Received: 1 March 2020 | Revised: 26 May 2020 | Accepted: 8 June 2020

DOI: 10.1111/iwj.13444

ORIGINAL ARTICLE

IWJ WILEY

Clinical study of Maggot therapy for Fournier's gangrene

Alicia Fonseca-Muñoz[1] | **Hugo E. Sarmiento-Jiménez**[2] | **Rafael Pérez-Pacheco**[1] | **Patricia J. Thyssen**[3] | **Ronald A. Sherman**[4]

[1]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR), Instituto Politécnico Nacional (IPN), Unidad Oaxaca, Santa Cruz Xoxocotlán, Mexico

[2]Department of Surgery, Clinica Hospital Bianni San Jose Oaxaca, Oaxaca, Mexico

[3]Department of Animal Biology, Institute of Biology, University of Campinas (UNICAMP), Campinas, Brazil

[4]Board Chair and Director, BioTherapeutics, Education & Research (BTER) Foundation, Irvine, California, USA

Correspondence
Ronald A. Sherman, MD, MSc Director, BioTherapeutics, Education & Research (BTER) Foundation, 36 Urey Court, Irvine, CA, 92617.
Email: rsherman@uci.edu

Funding information
Consejo Nacional de Ciencia y Tecnología

Abstract

Fournier's Gangrene is a fulminating necrotizing fasciitis of the perineum and genitalia. Standard treatment involves immediate excision of all necrotic tissue, aggressive antibiotic coverage, and supportive medical care. Still, the infection is commonly fatal or disfiguring. Wound treatment with disinfected blowfly larvae (maggot debridement therapy or MDT) has been shown to be highly effective, with multiple studies demonstrating effective debridement, disinfection, and promotion of granulation tissue. MDT also has been associated with preservation of viable tissue and minimised blood loss. This report describes a prospective clinical study of MDT for Fournier's gangrene aimed to test the hypothesis that early use of maggots could decrease the number of surgical treatments required to treat Fournier's gangrene. Subjects were provided with one initial surgical excision, followed by debridement using only medical grade *Lucilia sericata* larvae. Only two subjects were enrolled, both diabetic men. Intensive care and culture-directed antimicrobial coverage were administered as usual. Maggot debridement was associated with the disappearance of necrotic tissue, control of infection and granulation tissue growth. In both subjects, wounds healed without requiring further surgical resection or anatomical reconstruction. Maggot therapy decreased the number of surgical procedures that otherwise would have been necessary, and led to favourable outcomes.

KEYWORDS

biotherapy, clinical study, Fournier's gangrene, *Lucilia sericata*, maggot therapy

1 | INTRODUCTION

Fournier's gangrene is a fulminating necrotizing fasciitis that develops mainly in the perineum and genitalia. It can extend to affect the inguinal region, thighs, and even the abdominal wall. This life-threatening condition is frequently caused by a synergistic polymicrobial infection.[1-6] The most common predisposing factors are age, diabetes, malnutrition, and other forms of immunosuppression.[7,8] Early diagnosis is essential because the infection progresses rapidly, leading to multi-organ failure and death in 30% to 50% of cases, or even more.[9-12] Initial treatment consists of broad-spectrum antibiotics and aggressive surgical resection of the necrotic tissue.[7,10-11] In fact, the infection—and therefore the resection—can be so aggressive that extensive anatomical reconstruction is often required after the infection is controlled and the necrotic tissue debrided.[7]

Maggot debridement therapy (MDT) is the use of disinfected blowfly larvae—usually *Lucilia sericata*—to treat problematic or non-healing wounds. MDT has been shown to be a highly effective and rapid method of debridement.[13-21] Medicinal maggots have three main actions on the wounds: debridement, disinfection, and

stimulation of new tissue growth.[22-23] Maggots debride wounds both by their physical "scrubbing" action on the wound and by secreting and excreting their digestive juices. The maggot's gut secretes a variety of proteolytic enzymes (including trypsin and chymotrypsin-like serine proteases) which liquefy necrotic tissue but appear to be incapable of digesting live tissue.[23] Secreted molecules also break down complement proteins C3 and C4 in a cation-independent manner,[24] thereby reducing complement activation by as much as 99.9%. Gram positive and negative bacteria, ingested by the maggots, are killed by the time they reach the mid hind-gut.[25] Medicinal maggots also kill microbes in the wound bed itself [26-28] due to the secretion of molecules like antimicrobial peptides,[29] lysozyme,[30] and deoxyribonucleases.[31] Maggot therapy may also kill microbes by inducing changes in the wound environment like pH.[32] Maggot secretions and excretions have even been shown to destroy *Staphylococcus aureus* and *Pseudomonas aeruginosa* biofilm, and prevent the formation of new biofilm.[31,33-37]

