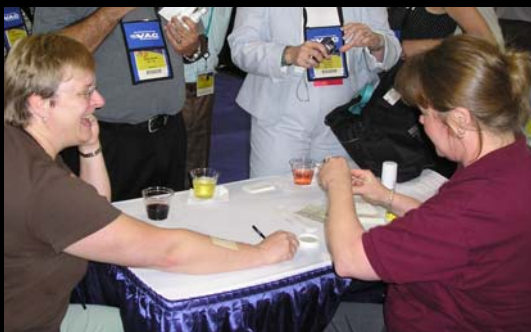




8th International Conference on Biotherapy



ABSTRACTS & HANDOUTS



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Abstracts and Handouts

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KEYNOTE ADDRESS

~ 1 ~

Title: Dr. William Stevenson Baer - A portrait
Author(s): Jolley A
Institution(s): BioTherapeutics, Education & Research Foundation

William Stevenson Baer's memorial, read at the April 18, 1931 meeting of the American Orthopaedic Association, stated, "To the world, he had represented all that was fine and admirable in a physician. In the medical profession, his name has, for years been associated with all of the enviable prominence which comes to the skillful, resourceful surgeon; the bold innovator whose promises have come true, the painstaking teacher whose lessons have been carried to the ends of the earth."

Though he was all of those things mentioned above, one of Baer's greatest legacies is that he was the first modern scientist in the medical profession to research, prescribe and implement bio-therapy procedures successfully. He is considered by many to be the "Grandfather" of modern biotherapy.

Baer's career was cut short by an untimely death, and his science was interrupted and superseded by the miracle of antibiotics. Much of his work was forgotten during the latter half of the 20th century, but the new renaissance in biotherapy has made Baer as relevant today as he was in 1930 when he first submitted his findings. In fact, Baer's life, his philosophy of medicine, and his work, both scientific and applied, makes him perhaps even more relevant today than he was in his own time.

Over the past two years, Baer's life, his scientific observations, and the history of his successful applications of biotherapy in hospital settings has been researched through access to his papers, professional letters, and memoirs from colleagues, students, and patients. His life has also been explored by visiting his residence and site of his practice, the Baltimore neighborhoods, hospitals, the William S. Baer School, and Johns Hopkins University (JHU) and Medical School where he was a professor and Chief of Orthopaedics for 31 years. During his entire career, he was affiliated with JHU, where he studied, worked, and practiced surgery.

By reviewing Baer's life and scientific work, we can better understand why biotherapy has evolved as it has. By studying his achievements in biotherapy and alternative medicine, we may be better prepared to move the field forwards.

BACTERIOPHAGE

~ 2 ~

Title: Characterization and implementation of bacteriophages as natural, self-replicating and self-limiting antibiotics
Author(s): Kutter E
Institution(s): Evergreen State College, Olympia, WA

Bacteriophages are ancient and ubiquitous; with an estimated 10³¹ phage on earth, we are constantly surrounded with them. They play key roles in maintaining microbial balance in virtually every ecosystem, as has been particularly well studied in the oceans, where they infect ¼ of the bacteria at any given time, and they were originally discovered in the stools of patients recovering from dysentery. In some parts of the world, the antibacterial power of phages has long been harnessed on a fairly routine basis to treat both enteric diseases and septic infections. However, in the Western world they were abandoned in the 1940s as the new antibiotic wonder-drugs became widely available. As antibiotics lose their power, it makes sense to again aggressively explore their therapeutic potentials, taking advantage of modern biological tools to select for high effectiveness, carefully characterize them singly and in cocktails under relevant physiological conditions, and carry out well-controlled clinical studies leading to broader acceptance and implementation of phage therapy. Among their advantages:

- Phages are both self-replicating and self-limiting.
- Applied locally, some enter the bloodstream and can be carried to distant sites of infection, even crossing the blood-brain barrier, and then multiply where they are needed.
- They continue multiplying and penetrating deeper as long as local infection is present, rather than decreasing rapidly in concentration below the surface, encouraging the development of resistance, as is seen with antibiotics.
- They can be targeted far more specifically than antibiotics to the particular problem bacteria, reducing dysbiosis.
- No serious side effects have been reported from application of phage, despite their very extensive oral and local use in patients, mainly in Eastern Europe but also in France and elsewhere.
- They are applicable prophylactically and in hospital sanitation, and have been shown to be especially useful in treating pathogens like MRSA, raising particular interest today.

This talk will provide a foundation in relevant phage biology, explore some of the historic applications of phage in the US in the 1940's as well as in the Republic of Georgia today, and discuss lessons learned in recent work with phages targeting *Pseudomonas aeruginosa* or *E. coli*. It will be of particular interest to explore their potential use in concert with other natural forms of therapy, such as maggot therapy for wound infections, where the phage could potentially supply the missing anti-*Pseudomonas* activity while the maggots assist in debridement and provide other antimicrobials.

Title: Clinical application of bacteriophages for recalcitrant bacterial infections

Author(s): *Gogokhia L^{1,2}, Gvasalia G²; Morales S²; Wolcott R

Institution(s): (1) University of Utah School of Medicine, Department of Pathology; (2) Tbilisi State Medical University, Department of General Surgery; (3) Special Phage Services/Sydney University of Technology; (4) Southwest Regional Wound Care, Lubbock TX

Bacteriophages are viruses that infect bacteria and are, arguably, the most ubiquitous organisms on earth. The therapeutic value of phages was recognized upon their discovery by d'Herelle while working with dysentery patients at the Pasteur Institute, and between the 1920's and 1940's phages were widely used to treat bacterial infections. Unfortunately, a combination of factors, including the discovery of antibiotics and a lack of understanding of phage biology, resulted in a decline in the use of phages as therapeutic tools in most of the world. However, they continued to be a standard component of treatment in parts of Eastern Europe, particularly the Republic of Georgia. The dramatic increase in antibiotic resistance has led to a resurgence of interest in phage therapy as a potential approach for treating recalcitrant bacterial infections. From a clinical perspective, phages present several advantages over current antimicrobial methods. They are safe and have a narrow spectrum of activity, thus removing 'targeted' problematic organisms while leaving most of the normal microflora intact. Phages multiply in the presence of susceptible bacteria, providing a self-sustaining therapy. This talk will present the general principles of phage therapy as practiced in the Republic of Georgia and in Texas trials, including important precautions. Detailed analyses of 2 cases of refractory infections with polymicrobial etiology, multi-resistance to antibiotics and severe co-morbidities will be presented. Complex use of local bacteriophage therapy, intensive wound debridement and systemic antibiotics over 8-12 weeks led to the eradication of infections and wound healing. In this context, a broad overview of the general procedures, limitations and precautions for the use of phage therapy to treat bacterial infections will be presented. Clinical applications in Sydney, Australia and at the Wound Care Center in Lubbock, Texas will also be discussed, including the first FDA approved phase 1 clinical trial in the US.

Title: New methods of nonsurgical periodontal treatment: perspectives and advantages of phage therapy.

Author(s): Gileva OS, *Libik TV, Bondarenko EA, Sadilova VA

Institution(s): Department of Oral Pathology and Physiotherapy, Perm State Academy of Medicine, Perm, Russian Federation

Introduction. Periodontal diseases are the most common oral conditions experienced by adults today. The main goal of periodontal therapy is to establish an oral environment compatible with periodontal health by the physical removal (syn. disposal, disruption) of the plaque biofilm and adjunctive (syn. obverse) chemical means if required. It is known that the major antimicrobial agents used in periodontology can cause oral and gastrointestinal dysbiosis, allergy, formation of antibiotic-resistance forms of periodontitis and can't be use for periodontal prophylaxis therefore "phage" therapy seems to be more perspective.

Objective. The aim of this 1-month (30-days) randomized controlled clinical and microbiological study was to compare the effectiveness of periodontal therapy with "SEXTAFAG" (pyobacteriophage polyvalent, FGUP NPO Microgen MZ RF Biomed) and 0,05% chlorhexidine mouth rinse in patients with gingivitis and mild periodontitis.

Methods. 45 patients with gingivitis and mild periodontitis were randomly assigned to one of the two treatment groups. The test group (N=21) received professional oral hygiene with application of "SEXTAFAG" in original techniques (Russian Federation Patent №2460, №2461, №2462 from 16.01.2009). The control group (N=24) received professional oral hygiene and 0,05% chlorhexidine mouth rinse twice a day. The following clinical parameters were assessed: plaque index (OHI-S), Papillary Marginal Attached Index (PMA), Papilla Bleeding Index (PBI) and Bleeding on Probing (BoP). A microbiological analysis was carried out to determine the efficacy of these means in changing the pathogenic flora. In addition, a multiplex polymerase chain reaction was carried out to confirm the presence of flora associated with chronic periodontitis.

Results. There was clinical improvement in both groups which correlated with an improvement in microbiological parameters; results were sustained for 30 days following therapy. The test group showed better clinical results which were demonstrated by clinical indexes and microbiological data.

Conclusion. According to this study phage therapy in complex periodontal treatment showed advantages over traditional 0,05% chlorhexidine mouth rinse, greater improvement in microbiological and clinical parameters.

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Title: A critical appraisal of worm therapy

Author(s): Pritchard D

Institution(s): School of Pharmacy, Nottingham University, UK

Worm therapy is medically prominent, through the use of whipworm ova in autoimmune disease and allergy, and through the sale of the human hookworm *Necator americanus* as a potential therapeutic agent.

This presentation will critically evaluate the epidemiological and experimental evidence which led to the adoption of helminth therapy, then summarise data from some completed trials.

Clearly, there are strengths and weaknesses associated with the evidence supporting worm therapy, leading to a number of pertinent questions.

Is the adoption of worm therapy the result of flawed thinking?

Alternatively, does worm therapy offer a therapeutic breakthrough to many patients, as yet unfulfilled?

If so, how can worm therapy be delivered to patients effectively, to maximise therapeutic benefit?

Title: Helmintherapy - A patient's journey

Author(s): Wade D

Institution(s):

I have suffered from Crohn's disease for over 22 years. In 2007, I ran out of drug options, so I decided to try helminth therapy. I purchased 10 *Necator Americanus* (hookworms) through an independent company, and therein began my experiment with worms.

Over the next 3 years, I experienced the successes of remission, and the frustrations of side effects (fever, diarrhea, edema, reactive arthritis). Repeated loss of my worms has necessitated repeated administrations of therapy. Most frustrating of all, however, has been the logistics of navigating the health care system to obtain treatment: physicians uninformed and unwilling to learn or explore; suppliers few and far between; a regulatory system placing large burdens on those who would research or produce medicinal helminthes. High financial costs squeeze everyone involved in helmintherapy . . . but especially the patients, who ultimately pay those costs.

These and other difficulties have led to my own experimentation, and have led me to share my experiences with other patients. Now, by sharing my journey with you clinicians and researchers in the field, I intend to energize you to continue your efforts in helmintherapy, and I intend also to convince you to be more creative and empathic in your approaches to treating our diseases in a way that we can live with.

MAGGOT THERAPY

~ 8 ~

~ 7 ~

Title: An accidental but safe and effective use of *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae) in maggot debridement therapy in Alexandria, Egypt

Author(s): *Tantawi TI¹, Williams KA², Villet MH³

Institution(s): (1) Department of Zoology, Faculty of Science, Alexandria University, Moharrem Bey, Alexandria, Egypt; (2) Durban Natural Science Museum, Durban, 4000 South Africa; (3) Southern African Forensic Entomology Research Laboratory, Department of Zoology & Entomology, Rhodes University, Grahamstown, 6140 South Africa

Introduction - The calliphorid fly *Lucilia cuprina* (Wiedemann) is known to cause serious malign myiasis in animals, whereas its sibling species *Lucilia sericata* (Meigen) is commonly a carrion breeder and is used in maggot debridement therapy (MDT). The present study reports an accidental involvement of *L. cuprina* in MDT in Alexandria, Egypt, that has proved to be safe and effective. In November 2008, the laboratory colonies of *L. sericata* (the species regularly used in MDT) at the Faculty of Science, Alexandria University were renewed by *Lucilia* flies collected as third instar larvae on exposed rabbit carcasses. Flies from the new colonies were identified as *L. cuprina* after being successfully used to heal the diabetic foot wounds of two patients at Alexandria Main University Hospital.

Objective - This work gives details of the molecular and morphological identification of the maggots, their occurrence in carrion in Alexandria, and preliminary evidence of the effectiveness of an Egyptian strain of *L. cuprina* in MDT.

Methods - Adults and larvae of *L. cuprina* were identified using molecular analysis and morphological features. Two cycles, each of four days, of free-range maggots of this species were applied to the wounds of two diabetic patients.

Results - Analysis of DNA sequences and adult and larval morphology revealed that these flies were and still are *L. cuprina*. The presence of *L. cuprina* in carrion in Alexandria and its use in MDT are new records. Maggots of this species were able to completely debride and close the neuroischemic ulcers of two patients.

Conclusion - Despite the safety of this strain of *L. cuprina* in MDT, entomologists rearing blow flies for the purpose of wound debridement should regularly maintain high quality assurance of their species' identity to avoid possible clinical complications that may result from the introduction of an unexpected and invasive species to their laboratory colonies.

