diagnosis by DNA barcoding. This is also the first clinically significant envenomation case in Jordan.

Materials and Methods: The spider was identified using DNA analysis which was extracted using a Qiagen DNeasy Blood and Tissue Kit following the standard protocol. PCR reactions were conducted in 20 µL volumes using 1 µL of DNA template, a final concentration of 0.5 µM of each primer. Cycling conditions consisted of an initial activation at 95°C for 15 min, then 35 cycles of denaturation at 95°C for 30 s, annealing at 45°C for 30 s and elongation at 72°C for 1 min with a final elongation at 72°C for 10. Successful PCR reactions were cleaned using Clean NA Clean PCR magnetic beads (GC Biotech) using the standard PCR cleanup protocol. Bi-directional sequencing was conducted at Genewiz UK.

Results: The species was identified using morphological and molecular data. The clinical process was evaluated including the symptoms, medical treatment, and the use of *Ferula assa-foetida* as self-medicated traditional medicine.

Discussion: The first record of this spider is very important for the contribution of the biodiversity of Jordan. In addition, due to its medical importance, the envenomation case and its treatment with Freula are important for local practitioners. Such medically important animals and related cases need to be documented for public health. It is necessary to recognize the existence of venomous spiders in Jordan, and to study their patterns of bites. We also propose a set of recommendations for communities and local hospitals including the necessity of hospitalization and antivenin administration for patients exhibiting serious symptoms.

Conclusion: It is important to document envenomation accidents, symptoms, and treatment protocol. Furthermore, traditional medicine practices should be reported as they can interfere with or even hinder medical treatment. The results of this article will certainly apply to a wider range of countries in the region.

Keywords: Envenomation, Ethnopharmacology, Jordan, *Latrodectus tredecimguttatus*, Spider Bite DNA Barcoding

Introduction

Latrodectism is defined as the specific set of symptoms caused by alpha-latrotoxin and the other components present in the venom of *Latrodectus* spiders, commonly known as black widows (Nicholson & Graudins, 2002). Symptoms have been widely reported in the scientific literature and are well understood, varying considerably depending on the level of envenomation as well as other factors, and can begin within minutes after the bite, to several hours (Sutherland, 1983; White, 1987). Among the symptoms of latrodectism, the most severe cases can present skeletal muscle pain, constricting, diaphoresis, and hypertension (Kobernick, 1984), being potentially lethal due to respiratory and cardiac arrest in children (Vutchev, 2001).

Our knowledge about black widow spiders' diversity, their distribution, and human interactions in Jordan is very limited and is confined to a single record of the white widow, *Latrodectus pallidus* (El-Hennawy 2006, Shakhatreh *et al*., 2021). This manuscript reporting the first clinically significant envenomation of *L. tredecimguttatus* in Jordan is therefore also the first scientific record of this species in the country, despite its distinct morphological features. No research group has yet been established in Jordan focusing on spiders in general or the characterization of spider toxins in particular. This lack of information might have caused health practitioners in Jordan to overlook symptoms of latrodectism, potentially delaying effective treatment.

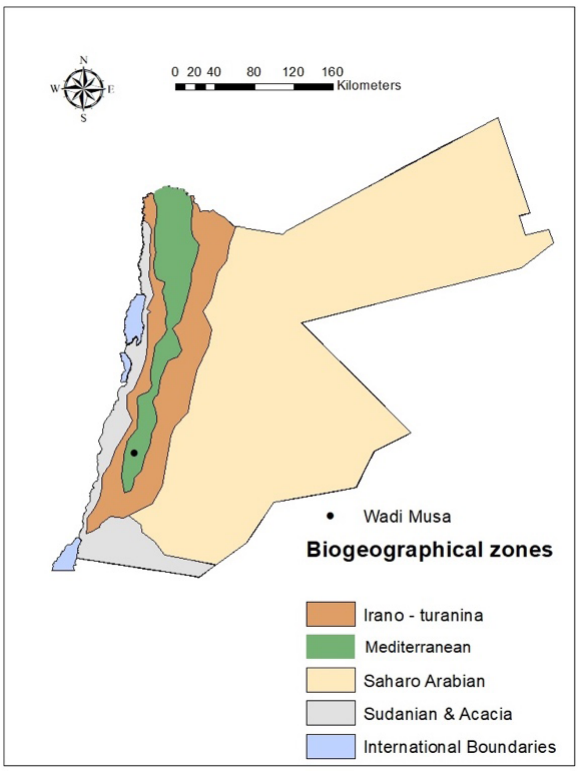

Figure 1. Location where the bite has occurred in relation to Jordan's biogeographical zones (Credit: Ehab Eid).

Materials and Methods

Site Description

The bite occurred in Wadi Musa, which is in the Ma'an governorate south of Jordan (Fig. 1), within the Mediterranean bio-geographical zone, which is characterized by annual rainfall ranges from 400 to 600 mm and a summer temperature rarely exceeding 30°C. The specimen of *L. tredecimguttatus* (Fig. 2) assumed to be responsible for the bite was found in Mediterranean vegetation consisting mostly of shrubs and bushes, dominated by *Rhamnus palaestinus*, *Artemisia herba-alba, Calycotome viliosa, Sarcopoterium spinosum,* and *Cistus* spp. This habitat type takes up a considerable portion of the country overlapping with multiple sites of sizable human presence and use.