The mechanisms by which maggot therapy might bring about hastened tissue growth have been more difficult to define. Maggot secretions have been shown to have a wide variety of effects on human tissue, including the stimulation of mitosis and/or migration of fibroblasts, endothelial cells, and even neural tissue.[23] Clinical evidence of wound healing has been conflicting, with some studies demonstrating clear evidence of hastened tissue growth[13,15-16] and others decidedly not.[19]

Maggots also have the capability to access the difficult to access areas of the wound for the surgeon, e.g., wound pockets and sinuses.[22,38,39.] This, combined with the ability of the maggots to dissolve necrotic tissue without harming viable tissue, makes maggot therapy particularly valuable for treating Fournier's gangrene and similar conditions in which the necrotising fasciitis penetrates deep into spaces containing vital structures – nerves, veins, and arteries. In the treatment of Fournier's gangrene, one of the greatest problems facing surgeons is the difficulty of removing all of the dead, infected tissue without harming the critical tissue nearby that is still viable.

Maggot therapy is not without its risks; but fortunately those risks are relatively small and uncommon. The most common risk is pain, due to the maggot's rough body (exoskeleton) and probing mouthhooks (modified mandibles) crawling over tender innervated flesh. The potent proteolytic enzymes that it secretes over the wound may also cause some discomfort. Since deep tissue wounds are usually devoid of functional sensory nerves, such pain is rarely a problem in patients with necrotising fasciitis. Significant haemorrhage has been reported to occur during maggot therapy.[40] This was likely the result of blood vessels whose necrotic or partially necrotic walls dissolved along with the rest of the regional necrotic tissue.[41]

Key Messages

- Fournier's gangrene (necrotizing fasciitis of the perineum and genitalia) carries a high rate of morbidity and mortality
- the subjects in this prospective study, surgically debrided upon initial presentation but debrided only by maggot debridement therapy (MDT) thereafter, survived their wounds without requiring any further surgical resection or reconstructive surgery, other than simple closure or split thickness skin grafting
- early use of MDT in Fournier's gangrene could decrease the number and extent of surgical debridements otherwise required

In order to reduce these adverse events and also reduce the disgust of handling live fly larvae ("yuk factor"), some therapists use bag-like dressings that completely contain the maggots[42] so that the larvae cannot wander freely. For superficial wounds like venous stasis ulcers, contained (bagged) maggots have been found to debride faster than control ("standard") non-surgical therapy, but not as quickly or efficiently as maggots with total access to the wound bed ("free-range maggots").[19]

MDT is used most commonly in wounds like pressure ulcers,[13,15] diabetic foot ulcers.[16,22,43] venous stasis ulcers,[19,44] for which there have been several successful clinical trials. When used in patients with deep tissue wounds like necrotising fasciitis,[45-49] maggot therapy usually has been initiated as a salvage procedure, after everything else has failed. Under such circumstances, even when it is effective, so much time and tissue has been lost that the outcomes may still be quite poor.[45] Without prospective studies of MDT for necrotizing fasciitis and with the ever-present "yuk factor" surrounding fly larvae, it is not surprising that clinicians and researchers have been reluctant to exchange the standard surgical resection for maggot debridement in life-threatening deep tissue infections like Fournier's gangrene.

If used earlier in the course of treatment, might maggot therapy be able to minimise the number and extent of surgical debridements, as previously suggested?[49] This report describes the first prospective clinical study of maggot therapy for Fournier's gangrene, carried out in order to test that hypothesis. Although the study ended

after enrolling only two study subjects and no control subjects, its results are presented here because they should be of interest to clinicians practicing wound care and trauma surgery.

2 | METHODS

2.1 | Inclusion criteria

Patients diagnosed with Fournier's Gangrene by the multidisciplinary team of medical specialists at San José Hospital, Oaxaca, Mexico were enrolled in the study if they provided written informed consent. The study and consent form were approved by the institutional review board as being in compliance with the World Medical Association's Declaration of Helsinki.

2.2 | Rearing medicinal flies and maggots

Colonies of *L. sericata* were established from adult flies collected in the field (Santa Maria el Tule Oaxaca, Mexico, 17°02′47″N, 96°38′11″O) using traps[50] containing raw bovine liver as bait. The collected specimens were taken to the laboratory, temporarily anaesthetised by cooling them for about 3 minutes at −20°C, identified,[51] and transferred to screened plastic cages in a climate-controlled room at 27°C ± 1°C, 60% ± 10% relative humidity, and a 12-hour photoperiod. Water and granulated sucrose were provided to the adult flies ad libitum. A portion of raw beef liver was used to stimulate oviposition. With the aid of a brush, the eggs were removed from the substrate, washed, disinfected,[52] and kept on filter paper in a climatic chamber at 27°C for 24 hours, until the larvae hatched. During this interval and prior to therapeutic application, some of the disinfected eggs were plated by spreading onto Petri dishes containing brain heart infusion agar and incubated at 37°C for 24 hours to ensure that there were no contaminants present.