Title: Maggot therapy in Iran

Author(s): *Mirabzadeh A¹, Ladani MJN², Brojerdi SS, Imani B¹

Institution(s): (1) Iranian Research Organization for Science and Technology; (2) Medical University of Baghiatallah

Background - Almost 1,000 years ago, the great Iranian scientist Avicenna commented that any wound on which larvae will appear certainly will heal. According to World Health Organization (WHO) every 30 seconds, a diabetic's foot is amputated because of a non-healing ulcer.

The annual rate of amputations in Iran is not known, but according to the Chair of the Iranian Diabetic Society, almost half of all Iranian diabetic patients eventually develop a foot ulcer severe enough to require hospitalization.

Methods - We studied the use of maggot debridement therapy (MDT) in three hospitals in Tehran, applying them to various wounds, including: 16 diabetic foot ulcers, 5 post-operative infections, 3 pressure ulcers ("bed sores"), 3 meningomyeloceles, 1 burn wound and 1 wound associated with Burger's diseases. Osteomyelitis was present in 22 of these wounds.

Results - We treated 29 patients with MDT; all successfully. The most common patient complaints associated with maggot therapy were bad odor and wound pain. In diabetic patients, wound healing took a little longer than it did in non-diabetic patients. Maggot treated wounds did not require closure with skin grafts, and no blemishing scars were observed. Prior to treatment with MDT, the average erythrocyte sedimentation rate (ESR) for the 22 patients with osteomyelitis was 100; after treatment, the average dropped to below 20 (normal). Clinically, the cure rate for those wounds with underlying osteomyelitis was 100%.

Discussion - MDT has been so successful in Iran that many diabetic patients and their doctors are now demanding this type of treatment, and we urgently need to raise larvae on a simple but much larger scale to meet the medical need.

Title: Maggot debridement therapy in Slovakia: The story of success

Author(s): *Kozánek M¹, Čambal M², Takáč P¹

Institution(s): (1) Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia; (2) 1st Department of Surgery, Medical Faculty Hospital, Comenius University, Bratislava, Slovakia

Maggot debridement therapy is a modern biotherapeutic method based on the use of necrophagous flies to treat chronic non-healing wounds. In last two decades, maggot debridement therapy became the routinely used therapeutic method and thousands of patients over the world had benefit of its healing power.

In Slovakia, maggot debridement therapy was successfully used for the first time in August 2003 at the Medical Faculty Hospital in Bratislava to clean the persisting wound of a patient suffering from diabetes. Sterile larvae of blowfly *Lucilia sericata* were prepared by the Institute of Zoology, Slovak Academy of Sciences. A year later, non-profit organization MEDALT was established with the aim to develop biotherapeutic methods and to introduce biotherapeutic methods in clinical praxis in Slovakia. The research and introduction of maggot debridement therapy was supported by the European Social Foundation through the project running in 2005-2008. In the frame of this project, modern laboratory for production of sterile larvae was developed, the system of sterile larvae delivery to any hospital in the country was established and improved monolayer “biobag” was designed. Tens of lectures for medical experts and common public as well as media presentations were organized. Thanks to these activities, maggot debridement therapy is routinely used in hospitals over the country.

Successful introduction of maggot debridement therapy evoked interest of practitioners for utilization of further biotherapies. Since 2009, Institute of Zoology, Slovak Academy of Sciences together with Medical Faculty of Comenius University in Bratislava cooperates on the common biomedical project supported by European Structural Funds. The aim of the project is to build a biotherapeutic centre serving for research and introduction of ichthyotherapy and hirudotherapy as well as continuing research and improvement of MDT. Simultaneously, therapeutically active molecules produced by organisms using in biotherapy are studied.

Title: The use of maggot debridement therapy in the treatment of chronic and acute wounds in hospitalized and ambulatory patients of the Hadassah University Hospital, Jerusalem

Author(s): *Mumcuoglu K¹, Gilead L², Ingber A³

Institution(s): (1) Department of Microbiology and Molecular Genetics, The Kuvim Center for the Study of Infectious and Tropical Diseases, The Institute for Medical Research Israel-Canada, The Hebrew University - Hadassah Medical School, Jerusalem, Israel; (2) Department of Dermatology and Venereology, Hadassah University-Hospital, Jerusalem, Israel

During the years 1996-2009, 662 wounds of 385 patients (158 females and 227 males) were treated with maggot debridement therapy (MDT) in 16 departments and units of the Hadassah University Hospital in Jerusalem, Israel. Overall, 221 patients were treated while they were hospitalized, 164 were treated as outpatients. In 94.6% of the patients, the wounds were located on the foot; 45.2% of these patients suffered from diabetes. The wounds had already existed 1-240 months (average 9.3; median 4 months). Sterile maggots of the green bottle fly, *Lucilia sericata*, were used for MDT. In 89.4% of the cases, maggots were placed directly on the wound using a cage-like dressing and left for 24 hrs, while in 10.6% of the patients, maggots were contained in tea-bag-like polyvinyl netting. The contained maggots were left on the wound for 2-3 days. The number of treatments varied between 1-19 (average: 2.5; median 2) and the duration of the treatment varied between 1-29 days (average: 3.7; median 3). In 318 patients (82.6%), complete debridement of the wound was achieved, while in 62 patients (16.1%), debridement was partial and in 5 (1.3%), MDT was ineffective. Overall, 36.6% of the patients reported increased pain during MDT.

Title: Pain related to maggot debridement therapy

Author(s): *Mumcuoglu K¹, Davidson E², Gilead L³

Institution(s): (1) Department of Microbiology and Molecular Genetics, The Kuvim Center for the Study of Infectious and Tropical Diseases, The Institute for Medical Research Israel-Canada, The Hebrew University - Hadassah Medical School, Jerusalem, Israel; (2) Department of Anesthesia & CCM, Hadassah University-Hospital, Jerusalem, Israel; (3) Department of Dermatology and Venereology, Hadassah University-Hospital, Jerusalem, Israel

During the years 1996-2009, 682 wounds of 418 patients were treated with maggot debridement therapy (MDT) in 8 hospitals and outpatient clinics in Jerusalem, Israel. Maggots were either placed directly on the wound (DA) using a cage-like dressing or they were confined within a tea-bag-like polyvinyl netting (TB) and applied to the wound. Overall, 37.8% of the patients complained from increased pain during MDT. Sixteen out of 39 patients (41%), who were treated with the TB technique, and 131 out of 379 patients (34.6%), who were treated with the DA technique, complained of increased pain and required treatment with analgesics before or during MDT. In five patients the treatment was discontinued due to pain. Pain control measures were undertaken in patients who were already in intense pain prior to the initiation of MDT and in those patients who reported intense pain during DA-MDT. Additional measures included application of MDT for shorter periods of time, use of TB rather than the DA method, use of smaller maggots and application of smaller number of maggots during the MDT session. Peripheral nerve block, oral and trans-cutaneous analgesic medication were used to decrease pain in some patients. Due to the fact that a full debridement was achieved in an average of 2-3 maggot cycles, which lasted 3-4 days, we believe it is worthwhile to treat patients with MDT, even though they may require strong analgesics, because conventional methods would take much longer to achieve the same degree of debridement.

Title: In search of pain-free MDT: Effects of lidocaine on the debridement capacity of medicinal maggots

Author(s): Sherman RA

Institution(s): BioTherapeutics, Education & Research Foundation

Introduction - Maggot debridement therapy (MDT) for treating problematic wounds has seen a profound renaissance since 1995. Adverse events are mild and uncommon, except pain or discomfort, generally experienced by patients with painful wounds to begin with, and only when the maggots have increased in size and activity (that is, after the first 24 hours of treatment). Generally, discomfort is well-managed with oral analgesics; but occasionally they are inadequate. A few therapists have attempted to mitigate MDT-associated pain with topical anesthetics such as lidocaine. To date, though, no clinical reports of topically administered pain medication have been published.

Objectives - These investigations were performed in order to determine whether, and under what conditions, medicinal maggots might survive exposure to lidocaine.

Methods - A series of three experiments were performed: 1) increasing concentrations of lidocaine were added every 4 hours to an in vitro model of necrotic wounds (liver-agar) with *Phaenicia (Lucilia) sericata* larvae; 2) 1% Lidocaine was added every 4 hours to the same wound model, and allowed to remain with the maggots only for 5 to 60 minutes; 3) larvae were fed liver-agar impregnated with increasing doses of 1% Lidocaine.

Results - When water or lidocaine were added to the in vitro model every 4 hrs and not drained, the fluid accumulated and survival was decreased from the fluid alone. Exposure to increasing concentrations of lidocaine resulted in further developmental delay and death of the larvae, with no survival in dishes to which concentrations 0.06% lidocaine were repeatedly added. When 1% lidocaine was added to the dishes for only short periods of time, then it had little measurable effect on the larvae unless it stayed in the dish for 30 minutes or more. Lidocaine (1%) mixed into the maggots' media had little effect on their maturation or survival until the concentration of lidocaine in the media reached 0.1%.

Conclusions - Lidocaine decreases larval growth and survival in a dose-related fashion. However, in low doses or for short periods of exposure, the lidocaine had little effect on maggot development or debridement capacity. Based on our findings, a clinical evaluation of topical lidocaine for MDT-associated wound pain is warranted.

Title: Pain release drugs in maggot therapy - Uncover the physiological interaction

Author(s): Heuer HL, *Heuer L

Institution(s): Agiltera GmbH & Co. KG, Am Krausberg 31, D-41542 Dormagen, Germany

Background: Maggot therapy (also known as Larval therapy, Biosurgery) is a valuable method of debridement. Patients and physicians consider the use of *Lucilia (Phaenicia) sericata* larvae as a quick and safe method for cleaning wounds. Over the last 20 years this therapy has spread from the US to all over the world, and today about 30,000 patients are treated each year in Europe and US. We therefore believe that maggot therapy is the most frequently used of all biotherapeutic modalities. Maggot therapy's benefits include: 1) fast & effective debridement, 2) nearly no side effects, and 3) very inexpensive.

Objective: In some cases pain occurs. We therefore undertook a detailed study of pain release drugs and have tested commonly used analgesics and local anesthetics.

Methods: In the laboratory, the growth a development of *L. sericata* larvae was observed after adding increasing doses (50 to 500 mg) of the following anesthetics to the media: benzocaine, fomocaine, lidocaine, prilocaine, and procaine.

Results: Most of the drugs caused decreased motility and abnormal development, with obvious dose-dependent effects. Using microscopy and motion pictures, we were able to uncover the physiological interaction of pain release drugs and maggots.

Discussion: Anesthetics tested in the laboratory impaired the motility and development of *L. sericata* larvae. Implications on maggot therapy will be discussed, along with suggestions for further research.

Title: Clinical and microbiological efficacy of MDT in management of pressure ulcers

Author(s): AbouZeid NA¹, William SG¹, Tantawi TI³, *Gohar YM⁴, Kotb MM⁵

Institution(s): (1) Medical-Surgical Nursing Department, Faculty of Nursing, Alexandria University, Egypt; (2) Medical Entomology, Zoology Department, Faculty of Science, Alexandria University, Egypt; (3) Medical Microbiology, Microbiology Division, Faculty of Science, Alexandria University, Egypt; (4) Department of Surgery, Faculty of Medicine, Alexandria University, Egypt

Introduction. Pressure ulcers remain a significant problem in both the acute and community health settings. Despite the many developments in wound care during the past two decades, there has been no significant decrease in pressure ulcer prevalence or any demonstrable improvement in overall outcomes. New treatment paradigms must be examined as we strive to reduce pressure ulcer morbidity. The past two decades have witnessed the resurgence in the use of maggot debridement therapy (MDT) in wound management mainly due to the increase of antibiotic resistance.

Objectives: To investigate the clinical (debridement and wound healing) and microbiological outcomes of MDT in the management of pressure ulcers.

Methods: Fourteen bed-bound patients with 14 sacral and ischial pressure ulcers of stage III and stage IV over a period of 14 months were included in the study at general medical surgical wards, critical care units, and emergency recovery room, Alexandria Main University Hospital. The patients were generally bed ridden and hospitalized for a mean duration of 11.28 weeks. The patients suffered from the following underlying medical conditions: cerebral vascular stroke, diabetes mellitus, and chronic obstructive pulmonary disease. All but two patients had decreased levels of consciousness. The patients also suffered from anemia and malnutrition. The blow fly *Lucilia sericata* was used for maggot therapy. Each ulcer was treated with one maggot cycle per week. The ulcers were observed weekly and swabbed for microbiology before and after application of each maggot cycle to investigate the bacterial burden, polybacterial population, and the type of bacteria in each ulcer.

Results: MDT was associated with a rapid rate of debridement, rapid growth of granulation tissue, and marked antimicrobial activity. Before MDT, the mean surface area of devitalized tissue was 58.81 cm² (range 8.25-131.25 cm²), whereas after MDT this mean significantly decreased to 15.35 cm² (range 0-40 cm²) (P=0.000307) during a mean period of 1.5 weeks (range 1-2 weeks). Three ulcers were completely debrided with one or 2 cycles of maggots. Before MDT, the mean surface area of granulation tissue was 16.03 cm² (range 0-80 cm²), whereas after MDT this mean significantly increased to 55.86 cm² (range 7.37-116 cm²) (P=0.000221) during a mean period of 2.14 weeks (range 1-10 weeks). Nine ulcers had > 50% of their size occupied by a red healthy granulation tissue. All ulcers exhibited a mixed bacterial population ranged from 3 to 7 microorganisms. The mean of initial bacterial burden was 4.86×10^8 CFU/ml exudate. After the first maggot cycle, this mean significantly decreased to 1.92×10^4 CFU/ml exudate (p=0.01814) below the 10^5 threshold of nature healing.