Figure 2. *L. tredecimguttatus* collected from the envenomation study site (Credit: Hussein Alnasarat)

Case Study

A 29-year-old male was admitted to the Queen Rania hospital in Ma'an governorate located in the southern part of Jordan on the 25th of June 2020 with a self-diagnosed spider bite. The patient stated that around 08:00 pm Jordan time, as he was placing his shoes to leave a picnic on a field, he felt a sudden stinging pain lasting for around 5 seconds in the middle of his right toe. The patient did not witness the bite taking place, but when removing his foot from the shoe noticed a spider on that same toe. The spider

was killed as it tried to escape and was collected by a friend who escorted him to the hospital along with the sample (analysis of this specimen is described below).

The patient described a clear sensation of pain starting immediately after the bite, but it was localized over the assumed bite area (the toe). However, the pain sensation gradually intensified and became very sharp, with the patient describing the feeling as if his foot had a fire ember on it. A tourniquet was self-applied below the knee and was in place for approximately 10 minutes, the time it took to reach the hospital after the incident. Once a nurse removed this ligament, the patient described the pain as spreading all over his leg and into the rest of the body.

Upon arrival, the patient was moved to the Intensive Care Unit as further systematic symptoms appeared, which included general weakness, dizziness, numbness, and generalized pain. Beyond the topical symptoms most probably because of the general pain situation, which included generalized pain in his body, there was complete paralysis and inability to move, slurred speech, numbness in the legs, and a constant tingling and heat in the foot.

Blood tests showed an increased level of liver enzymes including Aspartates aminotransferase and alanine aminotransferase, indicating toxicity and inflammation in the liver, while also displaying elevated levels of white blood cells, especially neutrophils. The patient also displayed an increased heart rate (90-125), high temperature (around 39°C), high blood pressure reaching 192/111 mmHg, and developing a skin rash that started 10 days after the bite.

The hospital treatment included administering glucose, antibiotics, and antipyretics. However, once the administered medications proved unable to mitigate the main symptoms, which remained severe even two days after the incident, the patient's family decided to resort to traditional medicine, specifically the use of *Ferula assa-foetida*. The resin of this plant was boiled in 200ml of water, and the patient drank from the entire dose only once, reporting intense sweat within 5 minutes, which lasted for six continuous days and decreased gradually. The patient continued his treatment at the hospital, where he left on the 28th of June 2020, while also reporting improvements in overall health and symptom mitigation daily. Ten days after the patient departed from the hospital, he presented a rash on his back, abdomen, and hands.

Analysis

The spider was promptly identified as a black widow, but the species identity was much harder to ascertain with accuracy, as no records of these species had been previously made and this was the first recorded bite of this group in the country. The animal that is inferred to have bitten the patient was killed on site, causing its morphology to be partially damaged, but its remains were collected and preserved in 70% ethanol, and later mounted with 5% glycerin in 50% ethanol on a microscope slide, to be analyzed and photographed. The specimen was not sexually mature and did not present any bifurcated spiders that are characteristic of *L. tredecimguttatus*.

An immature specimen sharing a similar habitus was later collected from the same site for molecular analysis. Similarly to the previous specimen, it did not present any dorsal bifurcated hairs. DNA was extracted using a Qiagen DNeasy Blood and Tissue Kit following the standard protocol. PCR reactions were conducted in 20 µL volumes using 1 µL of DNA template, a final concentration of 0.5 µM of each primer (LCO1490, HCO2198 (Folmer *et al*., 1994) and 4 µL of 5×HOT FIREPol Blend Master Mix Ready to Load with 3.0 mM $MgCl_2$ (Solis BioDyne). Cycling conditions consisted of an initial activation at 95°C for 15 min, then 35 cycles of denaturation at 95°C for 30 s, annealing at 45°C for 30 s and elongation at 72°C for 1 min with a final elongation at 72°C for 10. Successful PCR reactions were cleaned using Clean NA Clean PCR magnetic beads (GC Biotech) using the standard PCR cleanup protocol. Bi-directional sequencing was conducted at Genewiz UK.

Results

The specimen was not sexually mature and did not present any bifurcated spiders that were characteristic of *L. tredecimguttatus*. According to DNA barcoding study results the sample has a 100% BLAST ID with two previously sequenced *Latrodectus tredecimguttatus* specimens (GenBank codes: KC414085 and KC414084; Garb & Hayashi, 2013). Although we would question the geographical origin of KC414084, as it is labeled as Namibia/S. Africa border, a region where it seems unlikely for this species to have been introduced into, and therefore labeling error appears more likely."