2.3 | Application of larvae (method of maggot debridement)

After the peri-wound skin was cleaned and dried, 1 × 6 cm adhesive dressing strips (Hypafix; Charlotte, North Carolina) were applied around the wound bed to allow adhesion of the primary and secondary dressings. Approximately 10 larvae per cm^2 were placed on the wound bed,[46] and the maggots were then covered by a sterile fine polyester mesh, taped to the previously placed adhesive strips. After the dressing was sealed, several gauze pads were placed on top to absorb the exudates that would eventually drain from the wound. The gauze pads were changed when they became soiled, to avoid asphyxiation or drowning of the maggots and to avoid maceration of the skin. The dressing was inspected every 4 to 8 hours to check maggot viability and to ensure that there were no escapes. Maggots were removed from the wound by flushing them with saline or clean water. They were sealed in biohazard bags until autoclaving and disposal. Between each 2- or 3-day cycle of MDT, the wounds were washed with sterile water.

3 | RESULTS

Two subjects were enrolled in this prospective clinical study. They each received only one immediate surgical debridement, along with antibiotic therapy directed according to microbial culture results. Thereafter, they received only MDT and no further surgical debridements. Neither subject withdrew from the study; neither subject required subsequent surgical debridement or reconstructive surgery; neither patient had a poor outcome.

3.1 | Case 1

Subject #1 was a 59 year-old hemiplegic man from Oaxaca de Juarez, Mexico. He had non-insulin-dependent diabetes mellitus for 30 years, hypertension for 5 years and renal failure requiring peritoneal dialysis for the prior 3 years. He presented with necrosis of the scrotum over the previous 15 days. The wound was fetid and exudative. The diagnosis of Fournier's gangrene was made, and the patient underwent aggressive surgical debridement with antibiotic coverage: metronidazole and cefotaxime. *Escherichia coli* and *S. aureus* were cultured from wound samples. Due to persistent sepsis, advancing necrosis, and the desire to preserve as much viable tissue as possible, at this point he was given the opportunity to participate in this trial, which he accepted.

His wounds were treated with three 48-hour cycles of MDT (approximately 300 larvae/treatment). After the first cycle, there was a notable decrease in exudate and odour, and granulation tissue began to appear along the wound bed. By the end of the third cycle, the wound was covered mostly by healthy granulation tissue (Figure 1). At this point, it was decided to treat the wound with honey-impregnated gauze. Five days later, the wound was grafted successfully. The patient was discharged after 20 days in the hospital.

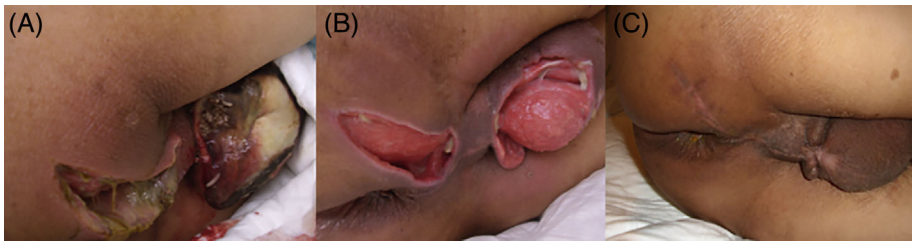

FIGURE 1 Subject #1. A, During maggot debridement therapy (MDT); B, wound bed with granulation tissue after MDT; C, approximately 2 weeks after punch grafting

3.2 | Case 2

Subject #2 was a 32 year-old man from Oaxaca, Mexico, recently diagnosed with diabetes mellitus. He was symptomatic (polydipsia, polyphagia), but had not yet started drug therapy. His history was also notable for cigarette smoking, alcoholism, myelomeningocele, prior blood transfusion, and lactose intolerance, but no recent trauma.

He presented to the emergency room complaining of 11 days of perianal pain, redness, and now fevers. Upon examination, he was found to be tachycardic and tachypnic, with perianal edema and a central ulcer draining fetid, purulent material. Hyperemia extended to the inguinal ligament bilaterally, and the tissue necrosis extended to the right flank. He was dehydrated, with a leukocytosis and a serum glucose of over 500 mg/dL. *E. coli* and *Candida albicans* were cultured from wound samples. Initial management included re-hydration, metabolic correction, broad-spectrum antibiotics (initially vancomycin, imipenem and metronidazole, subsequently narrowed to levofloxacin and metronidazole) and an aggressive surgical resection.

The patient's metabolic and fluid status stabilised, but further debridement was needed. After consenting to participate in this study, he received a total of eight cycles of MDT (approximately 500 larvae/treatment). Following the final cycle of MDT, no necrotic tissue remained, wound cultures were clear of pathogens, and the wound base was covered with abundant healthy granulation tissue (Figure 2). At this point, it was decided to treat the wound with honey-impregnated gauze for another 10 days. Finally, the wound was sutured closed. The total hospital stay was 52 days.