Conclusion: The application of disinfected larvae of *Lucilia sericata* to infected, non-healing pressure stage III and stage IV pressure ulcers resulted in the rapid removal of necrotic tissue, disinfection, and enhancement of the healing process. Microbiological outcomes demonstrate that maggot therapy is an efficient antimicrobial treatment. One or two cycles of maggot therapy was associated with a reduction in the bacterial load to below the 10^5 threshold which permits healing. MDT could alleviate the suffering in patients with bed sores in Egypt.

Title: Maggot debridement therapy for treating severe diabetic foot ulcers in Japan

Author(s): *Mitsui H, Kawabata T, Ugaki S, Fujii Y, Sakurai S, Sano S

Institution(s): Okayama University Graduate School of Medicine and Dentistry, Department of Surgery, Division of Cardiovascular Surgery

Introduction: Although the effectiveness of maggot debridement therapy (MDT) for treating intractable diabetic foot ulcers has been advocated in Europe and United States, it has not been widely used in Japan. We have used this type of therapy in the treatment of diabetic foot ulcers for the first time in Japan in 2006, and have gained much experience over the next 4 years.

Methods: We have retrospectively analyzed the outcomes of MDT for 136 foot wounds in 122 patients: 116 neuropathic foot ulcers (Ankle-Brachial Index (ABI) < 0.9) and 20 ischemic foot ulcers ($0.9 > \text{ABI} > 0.5$). Sterile 3rd instars of *Lucilia sericata* (7-9 mm) were administered to these wounds 3 - 8 times (mean: 5.3), usually twice a week, with our special dressing to facilitate effective and comfortable debridement of the ulcer bed.

Results: Of the 116 diabetic foot ulcers, 103 (89%) healed after 3 months, whereas the remaining 13 wounds required major amputation within 3 months. Of the 20 ischemic foot ulcers (most of which were claimed by the referring physicians to require major amputation), 13 (65%) healed but 7 required major amputation within 3 months. During these treatments, there were no complications related to MDT. The patients who failed to respond to MDT had the same co-morbidities (chronic renal failure and low cardiac output), and all underwent amputation due to severe sepsis.

Conclusions: We concluded MDT is an effective tool for wound bed preparation of diabetic ulcers. Maggot debridement therapy has the following benefits: 1) no contra-indication, 2) anesthesia is unnecessary, 3) cost-effective compared to conventional therapy, 4) long history and enough evidence of effectiveness for diabetic ulcer treatment in western countries. We may need different strategies to treat ulcers of chronic renal failure and low cardiac output patients to control infection and not to develop severe sepsis. We are now promoting MDT to save the feet of diabetic patients from amputation in Japan.

Title: Uncommon applications of maggot therapy for common and problematic wounds

Author(s): Blum K¹, *Mendez S²

Institution(s): (1) West Suburban Medical Center, Oak Park, IL; (2) Providence Holy Family Hospital, Spokane, WA

Despite the progress in wound care and wound care products over the past 20 years, many wounds today still fail to respond to conventional therapy. Maggot therapy is often used for recalcitrant wounds, especially diabetic foot ulcers, pressure ulcers and venous stasis ulcers. We evaluated the outcomes of maggot therapy in our patients with less common indications: pyoderma gangrenosum, necrotic tumor and vasculitis. Maggot debridement resolved the wound infections, treated the recalcitrant biofilm and improved our patients' quality of life. Based on our experience and a few published reports, maggot therapy appears to be useful for many more types of complicated and non-healing wounds than are listed on the package insert.

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Title: The Greenbottle Pharmacy Project: Next generation wound debridement products

Author(s): David Pritchard

Institution(s): School of Pharmacy, Nottingham University, UK

A recent clinical trial (Dumville JC et al (2009) Larval Therapy for Leg Ulcers (VenUS II): Randomised Controlled Trial. BMJ 338:773) demonstrated the value of maggots as wound debridement agents although the treatment was unpleasant and painful to many patients.

With Wellcome Trust, Technology Strategy Board (TSB), Engineering and Physical Sciences Research Council (EPSRC) and Defence Science and Technology Laboratories (DSTL) funding, we identified a chymotrypsin from the secretions of the greenbottle maggot *L. sericata* which completely digests eschar from human venous leg ulcers ex vivo, making it a prime candidate for development as a next generation wound management product.

Consequently, the enzyme was cloned and expressed at research scale and shown to mimic the effects of the whole organism in ex vivo debridement assays, where slough and eschar from venous leg ulcers were analysed proteomically.

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Title: The antibacterial activity of medicinal maggots of the blow fly *Lucilia cuprina* against multidrug-resistant bacteria frequently infected diabetic foot ulcers in Alexandria, Egypt: A preliminary in vitro study

Author(s): *Gohar YM¹, Tantawi TI², El-Ghaffar HA², El-Shazly BMA²

Institution(s): (1) Microbiology Section; (2) Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt

Introduction The antibacterial activity of maggots (larvae) of the blow fly *Lucilia sericata* (the species frequently used in maggot therapy) is well documented both in vivo and in vitro studies. Recently, the larvae of *Lucilia cuprina* (the sibling species of *L. sericata*) has proved to be safe and effective for treating infected, nonhealing diabetic foot wounds at Alexandria Main University Hospital. Therefore, it was found necessary to investigate the antibacterial activity of this species.

Objectives 1- To investigate the antibacterial activity of *L. cuprina* larvae against some multidrug-resistant bacteria isolated from the foot ulcers of diabetic patients. 2- To partially characterize the antibacterial agents extracted from these larvae.

Methods. Whole body and gut extracts, excretions/secretions, and hemolymph of sterile, actively feeding third instars of *L. cuprina* were gained, lyophilized, and ultra-filtered with 10 and 3 kDa molecular weight cut-off membranes. Same extracts were accessed by the same procedures from sterile larvae after inoculation with each of the following six bacteria; *M. luteus*, *S. aureus*, *St. pyogenes*, *Ps. aeruginosa*, *P. mirabilis*, and *Ser. marcescens*. Antibacterial activity of the extracts obtained from sterile and inoculated larvae were investigated against these bacteria using the drop diffusion test on Petri dishes by the double-layer technique.

Results. All whole body extracts from sterile or inoculated larvae of *L. cuprina* exhibited antibacterial activity with different degrees against the six tested bacteria. Three fractions with molecular weights of >10, <10, and 3-10 kDa from larval guts were found active against bacteria. Antibacterial activity was more effective against Gram-positive bacteria than against Gram-negative bacteria. Extracts of inoculated larvae possessed a slightly higher antibacterial activity than those of non-inoculated larvae. Work concerning the antibacterial activity of excretions/secretions and haemolymph is still under investigation.

Conclusion. The results of this preliminary study indicate that the larvae of *L. cuprina* exhibit antibacterial activity against these virulent bacteria and therefore could have beneficial therapeutic effects when used in maggot therapy.

Title: Introducing maggot therapy in Slovenia; antibacterial activity of larval excreta/secret of *Lucilia sericata*

Author(s): *Jaklič D¹, Lapanje A^{1,2}, Smrke D³, Gunde-Cimerman N¹

Institution(s): (1) Department of Biology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia; (2) Present address: Institute of Physical Biology Ltd., Toplarniška 19, SI – 1000 Ljubljana, Slovenia; (3) University Medical Centre Ljubljana, Zaloška cesta 2, SI - 1000 Ljubljana, Slovenia

Larvae of the *Lucilia sericata* fly have been used for healing of chronic and infected wounds since ancient times. Clinical use and successful healing of infected wounds was first described in 1931 and became before the Second World War the main way of treatment of infected wounds. After the appearance of antibiotics, medicinal use of larvae drastically decreased, however it was revived again in the nineties.

In this study sterile larvae of *L. sericata* fly were used for treating 32 wounds of 30 patients. For investigation of larval excreta/secret (ES) antibacterial activity, special method for collecting sufficient amount of ES was developed. The antibacterial activities were investigated in vivo studies, by comparing bacterial diversity in wounds before and after the larvae application. Microbiological smears were collected before and after the application of the larvae on the wounds. Microorganisms were then isolated and identified. During larval therapy healing improvement, patient's general state, potential presence of pain, mobility, patient's independence and psychological reaction of the therapy were monitored.

Using maggot therapy 24 (75 %) out of 32 wounds were cleaned and healed. In the case of combined arterio-venous leg ulcers one wound was completely healed and eight of them were cleaned of necrosis and infection. In the case of a venous leg ulcer, six diabetic ulcers, eight cases of chronic postoperative wounds and pressure ulcers, wounds were completely cleaned after the treatment. Our analyses of microbial diversity of wounds had revealed that the bacteria present can be divided into two main groups. The first group could be described as highly susceptible. The second group consists of bacteria that either remained unaffected or even increased in occurrence. This group consists mainly of Gram-negative, as well as some selected Gram-positive bacteria. These results were supported with in vitro tests of ES active against clinically important bacteria, such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and other, mainly Gram-positive bacteria.

The method of healing chronic wounds with *L. sericata* larvae was successfully introduced into Slovenia. Maggot therapy is highly recommended for the treatment of wounds infected with Gram-positive bacteria and less so for wounds infected with Gram-negative bacteria. Thus, ES contain a complex mixture of substances that synergistically inhibit the growth of microorganisms, as well as promote the healing process of infected wounds.

Title: New antimicrobial factors of larval extracts of the blowfly *Lucilia sericata*

Author(s): *Takac P¹, Majtan J¹, Novak P², Bohova J¹, Cambal M³, Kozanek M¹

Institution(s): (1) Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia; (2) Institute of Microbiology, Academy of Sciences of the Czech Republic, Praha, Czech Republic; (3) 1st Department of Surgery, University Hospital and Faculty of Medicine, Comenius University, Bratislava, Slovakia

Introduction - Maggot therapy, also known as biosurgery, is an old method for the healing of chronically infected wounds. The rediscovery of maggot debridement therapy into the clinical practice has prompted further research into the antibacterial effectiveness of the blowfly, *Lucilia sericata*. In the recent time, several scientific groups described the presence of some factors in the extracts of maggots with different molecular masses. An important class of these compounds are the group of insect defensins. The novel defensin, named lucifensin was discovered only recently.

Objective - The main focus of our investigation was to describe the isolation of the novel ubiquitous antibacterial peptides in the whole Larval extracts of the blowfly *Lucilia sericata*.

Methods - Larvae of the blowfly *Lucilia sericata* in the middle of the third instar were homogenized and extracted with methanol : water : acetic acid. The water extract indicated the antibacterial activity against *Micrococcus luteus*. For the further purification Heparin HP and CM-Sephrose FF separation columns were used. The active fraction was subsequently loaded to RP-HPLC system with ACN linear gradient. The final purified active fraction was analysed by Mass spectral analysis. Mass spectra were acquired in positive ion mode on an APEX-Ultra FTMS instrument equipped with a 9.4 T superconducting magnet and a Dual II electrospray ionization (ESI) ion source (Bruker Daltonics, Billerica, MA). ECD was performed with an indirectly heated hollow cathode operated at 1.9 Amps of heater current (Heatwave, Crescent Valley, BC, Canada). All MS and MS/MS spectra were acquired in the positive ion mode with 512k data points and 128 time-domain transients. Interpretation of the mass spectra were performed by a software package DataAnalysis 4.0 (Bruker Daltonics, Billerica, MA), and manually.

Results - Mass spectral analysis of the active antibacterial fraction revealed again the already known lucifensin measured by ESI-QTOF MS, with the calculated m/z value of 4,113.89 kDa, but at the same time we identified the supposed isoform of lucifensin with m/z value of 4,063.118 kDa. In the same fraction additionally we detected and partially sequenced the novel peptide with m/z value of 2,758,427 kDa.

Conclusion - If the wound is infected with an antibiotic-resistant bacterial strain, it becomes difficult or impossible to treat the underlying infection and for any healing to occur. Our results indicate the discovery of new antimicrobial peptide for the treatment of non-healing wounds.

Title: Healing properties of maggot therapy - What is the evidence?

Author(s): Sherman RA

Institution(s): BioTherapeutics, Education & Research Foundation

Background - The wound-healing effects of maggot therapy have been touted for 80 years. Yet there remain questions today, especially in light of a prospective clinical trial demonstrating no faster wound healing with maggot debridement than without.

Methods - Clinical and laboratory studies over the past 20 years were reviewed to examine the extent of data associated maggot therapy with wound healing. An unreported phase 2 research subject from 1990 will be presented in which wound healing was followed over time, with and without maggot therapy.

Results - Recent clinical studies support the observations of maggot therapists 80 years ago: maggot debridement is relatively fast, safe and effective, but does not lead to faster wound healing. When maggot therapy is continued beyond the point of wound debridement --- that is, when maggot therapy is used on relatively clean but non-healing wounds --- wound healing is hastened.