Discussion

Knowledge about the black widow spiders in Jordan is still very limited and is confined to a single record of the white widow spider; *Latrodectus pallidus* (El-Hennawy, 2006; Shakhatreh *et al*., 2021). This article has expanded our

knowledge on new species of widow spiders from Jordan and added details for the first time on an envenomation case and using folk medicine for treatment.

The incidents of envenomation by spiders are very limited worldwide. Watson *et al*. (2003) reported no lethal cases by *Latrodectus* envenomation according to the American Association of Poison Control Centers since its first annual report in 1983 until 2004. However, lethal cases involving *L. tredecimguttatus* have been reported in Spain (González *et al*., 2001), Greece (Pneumatikos *et al*., 2003), and through Albania (Hoxha, 2006). As the first case reported from Jordan, it should give healthcare practitioners pause to consider spiders as a potential source of envenomation, by terrestrial animals, on par with snakes and scorpions that have more commonly been reported in the region. This will require them to disambiguate among these species and recognize the signs and symptoms as quickly as possible to apply the appropriate health care for the patients. This process could be expedited through systematic data collection from patients that suspect to have been bitten, including accompanying escorts, and by requesting to observe the spider, ideally, as a preserved specimen, when it is safe to collect, or via photographic record. Although preserved specimens can be an ideal source of reliable information for health practitioners, black widow spiders can be difficult to identify at the species level, particularly in their immature stages, when some diagnostic characters are missing. While some characters that have been considered distinctive in the literature, i.e., the presence of bifurcated dorsal hair in *L. tredecimguttatus*, might be less reliable than once assumed. As seen in the case presented here, DNA analysis has proven to be an effective way to overcome some of these taxonomic challenges, but it will unlikely provide a timely answer to aid treatment. Fortunately, black widows can be quite distinctive from other spiders and several antivenoms have been developed against *Latrodectus* spp. in various parts of the world (Bildik *et al*., 2021). Furthermore, a case reported in Italy showed that the antivenom of *L. mactans* (Antivenom *L. mactans*, Merck Sharp & Dohme®) is effective to treat the bite of *L. tredecimguttatus* (Di Paola *et al*., 2020). Therefore, a species-specific identification might not be necessary from a medical standpoint to initiate treatment.

Best practices for first aid and medical treatment of latrodectism are relatively well understood, however, the application of tourniquets can still be in use (as in the case study presented here) despite having no scientific evidence to support this practice and having been discouraged since the early 80's (Sutherland & Duncan, 1980). Meanwhile, health practitioners in several regions of the world likely over diagnose spider bites (i.e., misidentifying a range of cutaneous reactions as bites), while at times failing to recognize the symptoms of latrodectism, potentially delaying targeted treatment (Rochlin *et al*., 2021). Additionally, medical treatment efficacy may be further confounded using traditional medicine, information that patients might potentially not always share.

The plant *Ferula assa-foetida* (commonly known as Asafetida) is mostly grown in Iran and Afghanistan (Mahendra & Bisht, 2012), but its use has been reported in several regions of the world, mostly in Asia, Southern Europe, and Northern Africa (Iranshahy & Iranshahi, 2011), used for its aromatic properties, as a food condiment (particularly in India and Iran) and in traditional medicine (Amalraj *et al*., 2017). The current traditional medicine literature reports *F. assa-foetida* being used in a wide variety of ways for a vast range of medical issues (Iranshahy & Iranshahi, 2011; Javaid *et al*., 2012; Mahendra & Bisht, 2012). These include mixing it with garlic to treat snakebites (Bhattacharjee, 2004), its gum being dissolved in olive oil as a topical treatment for insect and snake bites (Mohammadhosseini, 2016) or reported to treat scorpion stings (Javaid *et al*., 2012), often misreported as scorpion bites. However, our bibliographical search only found recorded uses of *F. assa-foetida* in Jordan, consumed as a sugar reduction to induce abortion (Lev & Amar, 2002).

Ferula assa-foetida has never been reported for the treatment of spider bites, but its overuse has been reported to cause diarrhea, tympanites, headaches, and dizziness (Eigner & Scholz, 1999), as well as prevent platelet adhesion, lowering blood pressure (Mohammadhosseini *et al*. 2019). Side effects that confuse diagnosis as they overlap with latrodectism and might mislead health practitioners about the effectiveness of treatment. We recorded for the first-time black widow spider as a cause of envenomation in Jordan, while recording the self-prescribed use of *F. assa-foetida* as traditional medicine in its treatment, hoping it will help communities and local hospitals to take appropriate steps, potentially including targeted anti-venom, while triggering efforts at regional level toward this group of animals.

Conclusions

It is very important that healthcare facilities recognize the existence of venomous spiders, recognize their patterns of bites, and distinguish the symptoms of *Latrodectus*

envenomation. This will help to provide immediate care to *Latrodectus* bite victims. In addition, understanding the distribution range is important since it helps to develop precautionary measures and reduce the risks of accidents to people, especially in rural communities, and tourists. Hospitalization and antivenin administration should be secured and reserved for patients exhibiting serious symptoms. This is important as people who report these factors should receive antivenin as soon as possible to avoid suffering envenomation complications.

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