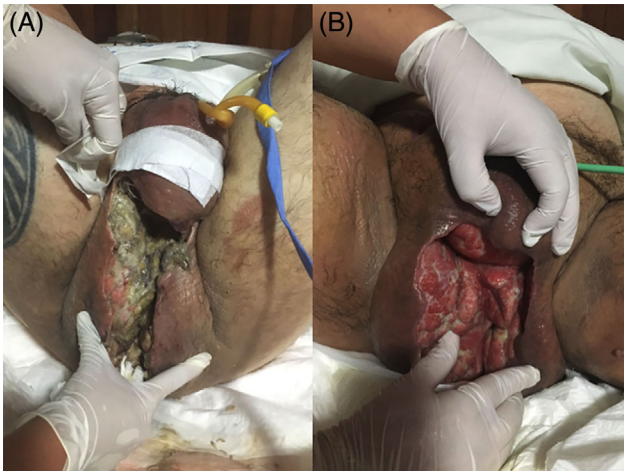

FIGURE 2 Subject #2. A, Wound during maggot debridement therapy (MDT); B, granulation tissue covering wound bed after MDT

4 | DISCUSSION

The efficacy of MDT for debridement and infection control has been demonstrated repeatedly in a variety of clinical and medical settings around the world.[13-21,26,43,45-49,52-69] Fournier's gangrene is a medical emergency with a high risk of death and disfigurement. Occasionally, maggot therapy has been called upon to help debride patients with Fournier's gangrene, but only as the "last resort," i.e., to prevent additional loss of blood and viable tissue when the wound encroached on vital structures, or when the patient was not able to tolerate anaesthesia or the other risks of the surgery.[45-46]

Several years ago, it was reported that two patients with Fournier's gangrene were successfully treated with MDT, but the therapy was not initiated until more than 2 weeks after hospital admission.[45] By that time, one of their patients needed extensive reconstructive surgery and the other ultimately succumbed to his "generalised debility." Still, the authors pointed out the benefits of MDT in terms of decreased bleeding, decreased loss of viable tissue, and decreased cost. More recent reports indicate that maggot therapy not only effectively debrides the necrotic tissue of patients with fasciitis, but also disinfects the wound, minimises tissue loss, and promotes the growth of granulation tissue.[46,59] In fact, the highly successful grafting after MDT is sometimes credited to the healthy granulation tissue following maggot debridement;[59] it may also be the result of the maggots' antimicrobial activity.[54]

Steenvoorde and colleagues[49] published their results after analysing the largest case series yet: 15 patients with necrotizing fasciitis treated with surgical debridement and antibiotic therapy in combination with MDT. They

found that early use of MDT was associated with a reduction in the number of surgical debridements. Herein, we have described the first prospective study to test whether maggot therapy really can reduce the number of surgical resections required. These results demonstrate that maggot therapy did indeed reduce the number of surgical procedures that would have otherwise been performed.

The growing literature of clinical studies and case reports clearly indicates that maggot therapy carries a very favourable risk–benefit ratio (Table 1). Furthermore, the cost of MDT is much less than the cost of surgery (facilities, supplies, and personnel time). Therefore, where medicinal maggots are readily available, no good reason remains to restrict the use of maggot therapy to that of a "last resort" modality. The results of this small but prospective clinical study demonstrate that there is great potential in the early initiation of maggot debridement in Fournier's gangrene, including reduced surgical resections and reduced morbidity.

Although the present study recruited only two subjects, and although no control subjects were included, our results of complete cure after one single surgical debridement followed by maggot debridement are as good or better than our historical experience treating these infected deep tissue wounds. Both subjects would have undergone one or more additional surgical resections had they not enrolled in this maggot therapy study (based on the study enrollment criteria), and one or both might have required reconstructive surgery after that. Given these results and the results of previous studies, we propose that the routine use of MDT after a single aggressive surgical resection, could be a safe and effective protocol for the treatment of Fournier's gangrene, and should be studied further.

TABLE 1 Advantages, disadvantages and limitations of maggot debridement therapy for the treatment of necrotizing fasciitis

Advantages	Disadvantages/limitations
Debridement more rapid than conventional dressings	Debridement not as rapid as surgical resection
Debridement more precise and specific than surgery/ avoids resection of viable tissue	May be difficult to make the cage dressing due the size and location/anatomy of the wound
Because viable tissue is spared, the need for reconstructive procedures is minimised	Maggots may escape if dressings are not secure and cause dermatitis to healthy skin, or if the dressings loosen from excessive exudate
No need for anaesthesia	
Reduce the number of surgical resections	Sometimes causes tickling, itching, or pain
Maggots can be applied without need for colostomy	Removal of maggots can be messy
Maggots can access the undermined areas (small spaces deep within the wound) without the need to open the area widely	Dressings must be placed so they do not interfere with bowel movement and urination
Minimal blood loss	Can cause anxiety in medical personnel and some patients
Decrease edema, exudate, and odour	