Recent laboratory studies suggest several different mechanisms that might all be playing a role in this maggot-induced growth-stimulating effect: increased cellular mitosis, angiogenesis, hastened migration over the wound, biofilm degradation and inhibition, direct antimicrobial activity, and increased local perfusion, just to name a few.

Conclusion - Clinical and laboratory evidence exists to suggest that maggot therapy can induce wound healing beyond the benefit seen simply with debridement. However, long-term, prospective randomized clinical trials have not yet been done to test the clinical relevance of such observations.

HIRUDOTHERAPY

Title: Modern hirudotherapy: Experimental background and clinical efficacy

Author(s): Gileva OS

Institution(s): Department of Oral Pathology and Physiotherapy, Perm State Academy of Medicine, Perm, Russian Federation

Hirudotherapy - the medicinal use of leeches (*Hirudo medicinalis*) has existed since high antiquity and still remains as an important part of many ethnomedical systems. In spite of the significant advances in our understanding about hirudotherapy and its performance, still its mechanism of action is not totally understood even now.

The objective of our study is to summarize the experience of modern medicine in hirudotherapy clinical effectiveness in different human diseases and disorders, based on more than 10-years of good clinical observations, experimental data and special literature overview.

The first part of the report will address some new experimental findings on anti-inflammatory, antiedemic, decongestive, immunomodulative and regenerative properties of leeching. Curative and preventive healing effects of aspirative and non-aspirative types of leeching will be described in experimental rat models. The report provides comprehensive information on a wide-range of clinical applications of leeches - in internal medicine, microvascular and reconstructive surgery, synostology, neurology, gynecology, ophthalmology, dermatology, cardiology, maxillofacial surgery and dentistry. The clinical part of the report also presents numerous case histories of patients who have had different diseases successfully treated with leeches. This presentation will include clinical photographs, line drawings and schematic figures to assist in our understanding of hirudotherapy. We will also focus on some actual problems associated with hirudotherapy: bioethics, temporal toxicity of leeches, bioutilization, hirudotherapy combination with other pharmaco- and physiotherapy methods and etc. Special attention will be paid to leeching side effects: why they occur, how to recognize them, and how to prevent or treat them. Cupping techniques will also be addressed. Finally, recommendations for using the leeches in clinical practice will be made, based on controlled randomized trials.

Title: Leech therapy in recent times

Author(s): Sviridova L

Institution(s):

Leech therapy is one of the most ancient treatment methods documented in the history of medicine. The fact that leeches have been used for more than 3000 years in the world's most important traditional medicine systems such as traditional European, Ayurvedic, and Chinese medicine is in itself proof of the repeated success of leech therapy.

In ancient Greek history, bloodletting was practiced according to the humoral theory, which proposed that when the four humors, blood, phlegm, black and yellow bile in the human body were in balance, good health was guaranteed. An unbalance in the proportions of these humors was believed to be in case of ill health. This treatment used by ancient physicians to balance the humors to rid the body of the plethora and applied leeches for symptomatic local treatment of febrile and inflammatory diseases.

Today, the therapeutic properties of leeching can be demonstrated from the objective, scientific perspective based on the known activity of the bioactive substances in leech saliva. Leech therapy will be continue to be useful in modern clinical medicine, initially because of the extremely effective results it achieved in plastic and reconstructive surgery and, more obviously, because of its proven effectiveness in treating chronic pain syndromes associated with generating joint disease.

In 2004, the Ricarimpex SAS brand of medicinal leech was cleared for marketing in the U.S. by FDA as "an adjunct to the healing of graft tissue when problems of venous congestion may delay healing, or to overcome problems of venous congestion by creating prolonged localized bleeding". The decision to classify medicinal as a medical device was based on the use of medicinal leeches prior to 1976, clinical experience, the manufacturing process, and the past clinical use. This allows Ricarimpex SAS to sell leeches through suppliers like Leeches USA to physicians and veterinarians, in the USA, according to section 801.109 of the Code of Federal Regulations.

In Europe, for example Germany, the sale of medicinal leeches was controlled by the drug authorities. The German Drug Law (AMG) from July 2004, passage that includes the medicinal leeches under the definition of a medical product designed for medical use reads: "Medicinal products are substances or combinations of substances used to cure, alleviate or prevent diseases, suffering, bodily injuries or pathological complaints." The AMG also includes the "bodies of living animals" as a substance group.

In Russia, the leech therapy is also approved by the Ministry of Health Protection of Russian Federation.

Leech therapy has a very broad range of uses in various fields of medicine. The efficacy of leeching is based on a combination of multiple effects resulting in blood circulation-enhancing, blood and lymph drainage, anticoagulation effect, improvement of an endocellular exchange, positive influence on metabolic activity, pain relief, segmental (reflex) effects, immunostimulating and immunomodulating action.

The current data from Complementary and Internal Medicine Centers, Rehabilitation Departments and Hirudotherapy Clinics different countries suggests that the simultaneous action of multiple mechanisms may be responsible for the clinical effectiveness of leeching in nonsurgical indications. The medicinal leeches were mainly used to treat heart and circulatory problems, chronic inflammation and pain management are the primary indication.

Many chronic diseases do not respond well to conventional treatments. These patients are gradually increase from less to more potent drugs. Besides increasing treatment costs and side effects, multidrug treatments also increased risk of drug interactions and thus side effects which further set of problems for the chronically ill patients. Often long-term drug treatment results in SAE that may require outpatient continuation of pharmacological treatment or hospitalization. Sometimes these complications end fatally. Considering this problem, alternative treatment options are of particular interest.

The most important elements of the successful practice of leech therapy are:

1. The right technique of leech application;
2. The knowledge of contraindications and standard procedures for minimizing the risk of treatment complications;
3. The knowledge of the biology of *Hirudo medicinalis* and anatomy of the human body.
4. The skills in the leech storage and management;
5. Individuals administering treatments with live animals must have special qualification.

For this reason, should to create practical training seminars to optimizing the success of medicinal leech therapy in clinical and private practice. Compared to other techniques of complementary and natural medicine, leeching can be learned relatively quickly.

In addition, leech therapy is associated with certain risks and to protect patient and leech therapist from legal repercussions we should consider very important subjects. The legal status of leech therapy under FDA regulation. Leech therapist qualifications must to have in order to administer the treatment. The patient has been properly informed about potential side effects... Leech therapist can be confronted with liability, regulatory, and criminal law problems.

Title: Hirudotherapy in the complex treatment of periodontitis and oral mucosal diseases in patients with systemic cardiovascular pathology
Author(s): Gileva OS, Gibadullina NV, Korobejnikova GA, *Libik TV

Institution(s): (1) Department of Oral Pathology and Physiotherapy, Perm State Academy of Medicine, Perm, Russian Federation; (2) UST Health Resort, Perm, Russian Federation

Background. Periodontal disease has a microbial aetiology and an inflammatory pathogenesis characterized by destruction of tooth-supporting, soft and hard tissues of the alveolus resulting in periodontal pockets and roots denuded of periodontal ligament, cementum and alveolar bone. The pathogenic links between systemic and periodontal diseases are well documented. Hypertension and its complications (acute myocardial infection, cardiosclerosis etc.), diabetes are considered to be the main systemic risk factors for advanced aggressive and generalized forms of periodontitis. Effective management of periodontitis in patients with cardiovascular diseases is an important part of their treatment. The decongestive, anti-inflammatory, analgesic, antiaggregative, thrombolytic and fibrinolytic effects of leeches indicate that hirudotherapy can be successfully used for management of advanced periodontitis in patients with cardiovascular pathology. Clinical effectiveness of leechtherapy for patients with gingivitis and periodontitis have been demonstrated in numerous non-randomized studies.

Objective. The aim of this open randomized controlled study with two parallel treatment groups was to evaluate the efficacy of using medicinal leeches in the complex treatment of advanced periodontitis compared with traditional treatment protocol without hirudotherapy.

Methods. The research protocol was approved by our institutional ethics committee. 79 patients from dental clinic of Perm State Academy of Medicine with advanced periodontitis were randomly included. The test group (50 patients, mean age $54,6 \pm 10,2$ years) received traditional periodontal treatment with application of leeches (*Hirudo medicinalis officinalis*, biofarm "Rospharmacy", conformity certificate №50-45-2797 of Moscow Regional Center of Certification and Quality Control of Drugs №1801) according to our original methods (Patents of Russian Federation №73200200014, 1/2002) and the control group (29 patients, mean age $60,1 \pm 7,4$ years) received traditional periodontal treatment alone. At baseline and one, 6 and 12 months after treatment the main clinical periodontal (OHI-S, PMA, PI, PBI, BoP, PIRI), microbiological and functional indices (WHO, 2001) were recorded.

Results. Patients of the test group showed significantly more expressed improvement according clinical and functional data. The earliest and stabile recovery of periodontium oxygen regimen was detected in test group of patients undergoing hirudotherapy.

Conclusions. Hirudotherapy engagement in complex treatment of periodontitis combined with cardiovascular pathology significantly improve periodontal and systemic status of these patients' cohort.

Title: An introduction to oriental hirudotherapy

Author(s): UI Huda SMN

Institution(s): Cyberjaya University College of Medical Sciences.

Introduction: The scientific name for the medicinal leech is *Hirudo medicinalis* - hence the name Hirudo. Leech therapy can be termed as a combination of traditional, complementary and alternative systems of medicine with the modern medicine on the platform of evidence based medicine for the betterment of humanity. The medicinal use of leeches' dates back to ancient Egyptians around 1300 BC; the Greek physician Galen (130 to 201 AD) commonly used leeches for bloodletting. The 19th century heralded the widespread use of leeches for bloodletting—leading to a leech shortage from 1825 to 1850 in France requiring the importation of leeches from America.

Discussion: Leeches have been used for bloodletting dating back to centuries. Wound healing, and stimulating blood flow at postsurgical sites. Use in Knee Osteoarthritis is being done, and there are lots of scientific scopes of gathering clinical information to make recommendations. In Malaysia, leech therapy is a part of traditional treatment and is common. There are many leech therapists and leech breeding farms spread all over the country. Cyberjaya University College of Medical Sciences & the International Islamic University of Malaysia have undertaken the scientific studies on leech therapy and its efficacy on the platform of evidence based medicine. The amazing healing worms "The Leeches" have more than 100 biologically active substances in their saliva that help in treatment of various diseases; therefore there is a long list of diseases that can be treated by this method, for example cardiovascular diseases - hypertension, arterioscleroses, hyper coagulation, stenocardia, myocardial infarct, varicose veins and arterial blood flow disorders in lower limbs and their complications, such as tropic lesions and edemas. Gynecological ailments - they reduce various inflammation processes in the uterus and its adnexa, and can be useful in cases of ovarian cysts, endometriosis, climacteric syndrome, various adhesions in the pelvis, and even in cases of frigidity, infertility and mastopathy. For men, the application of leeches is successful in treating prostate and scrotum diseases, erectile dysfunctions, bladder and urinary tract inflammations. TAO (Thrombo Angitis Obliterans) is taken as a classical example to highlight the importance of Leech therapy.

Conclusion: Studies have shown that patients who have already tried this method of treatment reported improvements in sleep quality, mood, work capacity and increased energy levels. Hirudotherapy is popular and acceptable in Malaysia and the current interest of the various medical universities and the Ministry of Health Malaysia in promoting research will help in regularizing this simple and effective treatment. Hirudotherapy (Leech therapy) may be a best interface for integrative medicine.

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Bee Venom Therapy

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Title: Review of the recent studies of bee venom therapy
Author(s): Kim C
Institution(s): Biomedical Center, CAM, Graduate School, CHA University, Seoul, Korea

The author will review the 4 most recent studies of the bee venom therapy:

1. In Vitro Evaluation of bee venom as an Inhibitor of Human Cytochrome p-450 Enzymes
2. Treatment by injection-acupuncture with bee venom combined by Chinese herbal medicine in patients with canine hind limb paralysis
3. Therapeutic effect of bee venom and dexamethasone in dogs with facial nerve paralysis.
4. A Multi-Center, Randomized, Double-Blind, Active-Controlled, Parallel Group Study to Evaluate the Dose Effect of Intradermal Injections of Bee Venom vs. Histamine in Subjects with Osteoarthritis of the Knee

Title: Evidence-based medical apitherapy
Author(s): Kochan A
Institution(s):

Abstract not available at time of printing.

Title: Principles of green medicine

Author(s): Cherbuliez T

Institution(s):

Introduction: Natural therapies have acquired increasing importance in the western medical world. However, experience has shown that a number of generally accepted concepts and techniques in western science do not correspond to the realities of the fields in which natural therapies take place (EXAMPLE?)

This theoretical paper presents key concepts that are relevant to natural therapies and nevertheless allow for the kind of rigorous observation, experimentation and conclusions that characterize western approaches to science. These concepts are Multi-molecularity; Synergy; Variability; Indetermination; and Harmony and Communication with the Environment. A particular attention will be given to “Placebos” and their major role in the thinking of those who use it. Through the example one particular natural medicine, Apitherapy (therapy with all the products of the honeybee hive), I show how these concepts can offer alternative research protocols suitable to green medicine, and elucidate certain problems inherent in western medical science. I argue the principles of green medicine can apply, to various degrees, to all the other approaches promoted by the International Biotherapy Society.