These results suggest that routine use of MDT after a single surgical resection might also be a cost-efficient protocol for treating Fournier's gangrene. Most published cost comparisons of maggot therapy versus conventional debridement therapy have looked at relatively low-cost, outpatient alternatives. While all of those studies demonstrated faster debridement with maggot therapy, the cost differences were only substantial in those studies in which maggot therapy decreased the number of office visits.[44] In those studies where both the maggot therapy arm and the control arm had similar numbers of office visits, the total cost differences between the two study arms were insignificant.[19] But when it comes to Fournier's gangrene, the standard of care (alternative to maggot debridement) is surgical debridement, and the cost difference between maggot therapy and surgery is substantial. The mean cost of treating a patient with Fournier´s gangrene is €25 109.[70] This cost includes hospitalisation (per day), drugs (antibiotics and pain medication, for example), and interventions (surgery and dressing materials). For lack of a control and sufficiently large study size, a direct cost comparison between surgical care alone and a single (initial) surgical debridement followed by MDT is not possible from our data. However, assuming that the duration of hospitalisation would be unchanged and that there would not be any significant difference in drug usage, the cost difference between repeated surgical debridements and maggot debridement would likely be significant. For example, in Canada, 2013, the cost of maggots was $69 per treatment (50 EUR today) while the cost for operating room time alone for a simple debridement under anaesthesia (not including the cost for staff and supplies) was $4604 (the equivalent of 3370 EUR today).[71] Since maggots do not need to be applied by a surgeon or even a physician, personnel costs of MDT would also be substantially lower than personnel costs associated with surgery. A cost–benefit analysis should be part of the next clinical study of maggot therapy for Fournier's gangrene.

Circumstances did not permit this clinical study to enrol more than two patients. But based on our favourable findings, we hope others will pursue this line of investigation with a much larger prospective trial, including a surgery-only control arm. In addition to evaluating the safety and efficacy of MDT, a larger study

could also address other clinically relevant differences between the two study arms: cost differences; differences in antibiotic usage; differences in efficacy or safety between MDT performed with free-range maggots versus contained maggots. Given the positive outcome of MDT in this study and in other cases reported in the literature, the next clinical studies should begin soon if we are to quickly lower the high morbidity and mortality of Fournier's gangrene as it is currently treated.

ACKNOWLEDGEMENTS

The authors express their appreciation to the Consejo Nacional de Ciencia y Tecnología (CONACYT-Mexico) for the financial scholarship that was provided towards the doctoral degree of the first author. This study was conducted as part of that doctoral work.

CONFLICT OF INTEREST

Ronald Sherman declares the following conflicts of interest: He is Co-Founder and Laboratory Director of Monarch Labs, which produces medicinal maggots and other animals. To minimise conflicts of interest, Dr Sherman is not compensated for his laboratory or administrative work; his wife is, however. Dr Sherman did not design nor fund the present study. He joined the study team after the first draft had been written, and his contribution was to assist with the critical analysis and writing of the report. Dr Sherman is also Director of the non-profit BioTherapeutics, Education and Research (BTER) Foundation. Dr Sherman's work for the BTER Foundation, like his work for Monarch Labs, is pro bono publica. The remaining authors declare no conflicts of interest.

REFERENCES

1. Clayton MD, Fowler JE Jr, Sharifi R, Pearl RK. Causes, presentation and survival of fifty-seven patients with necrotizing fasciitis of the male genitalia. *Sur Gynecol Obstet*. 1990;170:49-55.
2. Laucks SS. Fournier's gangrene. *Surg Clin North Am*. 1994;74:1339-1352.
3. Morpurgo E, Galandiuk S. Fournier's gangrene. *Surg Clin North Am*. 2002;82:1213-1224.
4. Corcoran AT, Smaldone MC, Gibbons EP, Walsh TJ, Davies BJ. Validation of the Fournier's gangrene severity index in a large contemporary series. *J Urol*. 2008;180:944-948.
5. Lujan Marco S, Budía A, Di Capua C, Broseta E, Jiménez Cruz F. Evaluation of a severity score to predict the prognosis of Fournier's gangrene. *BJU Int*. 2010;106:373-376.
6. Erol B, Tuncel A, Hanci V, et al. Fournier's gangrene: overview of prognostic factors and definition of new prognostic parameter. *Urology*. 2010;75:1193-1198.
7. Eke N. Fournier´s gangrene: a review of 1726 cases. *Br J Surg*. 2000;87:718-728.
8. Moslemi MK, Sadighi Gilani MA, Moslemi AA, Arabshahi A. Fournier gangrene presenting in a patient with undiagnosed rectal adenocarcinoma: a case report. *Cases J*. 2009;2:9136.
9. Kaiser R, Cerra F. Progressive necrotizing surgical infections-a unified approach. *J Trauma*. 1981;21:349-355.
10. Spirnak JP, Resnick MI, Hampel N, Persky L. Fournier´s gangrene: report of 20 patients. *J Urol*. 1984;131:289-291.
11. Laor E, Palmer LS, Tolia BM, Reid RE, Winter HI. Outcome prediction in patients with Fournier's gangrene. *J Urol*. 1995;154:89-92.
12. Aridogan IA, Izol V, Abat D, Karsli O, Bayazit Y, Satar N. Epidemiological characteristics of Fournier's gangrene: a report of 71 patients. *Urol Int*. 2012;89:457-461.
13. Sherman RA, Wyle F, Vulpe M. Maggot therapy for treating pressure ulcers in spinal cord injury patients. *J Spinal Cord Med*. 1995;18:71-74.
14. Markevich YO, McLeod-Roberts J, Mousley M, Melloy E. Maggot therapy for diabetic neuropathic foot wounds. *Diabetologia: Proceedings of the 36th Annual meeting of the European Association for the Study of Diabetes* 2000;43 Suppl 1:A15.
15. Sherman RA. Maggot versus conservative debridement therapy for the treatment of pressure ulcers. *Wound Repair Regen*. 2002;10:208-214.
16. Sherman RA. Maggot therapy for treating diabetic foot ulcers unresponsive to conventional therapy. *Diabetes Care*. 2003;26:446-451.
17. Steenvoorde P, Jacobi CE, Van Doorn L, Oskam J. Maggot debridement therapy of infected ulcers: patient and wound factors influencing outcome—a study on 101 patients with 117 wounds. *Ann R Coll Surg Engl*. 2007;89:596-602.
18. Paul AG, Ahmad NW, Lee HL, et al. Maggot debridement therapy with *Lucilia cuprina*: a comparison with conventional debridement in diabetic foot ulcers. *Int Wound J*. 2009;6:39-46.
19. Dumville JC, Worthy G, Bland JM, et al. Torgerson DJ; VenUS II team. Larval therapy for leg ulcers (VenUS II): randomised controlled trial. *BMJ*. 2009;338:b773.
20. Marineau ML, Herrington MT, Swenor KM, Eron LJ. Maggot debridement therapy in the treatment of complex diabetic wounds. *Hawaii Med J*. 2011;70:121-124.
21. Mudge E, Price P, Walkley N, Harding KG. A randomized controlled trial of larval therapy for the debridement of leg ulcers: results of a multicenter, randomized, controlled, open, observer blind, parallel group study. *Wound Repair Regen*. 2014;22:43-51. Erratum in: Wound Repair Regen. 2014;22:290.
22. Mumcuoglu KY, Ingber A, Gilead L, et al. Maggot therapy for the treatment of diabetic foot ulcers. *Diabetes Care*. 1998;21:2030-2031.
23. Sherman RA. Mechanisms of maggot-induced wound healing: what do we know, and where do we go from here? *Evid Based Complement Alternat Med*. 2014;2014:592419.
24. Cazander G, Schreurs MW, Renwarin L, Dorresteijn C, Hamann D, Jukema GN. Maggot excretions affect the human complement system. *Wound Repair Regen*. 2012;20(6):879-886.
25. Mumcuoglu KY. Clinical applications for maggots in wound care. *Am J Clin Dermatol*. 2001;2:219-227.
26. Contreras-Ruiz J, Fuentes-Suárez A, Arroyo-Escalante S, et al. Estudio comparativo de la eficacia de la larvaterapia (LT) para desbridar y controlar la carga bacteriana en úlceras venosas comparado con desbridamiento quirúrgico y aplicación de un antimicrobiano tópico. [Comparative study of the efficacy of larva therapy for debridement and control of bacterial burden compared to surgical debridement and topical application of an antimicrobial]. *Gac Med Mex*. 2016;152(Suppl 2):78-87.