Conclusion: This different conceptual and relational approach aims to define the therapeutic parameters by which green medicine has its indications alongside western medicine.

Title: The therapeutic application of bee venom therapy for pain management

Author(s): Kleronomos C

Institution(s): Salem Comprehensive Pain Center

This presentation will provide an overview of the therapeutic application of BVT in an integrated clinical setting. It will discuss the rationale for diagnostic application and outline a basic protocol. A review of the mechanisms of action from a pharmacodynamic, pharmacokinetic and Traditional herbal perspective will be included.

Outline:

1. Introduction
2. Complexities of Pain Management
3. Venom mechanisms
4. Applying BVT to existing models
5. Basic protocol
 - a. Includes integrative algorithm
6. Case study
7. Questions

Ichthyotherapy

~ 30 ~

Title: Ichthyotherapy for skin disease

Author(s): Grassberger M

Institution(s): Institute for Pathology and Microbiology, Rudolfstiftung Hospital Vienna, Austria

Ichthyotherapy (therapy with the so-called “Doctorfish of Kangal”, *Garra rufa*) is a biotherapeutic modality for skin ailments, that received a considerable amount of media attention during the last years. However, this is in contrast to the rather scarce published scientific studies about this treatment option.

So far ichthyotherapy has shown promising results in an uncontrolled pilot study, which evaluated the efficacy and safety of ichthyotherapy in combination with short-term UVA sunbed radiation in the treatment of psoriasis under controlled conditions.

This presentation aims to provide an overview of the historic development of the ichthyotherapy treatment, the currently known mechanisms of action, the scientific aspects of the appropriate fish species and the possible hazards involved when rigorous hygienic standards are ignored or the wrong species is used.

Additionally new treatment results of patients diagnosed with Ichthyosis are presented and new data on patient reported outcomes using quality of life questionnaires are presented.

Finally, best practice standards regarding hygienic issues and patient safety are suggested and key issues for future research are addressed.

Title: The proposed use and application of ichthyotherapy in the health and wellness industry

Author(s): *MacIntyre PA, *Bradnam RA, Exelby KJ

Institution(s): Pillar & Post Inn Spa and Conference Center

Background - Ichthyotherapy has limited availability in North American due to legislative restrictions.

Objective – To develop fish pedicure systems for commercial use in the health and wellness industry.

Methods – Phase 1: We describe our development and journey throughout the process, ie researching and visiting existing operations. Although the *Garra Rufa* pedicure system was available on the Internet, we chose to develop our own system in an attempt to improve upon what is common in the market. Lastly, we began a media campaign to further expose the product.

Phase 2: The Niagra Regional Health gave us an order not to open or provide the service. We sought legal advice and appealed the order. We subsequently withdrew our appeal with the agreed provisions that we preserved the right to revisit the issue in the future. At our sole cost, we began investigating research and studies.

Phase 3: The Ontario Provincial Government took a new position; outside of our regional agreement to further our research, fish pedicures are now banned in the Province of Ontario. These new legislative requirements led us to wonder: Are we overregulated due to the need to be right regardless of new research or scientific evidence, or are we under-regulated? Does the Ontario Governance and regulation stifle Entrepreneurship?

The current justifications for the ban are as follows: already existing Spa Service regulations define the fish as a tool and instrument; Section 13 of the Health Protection and Promotion Act states that the fish must be sterilized before use; under the Ministry of Natural Resources Fish and Wildlife Conservation Act, *Garra Rufa* are not included on the list of eligible species for culture under the authority of the aquaculture license in Ontario; water quality parameters are non-existent; UV filters – the difference between sanitizers and sterilizers; existing water quality standards regarding pools, drinking water, and public beaches; and concerns that *Garra Rufa* represent an invasive species that could threaten ecological balances. To meet these concerns, we changed our equipment and pathogen management.

Phase 4: Our future, current status, and next steps.

Veterinary Biotherapy

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Title: Veterinary Biotherapy

Author(s): Ben-Yakir S

Institution(s):

Abstract not available at time of printing.

Title: Assessment of larval therapy on four clinical cases of animals in Bogota-Colombia

Author(s): *Bello F¹, Rey M², Castañeda A², González J², Acero V², Segura A¹

Institution(s): (1) Laboratory of Medical Entomology, Faculty of Mathematics and Natural Sciences, Rosario University, Bogotá, Colombia; (2) Facultad of Veterinary Medicine, University of La Salle, Bogotá, Colombia.

Introduction. Larval therapy is a natural, simple, safe and highly successful method used in the management of chronic infected wounds which effects may be categorized into three main areas: debridement, disinfection and bacterial death, and stimulation of wound granulation and repair. Most studies of maggot therapy have been done on humans but less research has been carried out with animals.

Objective. The main purpose of this work was to assess the use of *Lucilia sericata* larvae in the treatment of infected wounds from four animal cases.

Methods. We used two bovines, a horse and a dog that were treated with larval therapy at the veterinary clinic from the University of La Salle, Bogotá, Colombia. A standardized protocol for disinfection of the embryonated eggs from *L. sericata* was carried out and when first instar larvae hatched they were placed in the animal wounds, covering them with bandages. Macroscopic evaluation of the wounds was based on a standardized test that looked at: odor, presence of exudates and inflammation.

Results. The action of the larvae on the animal infected wounds removed the necrotic tissue, controlled the infections and favored the formation of new granulation tissue, all leading to the healing of the lesions in 15 days or less. Optimal scores were obtained at 9 and 12 days of treatment, while the best rating for the new granulation tissue was established on the 9th.

Conclusions. Our results demonstrated the effectiveness of the larvae of *L. sericata* in the treatment of infected wounds in animals. Based on this, larval therapy is presented as an effective alternative for the treatment of wounds in several animal species.

Title: BioDiagnosis & Canine Olfactory Detection

Author(s): *Guest CM, Church JCT

Institution(s): Cancer and Bio-dection Dogs, Westcott Venture Park, Westcott, Aylesbury Bucks

Our first study, entitled 'Canine Olfactory Detection of Human Bladder Cancer a Proof of Principle', was published in the British Medical Journal September 2004. This was the first scientifically robust study to support anecdotal reports that dogs may be able to identify the odour of cancer. Retired orthopaedic surgeon, Dr John Church, initiated this work at the end of 2002. His interest arose from an anecdotal story published in the Lancet in 1989, which described a woman whose pet dog showed a persistent interest in a mole on her leg. This proved to be a malignant melanoma, the most serious form of skin cancer. Cancers being identified by dogs have not been restricted to skin cancer, but also include cancer of the bowel, cervix, and breast.

The notion that dogs can smell cancer is not far fetched. Over the centuries, physicians have been aware that many diseases have a characteristic odour. Dogs are renowned for their sense of smell. Cancer cells are known to produce chemical compounds that differ from those made by normal cells. It is therefore not unreasonable to think that some may have distinctive odours. We intend to use the olfactory capabilities of our dogs to identify these odours. It is anticipated, in the long-term, that these findings will lead to the production of an electronic nose machine that GPs can use in surgeries. We believe from current information that this may well be a possibility.

Hypoglycaemia (low blood sugar) is an acute daily problem for people living with diabetes. Hypos are frightening and distressing, symptoms can vary from confusion to seizures and can be life threatening. The charity has successfully trained a number of dogs to recognise both high and low blood sugar levels. These levels, we believe, give off a different scent compared with blood sugars that are within the normal range. Dogs with their incredible sense of smell have the ability to detect these changes. When outside the normal range they alert their owners and can get help even before symptoms of hypo (low blood sugar) or hyperglycaemia (high blood sugar) are felt. We collect essential information in relation to the accuracy and sensitivity of all trained and placed dogs and results indicate that the dogs are highly reliable. We intend to publish this data in peer reviewed journals very shortly. In addition we also train Medical Assistance Dogs, to detect and alert to other potentially fatal conditions such as Addison's crisis. The work of training dogs to assist in the diagnosis and management of human disease through the detection of minute changes in our odour is in its infancy. However we believe the potential is vast.

Title: Overview of service animals, equine assisted activities and equine assisted therapies

Author(s): McKenzie S

Institution(s): Little Bit Therapeutic Riding Center

This presentation will be an introduction to Animal Assisted Therapy, Animal Assisted Activities, Equine Assisted Therapy (specifically Hippotherapy), and Equine Assisted Activities. Animal Assisted Therapies are those that is a goal-directed intervention in which an animal is incorporated as an integral part of the clinical health-care treatment process. Animal Assisted Activities are those that provide opportunities for motivational, educational and/or recreational benefits to enhance a person's quality of life. Equine Assisted Therapies are treatments that incorporate equine activities and/or the equine environment. Rehabilitative goals are related to the patient's needs and the medical professional's standards of practice. Equine Assisted Activities are equine activities that are recreational/education activities designed for people with disabilities or diverse needs. Hippotherapy is a physical, occupational, and speech-language therapy treatment strategy that utilizes equine movement as part of an integrated intervention program to achieve functional outcomes. Recent research on Hippotherapy has demonstrated that Hippotherapy can improve trunk strength, balance, and coordination leading improvements in functional mobility. Needs for continued research include larger subject size and research designs that include randomized control groups. In conclusion this will be an overview of the use of animals in therapy and recreational activities and current research that has been conducted.

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Title: New devise used like fence dressing on chronic wounds treated with maggot therapy

Author(s): *Torres-Pabon ML¹, Bello F²

Institution(s): (1) Universidad del Rosario, Hospital Universitario Mayor, Mederi, Bogotá, Colombia; (2) Laboratory of Medical Entomology, Faculty of Mathematics and Natural Sciences, Rosario University, Bogotá, Colombia

Introduction: Maggot therapy has been used for the debridement of wounds for several hundred years. A plethora of literature is available on maggot therapy. Maggot therapy has been employed effectively to treat a wide spectrum of wounds including venous and arterial leg ulcers, osteomyelitis, necrotizing fasciitis, traumatic necrotic leg wounds, primary burns, pressure sores and amputation sites including digital amputations in diabetic feet. Larval therapy has also been used for the treatment of a variety of chronic wounds, including sacral and leg ulcers of assorted etiologies. Standard maggot dressing enables the larvae migrate out of the wound, causing discomfort to the patient and reducing treatment effectiveness.

Objective: To evaluate the effects of a proposed device for fence dressing and its ability to improve the healing of chronic wounds.

Methods: A descriptive study of several cases on 10 patients with 14 chronic ulcers was carried out at Hospital Universitario Mayor. A new dressing device for maggot therapy was applied to those patients. Before the application of the dressing device, the wounds were measured. A 'fence dressing' was made of foam rubber, with a fine nylon mesh adhered to the external side. The other side has a double-sided adhesive tape, fixed to a masking tape. The mesh allows larvae to breathe, and the masking tape protects the skin, once the larvae are scattered on the wound. The fence dressing helps to confine the larvae to the wound.

Results: 'The fence dressing' improved the outcomes of maggot therapy, because it prevented the spreading of larvae outside of the wounds. Besides, this procedure helps protecting peripheral skin, concentrating the action of larvae in the wound area, and avoiding adverse emotional reactions from patients when they see the leaked larvae.

Conclusion: The fence dressing provides care to the peripheral skin surrounding the wound, a better use of larvae, which are limited to the wound by the fence, and improves service quality.

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Title: Lucifensin, the key antimicrobial player of maggot therapy and its possible applications

Author(s): *Čeřovský V, Slaninová J, Ždárek J, Monincová L, Fučík V

Institution(s): Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic

From the extracts of various tissues (gut, salivary glands, fat body, haemolymph) of the green bottle fly (*Lucilia sericata*) larvae and from their excretions/secretions we have recently isolated and characterized novel homolog of insect defensin designated lucifensin (*Lucilia* defensin). This is 40 residues and three intramolecular disulfide bridges peptide which is very similar to other dipteran defensins. We assume that lucifensin is the key antimicrobial component that protects the larvae when they are exposed to the highly infectious environment of a wound during the medicinal process known as maggot therapy. We also believe that lucifensin is one of the crucial disinfectants of the wound produced by the maggots which contributes to the healing process. Here we present the total chemical synthesis of lucifensin by means of the solid phase peptide synthesis method utilizing both the "step by step" strategy and the segment condensation. The oxidative folding of purified linear peptide performed under open air resulted in the correct pattern of disulfide bridges identical to that of natural peptide. This was examined by the identification of the fragments resulting from the thermolysin digestion of lucifensin by means of mass spectrometry. In antimicrobial assay using a set of different bacteria lucifensin shows activity preferentially against Gram-positive bacteria including *Staphylococcus aureus*. The discovered lucifensin may have a potential as a new agent for topical therapeutic applications in the treatment of serious surface infections. A promising aspect in the treatment of non-healing wounds could be the combination of conventional antibiotics with lucifensin and/or the use of this antimicrobial peptide as a supportive means to bolster the healing effect of maggot therapy.

Title: Medical and veterinary maggot therapy in New Zealand

Author(s): Bishop D

Institution(s): 44 Blue Mountains Road, RD 1, Upper Hutt, New Zealand

In December 2003 MRSA, a diabetic, was treated in her home for a foot ulcer which developed during a recent stay in hospital. This was the first patient to be treated in New Zealand using sterile larvae. Since then larvae have been supplied to five hospitals and four veterinary clinics with equine practices. *Lucilia sericata*, from a long established laboratory colony are used to produce the larvae. The larvae are supplied as free-range, single vial application. The wounds, treatment applications and outcomes are summarized.