27. Tantawi TI, Gohar YM, Kotb MM, Beshara FM, El-Naggar MM. Clinical and microbiological efficacy of MDT in the treatment of diabetic foot ulcers. *J Wound Care*. 2007;16(9):379-383.
28. Bowling FL, Salgami EV, Boulton AJ. Larval therapy: a novel treatment in eliminating methicillin-resistant *Staphylococcus aureus* from diabetic foot ulcers. *Diabetes Care*. 2007;30(2): 370-371.
29. Cerovský V, Zdárek J, Fucík V, Monincová L, Voburka Z, Bém R. Lucifensin, the long-sought antimicrobial factor of medicinal maggots of the blowfly *Lucilia sericata*. *Cell Mol Life Sci*. 2010;67(3):455-466.
30. Valachova I, Takac P, Majtan J. Midgut lysozymes of *Lucilia sericata*—new antimicrobials involved in maggot debridement therapy. *Insect Mol Biol*. 2014;23(6):779-787.
31. Brown A, Horobin A, Blount DG, et al. Blow fly *Lucilia sericata* nuclease digests DNA associated with wound slough/eschar and with *Pseudomonas aeruginosa* biofilm. *Med Vet Entomol*. 2012;26:432-439.
32. Sherman RA, Mumcuoglu KY, Grassberger M, Tantawi TI. Maggot therapy. In: Grassberger M, Sherman RA, Gileva OS, CMH K, Mumcuoglu KY, eds. *Biotherapy—history, principles and practice: a practical guide to the diagnosis and treatment of disease using living organisms*. Dordrecht: Springer Science +Business Media; 2013:5-29.
33. van der Plas MJ, Jukema GN, Wai SW, et al. Maggot excretions/secretions are differentially effective against biofilms of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *J Antimicrob Chemother*. 2008;61(1):117-122.
34. van der Plas MJ, Dambrot C, Dogterom-Ballering HC, Kruithof S, van Dissel JT, Nibbering PH. Combinations of maggot excretions/secretions and antibiotics are effective against *Staphylococcus aureus* biofilms and the bacteria derived therefrom. *J Antimicrob Chemother*. 2010;65(5):917-923.
35. Cazander G, van Veen KE, Bouwman LH, Bernards AT, Jukema GN. The influence of maggot excretions on PAO1 biofilm formation on different biomaterials. *Clin Orthop Relat Res*. 2009;467(2):536-545.
36. Cazander G, van de Veerdonk MC, Vandenbroucke-Grauls CM, Schreurs MW, Jukema GN. Maggot excretions inhibit biofilm formation on biomaterials. *Clin Orthop Relat Res*. 2010;468(10):2789-2796.
37. Gordya N, Yakovlev A, Kruglikova A, et al. Natural antimicrobial peptide complexes in the fighting of antibiotic resistant biofilms: *Calliphora vicina* medicinal maggots. *PLoS One*. 2017 Mar 9;12(3):e0173559.
38. Wollina U, Karte K, Herold C, Looks A. Biosurgery in wound healing—the renaissance of maggot therapy. *J Eur Acad Dermatol Venereol*. 2000;14:285-289.
39. Sherman RA, Shapiro CE, Yang RM. Maggot therapy for problematic wounds: uncommon and off-label applications. *Adv Skin Wound Care*. 2007;20:602-610.
40. Steenvoorde P, van Doorn LP. Maggot debridement therapy: serious bleeding can occur report of a case. *J Wound Ostomy Continence Nurs*. 2008;35(4):412-414.
41. Sherman RA. Maggot therapy for foot and leg wounds. *Int J Low Extrem Wounds*. 2002;1(2):135-142.
42. Grassberger M, Fleischmann W. The biobag—a new device for the application of medicinal maggots. *Dermatology*. 2002;204 (4):306.
43. Pinheiro MA, Ferraz JB, Junior MA, et al. Use of maggot therapy for treating a diabetic foot ulcer colonized by multidrug resistant bacteria in Brazil. *Indian J Med Res*. 2015;141:340-342.
44. Wayman J, Nirojogi V, Walker A, Sowinski A, Walker MA. The cost effectiveness of larval therapy in venous ulcers. *J Tissue Viability*. 2000;10:91-94.
45. Teich S, Myers RA. Maggot therapy for severe skin infections. *South Med J*. 1986;79:1153-1155.
46. Angel K, Grassberger M, Huemer F, Stackl W. Madentherapie bei Fournierscher Gangränerste Erfahrungen mit einer neuen Therapie. *Aktuel Urol*. 2000;31:440-443.
47. Dunn C, Raghavan U, Pfleiderer AG. The use of maggots in head and neck necrotizing fasciitis. *J Laryngol Otol*. 2002;116:70-72.
48. Preuss SF, Stenzel MJ, Esriti A. The successful use of maggots in necrotizing fasciitis of the neck: a case report. *Head Neck*. 2004;26:747-750.
49. Steenvoorde P, Jacobi C, Wong C, Jukema G. Maggot debridement therapy in necrotizing fasciitis reduces the number of surgical debridements. *Wounds*. 2007;19:73-78.
50. Moretti TC, Thyssen PJ, Solis DR. Breeding of the scuttle Fly *Megaselia scalaris* in a fish carcass and implications for the use in forensic entomology (Diptera: Phoridae). *Entomol Gen*. 2009; 31:349-353.
51. Grella MD, Thyssen PJ, Chave taxonômica interativa para espécies de dípteros califorídeos (Infraordem: Muscomorpha) do Brasil. 2011. http://keys.lucidcentral.org/keys/v3/calliphoridae_brazil. Accessed January 9, 2019.
52. Figueroa I, Flores J, Rodríguez S. Método de cultivo de larvas de moscas *Lucilia sericata* para terapia larval. *Parasitol Latinoam*. 2007;62:79-82.
53. Jukema GN, Menon AG, Bernards AT, Steenvoorde P, Taheri Rastegar A, van Dissel JT. Amputation-sparing treatment by nature: "surgical" maggots revisited. *Clin Infect Dis*. 2002;35: 1566-1571.
54. Sherman RA, Shimoda KJ. Presurgical maggot debridement of soft tissue wounds is associated with decreased rates of postoperative infection. *Clin Infect Dis*. 2004;39:1067-1070.
55. Armstrong DG, Salas P, Short B, et al. Maggot therapy in "lower-extremity hospice" wound care fewer amputations and more antibiotic-free days. *J Am Podiatr Med Assoc*. 2005;95: 254-257.
56. Figueroa L, Uherek F, Yusef P, López L, Flores J. Experiencia de terapia larval en pacientes con úlceras crónicas. *Parasitol Latinoam*. 2006;61:160-164.
57. Echeverri MIW, Álvarez CR, Higuita SEH, Idárraga JCW, Franco MME. *Lucilia eximia* (Diptera: Calliphoridae), una nueva alternativa para la terapia larval y reporte de casos en Colombia. *Iatreia*. 2010;23:107-116.
58. Felder JM, Hechenbleikner E, Jordan M, Jeng J. Increasing the options for management of large and complex chronic wounds with a scalable, closed-system dressing for maggot therapy. *J Burn Care Res*. 2012;33:70-76.
59. Tantawi TI, Williams KA, Villet MH. An accidental but safe and effective use of *Lucilia cuprina* (Diptera: Calliphoidae) in maggot debridement therapy in Alexandria, Egypt. *J Med Entomol*. 2010;47:491-494.
60. Campbell N, Campbell D. A retrospective, quality improvement review of maggot debridement therapy outcomes in a foot and leg ulcer clinic. *Ostomy Wound Manage*. 2014;60:16-25.

61. Borst GM, Goettler CE, Kachare SD, Sherman RA. Maggot therapy for elephantiasis nostras verrucosa reveals new applications and new complications: a case report. *Int J Low Extrem Wounds*. 2014;13:135-139.
62. Bohac M, Cambal M, Zamborsky R, Takac P, Fedeles J Sr. Maggot therapy in treatment of a complex hand injury complicated by mycotic infection. *Bratisl Lek Listy*. 2015;116:671-673.
63. DeFazio MV, Felder JM 3rd, Economides JM, Attinger CE. Home improvement in maggot therapy: designing a simple, cost-effective, closed-system habitat to facilitate Biodébridement of complex distal lower extremity wounds. *Plast Reconstr Surg*. 2015;136:722e-723e.
64. Martínez Faura MD, Franco Romero M, Ordoño Martínez C, García Mallo ME. Maggot therapy: case study in home care. *Rev Enferm*. 2016;39:26-29.
65. Nigam Y, Morgan C. Does maggot therapy promote wound healing? The clinical and cellular evidence. *J Eur Acad Dermatol Venereol*. 2016;30:776-782.
66. Mirabzadeh A, Ladani MJ, Imani B, Rosen SA, Sherman RA. Maggot therapy for wound care in Iran: a case series of the first 28 patients. *J Wound Care*. 2017;26:137-143.
67. Nasoori A, Hoomand R. Maggot debridement therapy for an electrical burn injury with instructions for the use of *Lucilia sericata* larvae. *J Wound Care*. 2017;26:734-741.
68. Nishijima A, Gosho M, Yoshida R, et al. Effective wound bed preparation using maggot debridement therapy for patients with critical limb ischaemia. *J Wound Care*. 2017;26:483-489.
69. Malekian A, Esmaeeli Djavid G, Akbarzadeh K, et al. Efficacy of maggot therapy on Staphylococcus aureus and *Pseudomonas aeruginosa* in diabetic foot ulcers: a randomized controlled trial. *J Wound Ostomy Continence Nurs*. 2019;46:25-29.
70. Jiménez-Pacheco A, Arrabal-Polo MÁ, Arias-Santiago S, Arrabal-Martín M, Nogueras-Ocaña M, Zuluaga-Gómez A. Gangrena de Fournier. Análisis descriptivo y coste económico-sanitario de nuestra serie de 37 casos. *Actas Dermosifiliogr*. 2012;103:29-35.
71. Woo KY, Keast D, Parsons N, Sibbald RG, Mittmann N. The cost of wound debridement: a Canadian perspective. *Int Wound J*. 2015;12:402-407. Epub 2013.

How to cite this article: Fonseca-Muñoz A, Sarmiento-Jiménez HE, Pérez-Pacheco R, Thyssen PJ, Sherman RA. Clinical study of Maggot therapy for Fournier's gangrene. *Int Wound J*. 2020;1–8. https://doi.org/10.1111/iwj.13444