Title: Maggot Therapy – Initial approaches to understand the influence of *Lucilia sericata* on bacterial wound communities

Author(s): *Saum SH, Kolter R

Institution(s): Department of Microbiology & Molecular Genetics, Harvard Medical School, Boston, Massachusetts, USA

Infected wounds are good examples of sites where bacterial biofilms can have deleterious effects on the host organism. Research on the basic biology of biofilms has shown that these are not just accumulations of bacteria enclosed in a matrix. Rather, biofilms are more akin to complex societies with numerous kinds of cell-to-cell interactions and different types of cellular differentiation. For many years, the Kolter Lab has been interested in understanding the molecular processes underlying the development of these communities in different systems and the way these societies change in response to different stimuli. Recently we have become interested in maggot therapy as a means to control bacterial biofilms in wounds. We are mainly interested in the influence of the maggots on different members of the wound ecosystems and the dynamics of the whole biofilm. First concepts and experiments will be presented.

Title: Antibacterial and antibiofilm activity of honey against wound bacteria

Author(s): *Majtan J^{1,2}, Bohova J¹, Takac P¹, Kozanek M¹, Majtan V²

Institution(s): (1) Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia; (2) Department of Microbiology, Slovak Medical University, Bratislava, Slovakia

Introduction - Chronic wound infections are responsible for considerable morbidity and significantly contribute to the escalation in the cost of health care. An important factor in the failure of a sore to heal is the presence of multiple species of bacteria, living cooperatively in highly organized biofilms. Honey could be a potential natural candidate to help eradicate of chronic wound infections through its antimicrobial and antibiofilm effects.

Objective - The goal of this study was to determine the antibacterial effect of two Slovak honeydew honeys collected from different regions of Slovakia and therapeutic manuka honey (UMF 15+) on 40 bacteria isolated from chronic wounds including methicillin-resistant *Staphylococcus aureus*. The most effective honey was selected and used for characterization of antibiofilm activity against wound strains with the highest biofilm-forming capacity.

Methods - The antibacterial activity of honey was determined using a broth dilution method. The concentration of honey used in study was within the range of 3.75% to 25% (w/v). Quantification of biofilm formation was carried out using a modified tube assay after 24 hours of incubation. Two sub-inhibitory concentrations (5 and 10 %) and two inhibitory concentrations (20 and 40 %) of honey were used to characterize the inhibition of biofilm formation and the ability to disturb already formed biofilm, respectively.

Results - The MICs for honeydew honey (Bardejov) ranged from 10% to 25%, while those for active manuka honey ranged from 12.5% to 25%. Honeydew honey (Bardejov) had the lower MICs than manuka honey against 22 of tested isolates. No differences in MIC values were found between honeydew honey and manuka honey in 12 isolates. Honeydew honey (Liptovsky Mikulas) showed weak antibacterial activity compare to honeydew honey (Bardejov) and manuka honey. *Proteus mirabilis*, *Escherichia coli* and *Enterococcus cloacae* isolates showed the strong ability to form biofilm. Honeydew honey was able to inhibit *E. coli* and *P. mirabilis* biofilm formation but it failed to disturb already formed biofilm. On the other hand, honeydew honey was not able to inhibit *E. cloacae* biofilm formation but successfully disturb already formed biofilm.

Conclusions - This study showed that Slovak honeydew honey has exceptional antibacterial activity against wound isolates and was more efficient than manuka honey (UMF 15+). Honeydew honey with antibacterial and antibiofilm activity could be used as a potential agent to eradicate of multi-drug resistance wound isolates.

Title: Bacteriophages targeting cystic fibrosis *Pseudomonas* strains

Author(s): *Sachs E, Donacour E, Coerver B, Sherman-Waldtochte A, Wieland S, Callahan D, Rory Johnson R, Casalini J, Ackermann H¹, Zemphira Alavidze Z², Brabban A, Kutter E

Institution(s): (1) Evergreen State College, Olympia Wa.; (2) Laval University, Quebec; (3) Eliava Institute, Tbilisi, Republic of Georgia

Pseudomonas aeruginosa is one of the most common causes of nosocomial infections, producing biofilms in wounds, burns and the lungs of cystic fibrosis patients that are particularly antibiotic resistant and difficult to treat. In some parts of the world, bacteriophages are routinely used therapeutically against *P. aeruginosa*, as well as other bacteria such as staphylococcus, streptococcus and enteric pathogens. From Olympias LOTT sewage treatment plant over 100 phages have been isolated targeting *P. aeruginosa* from dog ear infections and/or clinical cystic fibrosis isolates from Seattle Children's Hospital. One of these phages hits over 90% of the 200 CF strains. Based on host-range patterns, seven were chosen to create a typing set to differentiate among the Children's Hospital strains and about 25 others were selected for further study of therapeutic potential, looking at genome size, high efficiency of plating on many strains, infection patterns, morphology, sequence information, stability and in some cases co-infection with other phages. The typing set is potentially useful during the process of selecting future treatment options and has, for example, helped monitor bacterial changes over 3 years with a local CF patient who is a candidate for phage treatment. Wounds and CF lungs include microaerobic and anaerobic environments, so we have been looking at anaerobic as well as aerobic infection patterns, which are often significantly different, in both lab and clinical strains. In parallel, through collaboration with colleagues at the Eliava Institute, Republic of Georgia, a number of individual *P. aeruginosa* phages have been isolated and characterized from pyophage (a complex commercial phage cocktail used routinely to treat purulent infections), to help them develop a cocktail that takes advantage of their years of phage selection and experience, while also being characterized to be acceptable internationally. Our Georgian colleagues are interested in exploring the possibility of using phage and maggot therapy in conjunction for dealing with severe wound infection, particularly since *P. aeruginosa* is one of the few bacteria maggots do not target and wounds often contain wide range of bacteria beyond those targeted in standard cocktails like pyophage.

Title: The usefulness of maggot therapy for diabetic foot ulcers at outpatient clinic

Author(s): Tadasu Okada¹, Hideya Mitsui²

Institution(s): (1) Mami Dermatology Clinic, 6-10, Mamigaoka, Kashiba City, Nara, Japan (2) Department of Cardiovascular Surgery, Okayama University Graduate School of Medicine and Dentistry, Okayama University Hospital, 2-5-1, Shikata-cho, Okayama City, Okayama, Japan

Introduction: There is no need to debate over the effectiveness of maggot therapy in treating various types of necrotic ulcers, as it has long been proven. Today the management of chronic non-healing diabetic foot ulcers and preventing limb loss is very important. Patients with diabetic foot ulcers usually have co-morbidity, but without serious circulatory disturbances, there is no need to be admitted to hospital for treatment. They can be treated with careful local treatment and general diabetes control at outpatient settings. In this point of view, maggot therapy at outpatient clinic may be very useful for neuropathic diabetic foot ulcers. In addition, maggot therapy can be useful even for ischemic diabetic foot ulcers, which have no indication for surgical or endovascular revascularization. Maggot therapy for them may be an alternative treatment which can be carried out at outpatient clinic.

Objective: To investigate whether patients endangered to limb loss can be successfully treated and achieved limb salvage by maggot therapy at outpatient clinic settings.

Patients and Methods: 5 cases in need of lower limb amputation or disarticulation in hospitals were treated only by maggot therapy at outpatient clinic. Sterile maggots (free-range) were administered to the wound two to three times weekly.

Results: 2 cases were neuropathic diabetic foot ulcers: 55-year-old male had the great toe of gangrene and dorsum of foot ulcers with MRSA. He was admitted to hospital to heal the foot ulcers, but had a little signs of wound healing for a month. 48-year-old male on dialysis had foot ulcer with *E. coli* and the second toe of gangrene in initial consultation, but a month later minor amputation was carried out with the second, third and fourth toes in hospital. 3 cases were ischemic diabetic foot ulcers: 53-year-old female had a painful leg ulcer with MRSA and below knee amputation on the opposing leg. 46-year-old male on dialysis had gangrene on the great and second toes with severe pain. 89-year-old female had painful gangrene on all toes with MRSA, *Pseudomonas aeruginosa*, and *Serratia* infected ulcer. All 5 cases were treated only by maggot therapy. The lower limbs of all cases were successfully saved from major amputations.

Recommendations: Diabetic foot ulcers without serious circulatory disturbances can be treated by maggot therapy at outpatient clinic. This therapy is often useful even for ischemic diabetic foot ulcers which have no indication for revascularization. Moreover, it is very useful for neuropathic diabetic foot ulcers at outpatient clinic. Therefore, it should be considered for neuropathic diabetic foot ulcers to prevent limb amputations not as a last resort, but as a first resort.

ICB-2010

Handouts

Debora Wade – A Patient's Journey – ICB 2010

I have Crohn's disease (CD) and have been experimenting with helmintherapy since December of 2007. I discuss my medical options, the many studies I found that supported the theory behind the therapy. I share how difficult it was to procure worms.

I purchased 10 hookworms through a commercial company in December 2007, and talk about my side effects (including fever, edema, and arthritis), but also my benefits, including a reduction in inflammation, weight gain, and general improvement in CD by week 16.

However, I added worms in weekly doses and slowly regressed. I discuss the difficulty in getting medical support.

In February 2009 I infected with 10 more hookworms. I improved again, and was in touch with a growing community of patients with diverse autoimmune diseases who were having success with helmintherapy. I share interviews of me and other doctors.

I discuss the current thinking of finding a pharmaceutical derived from the worm, and I relate patients' eagerness to experiment with the live worm now.

I discuss my learning how to do my own egg counts, and how I got 6 months efficacy after each infestation. I redosed on September 2009 and continued to do well until March of 2010.

I tell how I lost my worm supplier when he was raided by the FDA and left the country.

I discuss our legal rights. What are patients allowed to do with their infections?

I list the current drug trials available for helmintherapy. I list the current commercial companies.

I added 15 more hookworms through a second commercial company in June of 2010. I have difficult side effects (diarrhea, pain) and use prednisone to control the inflammation.

On week 8, I added 2500 trichuris suis ova (TSO) every 2 weeks for a total of 3 doses, which caused severe regression. It is now 6 weeks later.

Where am I now? What are my new drug options? Should I pursue helmintherapy?

How can patients influence helmintherapy? How can we prove or disprove the hygiene hypothesis? Can we help fund the research? How can we safely make the worms available?

The worm journey continues...

A copy of this presentation can be found at:

<http://waitingforthecure.com/I/2010/11/11/icb-2010-presentation>

HANDOUTS

An Accidental But Safe and Effective Use of *Lucilia cuprina* (Diptera: Calliphoridae) in Maggot Debridement Therapy in Alexandria, Egypt

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Introduction

- The calliphorid fly *Lucilia cuprina* (Wiedemann) is known to cause serious malign myiasis in animals, whereas its sibling species *Lucilia sericata* (Meigen) is commonly a carrion breeder and is used in maggot debridement therapy (MDT).
- The present study reports an accidental involvement of *L. cuprina* in MDT in Alexandria, Egypt, that has proved to be safe and effective.
- In November 2008, the laboratory colonies of *L. sericata* (the species regularly used in MDT) at the Faculty of Science, Alexandria University were renewed by *Lucilia* flies collected as third instar larvae on exposed rabbit carcasses.
- Flies from the new colonies were identified as *L. cuprina* after being successfully used to heal the diabetic foot wounds of two patients at Alexandria Main University Hospital.

Objectives

This work gives details of the molecular and morphological identification of the maggots, their occurrence in carrion in Alexandria, and preliminary evidence of the effectiveness of an Egyptian strain of *L. cuprina* in MDT.

Methods

- Adults and larvae of *L. cuprina* were identified using molecular analysis and morphological features.
- Two cycles, each of four days, of free-range maggots of this species were applied to the wounds of two diabetic patients.

Results

- Analysis of DNA sequences and adult and larval morphology revealed that these flies were and still are *L. cuprina*.
- The presence of *L. cuprina* in carrion in Alexandria and its use in MDT are new records.
- Maggots of this species were able to completely debride and close the neuroischemic ulcers of two patients.

Lessons Learned and Discussion

- Any new population of a particular fly species must be taxonomically identified before establishing a colony of it in the laboratory, especially where sibling species coexist. The authors of the present study are aware that if the introduced species had been applied to the wounds of their patients and found to be invasive, the patients may have suffered. MDT is essentially a controlled therapeutic myiasis in which the primary role of the medical entomologist is to select a safe and effective species and strain.
- Entomologists should regularly verify and monitor the blow fly species composition in their local habitats because it may change due to exotic species introductions or global climate change.
- The present study highlights the question of whether different strains of the same species can be more or less invasive, safe, or effective.
- Despite the safety of this strain of *L. cuprina* in MDT, entomologists rearing blow flies for the purpose of wound debridement should regularly maintain high quality assurance of their species' identity to avoid possible clinical complications that may result from the introduction of an unexpected and invasive species to their laboratory colonies.
- The use of fly species other than *Lucilia sericata* in maggot therapy should be encouraged.

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Handouts on

The Clinical and Microbiological Efficacy of Maggot Debridement Therapy in the Management of Pressure Ulcers

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Introduction:

- Pressure ulcers remain a significant problem in both the acute and community health settings. Despite the many developments in wound care during the past two decades, there has been no significant decrease in pressure ulcer prevalence or any demonstrable improvement in overall outcomes.
- Pressure ulcer is defined as any skin lesion caused by unrelieved pressure, usually over a bony prominence, which results in damage to underlying tissue.
- New treatment paradigms must be examined as we strive to reduce pressure ulcer morbidity. The past two decades have witnessed the resurgence in the use of maggot debridement therapy (MDT) in wound management mainly due to the increase of antibiotic resistance.

Objectives:

To investigate the clinical (debridement and wound healing) and microbiological outcomes of MDT in the management of pressure ulcers.

Methods:

- Fourteen bed-bound patients with 14 sacral and ischial pressure ulcers of stage III and stage IV over a period of 14 months were included in the study at general medical surgical wards, critical care units, and emergency recovery room, Alexandria Main University Hospital.
- The patients were generally bed ridden and hospitalized for a mean duration of 11.28 weeks.
- The patients suffered from the following underlying medical conditions: cerebral vascular stroke, diabetes mellitus, and chronic obstructive pulmonary disease. All but two patients had decreased levels of consciousness. The patients also suffered from anemia and malnutrition.
- The blow fly *Lucilia sericata* was used for maggot therapy. Each ulcer treated with one maggot cycle per week.

- The ulcers were observed weekly and swabbed for microbiology before and after application of each maggot cycle to investigate the bacterial burden, poly-bacterial population, and the type of bacteria in each ulcer.

Results:

- MDT was associated with a rapid rate of debridement, rapid growth of granulation tissue, and marked antimicrobial activity.
- Before MDT, the mean surface area of devitalized tissue was 58.81 cm² (range 8.25-131.25 cm²), whereas after MDT this mean significantly decreased to 15.35 cm² (range 0-40 cm²) ($P=0.000307$) during a mean period of 1.5 weeks (range 1-2 weeks).
- Three ulcers were completely debrided with one or 2 cycles of maggots. Before MDT, the mean surface area of granulation tissue was 16.03 cm² (range 0-80 cm²), whereas after MDT this mean significantly increased to 55.86 cm² (range 7.37-116 cm²) ($P=0.000221$) during a mean period of 2.14 weeks (range 1-10 weeks).
- Nine ulcers had >50% of their size occupied by a red healthy granulation tissue. All ulcers exhibited a mixed bacterial population ranged from 3 to 7 microorganisms.
- The mean of initial bacterial burden was 4.86×10^8 CFU/ml exudate. After the first maggot cycle, this mean significantly decreased to 1.92×10^4 CFU/ml exudate ($p=0.01814$) below the 10^5 threshold of natural healing.

Conclusion:

- The application of disinfected larvae of *Lucilia sericata* to infected, non-healing stage III and stage IV pressure ulcers resulted in the rapid removal of necrotic tissue, disinfection, and enhancement of the healing process.
- Microbiological outcomes demonstrate that maggot therapy is an efficient antimicrobial treatment.
- One or two cycles of maggot therapy was associated a reduction in the bacterial load to pressure ulcers below the 10^5 CFU/ml wound exudates threshold which permits healing. MDT could alleviate the suffering in patients with bed sores in Egypt.

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Handouts on

The antibacterial activity of medicinal maggots of the blow fly *Lucilia cuprina* against multidrug-resistant bacteria frequently infected diabetic foot ulcers in Alexandria, Egypt: A preliminary *in vitro* study

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Introduction:

The antibacterial activity of maggots (larvae) of the blow fly *Lucilia sericata* is well documented both *in vivo* and *in vitro* studies. Recently, the larvae of *Lucilia cuprina* has proved to be safe and effective for treating infected, non-healing diabetic foot wounds. Therefore, it was found necessary to investigate the antibacterial activity of this species.

Objectives:

- 1- To investigate the antibacterial activity of *L. cuprina* larvae against some multidrug-resistant bacteria isolated from the foot ulcers of diabetic patients.
- 2- To partially characterize the antibacterial agents extracted from these larvae.

Methods:

Whole body and gut extracts, excretions/secretions, and hemolymph of sterile, actively feeding third instars of *L. cuprina* were gained, lyophilized, and ultra-filtered with 3 and 10 kDa molecular weight cut-off membranes. Same extracts were accessed by the same procedures from sterile larvae after inoculation with each of the following six diabetic foot bacteria; *Micrococcus luteus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Serratia marcescens*. Two methods of extraction were applied (TFA-aprotinin & Acetonitrile/water). Antibacterial activity of the extracts obtained from sterile and inoculated larvae were investigated against these bacteria using the drop diffusion test on Petri dishes by the double-layer technique. Shelf-life of each of the extracted fractions (1, 2 days and 2 weeks) was determined.

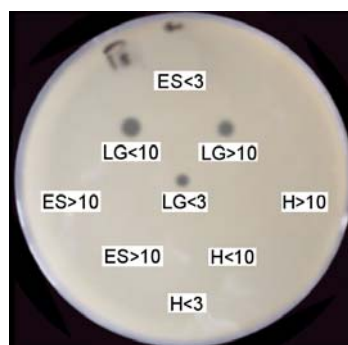


































































































Fig. 1. Drop diffusion test on Petri dishes by the double-layer technique showing the activity of different fractions with different molecular weights against *Ps. aeruginosa*

Results:

All whole body extracts from sterile or inoculated larvae of *L. cuprina* exhibited antibacterial activity with different degrees against the six tested bacteria. Three fractions with molecular weights of <3, 3-10 and >10 kDa from larval guts were found active against bacteria. Antibacterial activity was more effective against Gram-positive bacteria than against Gram-negative bacteria. Extracts of inoculated larvae possessed a slightly higher antibacterial activity than those of non-inoculated larvae. Method of extraction is critical for the bioactivity of the larval extracts. Shelf-life of larval extracts significantly depends upon the method of extraction and microbial flora in the ulcers.

Work concerning the antibacterial activity of excretions/secretions and haemolymph of *L. cuprina* is still under investigation.

Table 1. Effect of Virulent Clinical Bacteria, Extraction Method and Storage Time on the Bio-Activity of the Maggot Extracts

Extraction Method	Maggots' Treatment (Clinical)	Test Bacterium											
		<i>S. aureus</i> Standard			<i>P. mirabilis</i> Standard			<i>Ps. aeruginosa</i> Standard			<i>K. pneumoniae</i> Clinical		
		1 D	2 D	2 W	1 D	2 D	2 W	1 D	2 D	2 W	1 D	2 D	2 W
TFA / Aprotinin	Sterile												
	<i>S. aureus</i>												
	<i>P. mirabilis</i>												
	<i>Ps. aeruginosa</i>												
Acetonitrile / Water	Sterile												
	<i>S. aureus</i>												
	<i>P. mirabilis</i>												
	<i>Ps. aeruginosa</i>												



= Active



= Inactive

Conclusion:

- 1- Antibacterial activity of the extracts of *L. cuprina* depends on the method of extraction and the bacterial flora in the diabetic foot..
- 2- Shelf-lives of the extracts of *L. cuprina* differ according to method of extraction and the composition of the maggot extract and its extraction conditions.
- 3- The results of this preliminary study indicate that the larvae of *L. cuprina* exhibit antibacterial activity against these virulent bacteria and therefore have beneficial therapeutic effects when used in maggot therapy.

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Domen Jaklič, Aleš Lapanje, Dragica Smrke, Nina Gunde-Cimerman

Introducing maggot therapy in Slovenia; antibacterial activity of larval excreta/secreta of *Lucilia sericata* fly larvae

Biosurgery-Milestones

- 1491 Hortus sanitatus
- 1557: A. Paré
- 1st World War: W. Baer

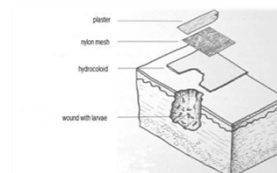
Milestones

- 1930-1940: 200 hospitals, 600 physicians, 5700 patients (USA)
- 1940 - decline
- 1980 – resurrection
- USA, Japan, Australia,
- Europe: Germany, Austria, Hungary, Sweden, Belgium, Ukraine, **Slovenia** (2005-2010)

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Use of larvae...

- Different types of chronic wounds:
 - Diabetic wounds
 - Postoperative wounds
 - Underlying osteomyelitis
 - Burns and insect's bites
 - ...



MDT in Slovenia

- 2005 collaborative project with University Medical Centre Ljubljana
- 32 wounds of 30 patients were treated
- Microbiological smears were collected before and after the application of the larvae on the wounds
- Monitoring
 - patient's general state,
 - potential presence of pain,
 - mobility,
 - patient's independence and
 - psychological reaction

Antimicrobial activity (from 1931-2010)

- Composition of larval E/S
 - allantoin, ammonium, urea, calcium carbonate, ammonium bicarbonate, enzymes, peptides...
- Antibacterial peptides ?
 - Innate immune system*
 - Stimulation: bacteria, wound conditions
 - 5 families
 - 21 – 40 a.a. residues



*Otvos, L., Jr. (2000). J Pept Sci 6(10): 497-511

Results (MDT)

- 24 (75 %) out of 32 wounds were cleaned and healed
- In the case of combined arterio-venous leg ulcers one wound was completely healed and eight of them were cleaned of necrosis and infection
- In the case of a venous leg ulcer, six diabetic ulcers, eight cases of chronic postoperative wounds and pressure ulcers, wounds were completely cleaned after the treatment

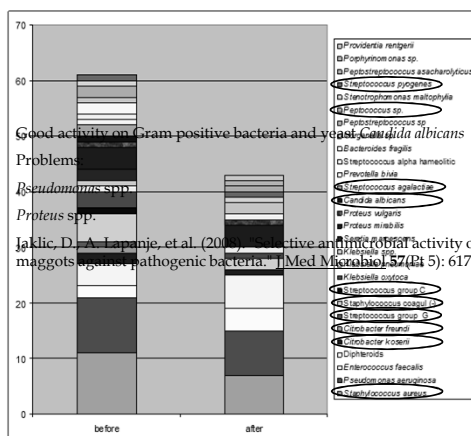
Results (MDT)

- 16 patients reported unpleasant feeling during larval treatment and two patients felt pain which diminished after the treatment with analgesics
- 17 (56,7 %) patients had to use crutches during biosurgical treatment and the same number of patients felt uncomfortable, frightened and depressed during the application of larvae

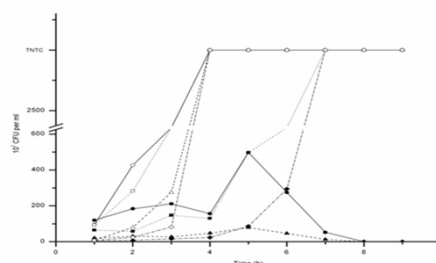
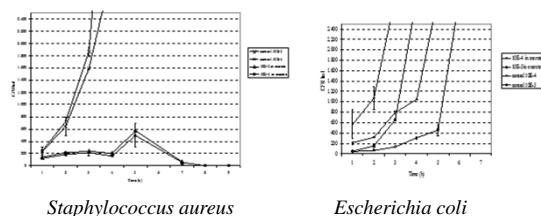
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Antimicrobial activities of ES

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Antibacterial activity of ES



In-vitro activity of larval ES in diluted cultures of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The viability of bacterial cells was checked every hour with plate spreading of 0.1 ml growth culture; colonies were plate counted after overnight incubation at 37 °C. ● *S. aureus* 10³ CFU/ml, treated with excretions; ○ control *S. aureus* 10³ CFU/ml; ▲ *S. aureus* 10³ CFU/ml, treated with excretions; △ control *S. aureus* 10³ CFU/ml (all in dotted lines). ■ *P. aeruginosa* 10³ CFU/ml, treated with excretions; ○ control *P. aeruginosa* 10³ CFU/ml; ◆ *P. aeruginosa* 10² CFU/ml, treated with excretions; ◇ control *P. aeruginosa* 10² CFU; TNTC too numerous to count

Conclusions & Perspectives

- successfully demonstrated in Slovenia
- Acceptance “?”, legislative issues
- *In vivo* activity of ES
- Antifungal activity of ES?

MDT works!



Before



... after classical larval therapy!

Leech Therapy in Recent Time

By: Svetlana Sviridova

Introduction

- Lana Sviridova, M.D., Professional Leech Therapist.
- Graduated from Medical School in Russia.
- Worked as a Medical Doctor in Rehabilitation Center (Russia).
- Completed Leech Therapy certificate program in 1994 (Russia).

In my clinical practice I observed many cases where leech therapy produce positive results:

- Venous diseases;
- Postoperative prevention of thrombosis;
- Symptomatic joint diseases;
- Chronic pain syndrome;
- Cardiovascular diseases.

Goals

- During last 2 years I collaborate with BTER Foundation:
 - Generating and documenting medical policies for Leech Therapy;
 - Main point to popularize Leech Therapy in the US;
 - Consolidate experts in this area;
 - Important role to achieving this should play Leech Therapy practical training seminar.

- Compared to other techniques of natural and complementary medicine, leeching can be learned relatively quickly, but individuals administering treatments with live animals must have special qualification.
- Leech Therapy practical training seminar will:
 - Contribute to optimizing the success of medicinal leech therapy in clinical practice;
 - The essential quality standards must be a main priority for this form of treatment.

- The most important elements of the successful practice of medicinal leeches are:
 - Technique of leech application;
 - Assessments of the indications for leech therapy;
 - Contraindications and standard procedure for minimizing the risk of treatment applications;
 - Knowledge of the biology of *Hirudo Medicinalis*;
 - Skills in the handling of leeches.

The major events in the history of
medicinal leech therapy

- The major event in the history of leech therapy was the discovery by J.B. Haycraft, professor King's College in Birmingham, that the throat and mouth of the leech contained a substance that prevented the blood from coagulating in 1884.
- The compound was isolated and named hirudin around 1903/04.

- A specific area of leech therapy was soon to be determined by the surgeon Termier. He recommended the direct application the leeches in 1922. This technique was called "hirudinization of the blood".
- In 1935 scientist Bottenberg established the general indications for leech therapy.
- In the 1960s, medicinal leech therapy achieved an international comeback, initially because of the spectacular results in plastic and reconstructive surgery for treatment of postoperative venous congestion and graft rejections.

- Since the 1980, leech therapy has regained recognition in the medical literature after initial publications by Upton's group and Mahaffey's team in Europe gave this treatment modality new impulse.
- It was a case report about the successful use of medicinal leeches to salvage the reattached ear of a boy in the US.

**What is the legal status leeches
nowadays?**

- There are different legal classifications of the medicinal leeches is used in USA and in the European Union.
- In USA the medicinal leech is considered as a “medical device”, but in European Union as a “drug/medicinal product”.
- The medical systems of the US and European Union are structured differently, as are those of different countries within the EU.

Food and Drug Administration

- In 2004, the medicinal leech was approved by the US Food and Drug Administration as “an adjunct to the healing of graft tissue when problems of venous congestion by creating prolonged localized bleeding” (quoted from the 510(k) summary of statement for medicinal leeches as a medical device and the product information from Leeches USA).

In EU (for example Germany)

- The leech was classified as a medicinal product by the German drug authorities of the Twelfth Amendment to German Drug Law in 2004.
- This law includes medicinal leeches under the definition of the medicinal product designed for medical use (section 2) quotes: “Medicinal products are substances or combination of substances used to cure, alleviate or prevent diseases, suffering, bodily injuries or pathological complaints”.

Medicinal leeches are used in various fields of medicine

- Medicinal leeches are used in various fields of medicine in Europe, Russia, India, Asia.
- The latest treatment results from Complementary and Internal Medicine Centers, Rehabilitation Departments and Hirudotherapy Clinics of different countries show that the simultaneous action of multiple mechanism of leech saliva may be responsible for the clinical effectiveness of leeching in nonsurgical indications.
- The latest research data confirm that leech therapy is drastically efficient method of treatment used in virtually all areas of medicine.

Example: Clinical research study at the Essen-Mitte Hospital (Germany)

- Symptomatic gonarthrosis (inflammation of the knee joint) is one of the best studied indication for leech therapy.
- The successful results confirm a larger randomized study at the Essen-Mitte Hospital.
- All 51 patients included in the study had long-standing X-Ray and clinically confirmed gonarthrosis of the knee.
- The patients were randomly assigned to group receiving a single leech treatment (24 patients) and topical diclofenac gel (27 patients).
- Symptoms were well documented using the Western Ontario and McMaster Universities Osteoarthritis Index.
- This Index was used to obtain a pain score, joint function score, morning stiffness score, and total score.

Conclusion

- During the entire study period (4 weeks), the Index of patients who received leech therapy were consistently better than those of patients in the control group.
- Quality of life, which was assessed at one month, was also significantly better in the leeching group.
- No serious adverse effects were observed.
- Moderate local itching that lasting for 2 to 3 days was frequently reported.

**Russia is one of the leading countries
in medical research and the use of
leeches**

- One of the oldest biofarm in the world near Moscow.
- Today this biofarm is International Center of Medicinal Leeches, led by Doctor of Biological Sciences, professor Niconov.
- This Center combines the scientific, and leech therapy industrial areas.

- Izolda Baskova is the President of the Russian Leech Therapist Association and leading researcher of the Moscow State University.
- In one of her interview she told: “ The main purpose of leech researchers is studying the action mechanism of medical leeches. Our research team obtained first data on its complex composition. We discovered the presence of a 150 proteins and several hundreds of low-molecular compounds, all of these correlated in the complex interaction with each other and human organism. These results are the subject of our future, is extremely interesting research work, which could go faster if scientists had known gene leeches”.

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General Review of Apitherapy

– C. MH. Kim, M.D. –

Apitherapy is the medicinal use of various products of *Apis Mellifera* – the common honeybee – including **raw honey, pollen, propolis, royal jelly, wax and venom**. Various studies attribute antifungal, antibacterial, anti-inflammatory, antiproliferative, and cancer-drug-potentiating properties to honey (Science News, 1993). In China, for example, raw honey is applied to burns as antiseptic and a painkiller. Recently, propolis (bee glue) has been identified as containing substance called caffeic esters that inhibit the development of precancerous changes in the colon of rats given a known calcinogen (Rao et al., 1998). In laboratory tests, propolis has exhibited a variety of interesting antimicrobial and anti-tumor properties, and is a potent antioxidant. Pollen is reported to be successful for treating allergies, and to increase their strength and stamina, and bee pollen is touted as an excellent food. Royal jelly, in animal studies, has anti-inflammatory, anti-hypertensive, and anti-tumor properties, and prolongs the life span of mice with reduced DNA damage. It is an incredibly nutritious food. Beeswax is a very natural substance that contains beneficial properties found in cosmetic products, and hair care products, and pharmaceutical products.

This section focuses on bee venom that treat chronic inflammatory illness because of the popularity of this treatment and the availability of related clinical research material.

Bee Venom Therapy

1. Introduction

Bee venom therapy (BVT, Apitherapy) has been used since ancient times. Ancient writers as diverse as Hesiod (800 BC), Aristophanes (450-388 BC), Varro (166-27 BC) and Columella (1st century AD) all wrote on the cultivation of the hive. Hippocrates (460-377 BC), the Father of Medicine, used it and call it *Arcanum* - a very mysterious remedy. Galen (131-201 AD), the Father of Experimental Physiology, mentioned it in his 500 treatises on medicine. Charlemagne (

742-814 AD) is said to have had himself treated with bee stings. The Koran (XVI:71) refers to bee venom in the following terms: "*There proceeded from their bellies a liquor wherein is a medicine for men.*" For apitherapy and the scientific understanding of bees, real progress began about 100 years ago when physician Phillip Terc of Austria advocated the deliberate use of bee stings in his work: *Report about a Peculiar Connection between the Beestings and Rheumatism.*

Today's proponents of apitherapy cite the benefits of bee venom for alleviating chronic pain and for treating many ailments including various rheumatic diseases involving inflammation and degeneration of connective tissue (e.g., several types of arthritis), neurological disease (migraine, peripheral neuritis, chronic low back pain), autoimmune disease (multiple sclerosis, lupus) and dermatological conditions (eczema, psoriasis, herpes virus infections).

In contrast, interest in bees has been sporadic in conventional medicine, focusing mainly on two areas unrelated to the therapeutic uses proposed above. These areas are: (i) the danger of hypersensitivity reactions, including anaphylactic shock, from the sting of insects of the genus *Apis*; (ii) the use of bee venom itself as immunotherapy for allergic reaction to such stings, especially to prevent life-threatening anaphylactic reactions in adults.

Apitherapy is still being widely used today, especially in China, Japan, Russia, Eastern Europe and South America, and it has approved a new biological drug in Korea (KFDA, May 2003), and a new biological drug for animals (KNVRQS, June 2009). In the US FDA/CBER, the final clinical Phase III study is on progress for inflammation and pain. It must, however be acknowledged that, in spite of this controversy, bee venom therapy has been the subject of many studies in animals as well as in human subjects. But, it is a fortune and good news that the Arthritis Foundation and Multiple Sclerosis Society of America recognized as BVT is a new complementary approach for those intractable disabling disorders.

It may be some time, however, before BVT is in general use in the world by the medical profession for above mentioned purpose. For those who cannot or do not want to wait, it is possible to use BVT, if one is willing to give it a chance, results will be striking in most cases. In general,

at least 80% of sufferers can expect good results if there is not too much irreversible damage done by the disease.

2. History

- **Hippocrates** (460-377 BC), the Father of Medicine, used it and called it *Arcanum* - a very mysterious remedy.
- **Roman, Pliny the Elder**, “Natural History”, written in about 14 BC.
- **Galen** (131-201 AD), “Prince of Physicians,” the Father of Experimental Physiology, mentioned it in his 500 treatises on medicine.
- **Charlemagne** (742-814 AD), King of Franks & Western Emperor who built the biggest empire in Western Europe since that of Rome, is known to have been treated with bee stings. It was thought at that time that bee stings cured all sorts of maladies and great store was laid by the venom’s curative healing properties.
- **Koran**, Chapter XVI, 71, reference to bee venom. “There proceeded from their bellies a liquor wherein is a medicine for men.”
- **Monfat** (1600-1634), prescribed bees for reducing kidney stones, the strengthening of the urinary tract and the better flow of the urine itself, as well as for a number of other conditions.
- **Dr. Desy’ardins** (1859, France), published the first scientific paper in the “*Abeille Medical* (Medical Bee Journal)” described successful treatment of rheumatic diseases. Also reported two cases of skin cancer which he was able to cure.
- **Professor Lukomsky** (1864, Russia) published in the “*Courier Medical*” about his success in the therapeutic effects of bee venom in rheumatic fever, gout, neuralgia and other diseases.
- Since 1985, many scientific papers by many physicians about their good results treating rheumatic patients with bee stings. Ro mention a few, Dr. Schwabe (Germany), Dr. Hale (England), Dr’s Marcy and Altschal (Germany), Dr. Goullon (France), Dr. C. Wolfe (Germany), and others.
- Dr. Philip Terc (Austria), the pioneer of the modern bee venom therapy, was the first physician to apply bee stings in a systemic way to the treatment of rheumatic diseases. In 1988, his first publication, “*Report about a Peculiar Connection Between the Beestings*

and Rheumatism.” During 25 years he had applied 39,000 bee stings to about 500 rheumatic patients without a complication, so called side effects or fatality. Most of these patients were lastingly benefited.

- Dr. Franz Kretschy (1928, Austria), invented a injectable form of the bee venom.
- Yoannovitch and Chahovitch (1932), “*Bulletin Academie de Medicine*,” treated experimental cancerous tumors with bee venom.

Over the past 50 years, research articles on bee venom have run over three thousands, mainly in European publications.

Some of the well-known apitherapist in the past (USA)

- Dr. Bodog F. Beck (New York)
- Dr. Raymond Carey (California)
- Dr. P.H. O’Connell (Connecticut)
- Dr. Joseph Broadman (New York)
- Dr. L.A. Doyle (Iowa)
- Dr. Joseph Saine (Montreal)
- Mr. Charles Mraz (Vermont)

The North American Apitherapy Society (NAAS) was formed in 1977 in Washington, D.C., and re-organized as **American Apitherapy Society** (AAS), and incorporated for *non-profit* organization in 1988.

- 1st and 2nd President: Christopher M.H. Kim, M.D. (*Founder*)
- 3rd and 4th President: Bradford Weeks, M.D.
- 5th, 6th and 7th President: Theodore Cherbuliez, M.D.
- 8th President: Andrew Kochan, M.D.
- 9th President: Dr. Frederique Keller

The Society serves their membership and the medical profession by: collecting information on apitherapy and maintaining a library for their members, which contains printed information, raw data, audio-visual materials, and a database; Informing the medical profession and the general public in matters relating to apitherapy, by publishing a subscription-based journal and conducting workshops.

3. Clinical Application (Apitox Therapy, BVT)

- **Allergy Skin Test**

The patients should be given a skin test before initiating treatment. Injections were given intradermally.

Apitox was diluted with injectable normal saline (1.0 mg / 1.0 mL) and 0.05 mL was injected intradermally in the flexor surface of the forearm. Local reactions were observed carefully for 15 minutes.

Local reactions to observe of the size of wheal, the size and shape of erythematous spreading, development of pseudopod, and etc. Systemic reactions are generalized itching, rashes, dizziness, shortness of breath, chills, fever and possible anaphylactic reactions.

1) Local Reaction

Criteria for local reactions described in the table.

Table . *Criteria for local reaction*

Grade	Erythema	Wheal
0	< 0.5 Cm	< 0.5 Cm
-	0.5 to 1.0 Cm	0.5 to 1.0 Cm
1+	1.1 to 2.0 Cm	0.5 to 1.0 Cm
2+	2.1 to 3.0 Cm	0.5 to 1.0 Cm
3+	3.1 to 4.0 Cm	1.1 to 1.5 Cm/ pseudopodia
4+	> 4.0 Cm	>1.5 Cm/ many pseudopodia

Check the injection site in 15 to 20 minutes after intradermal injection of Apitox 0.05 mL with a concentration of 1.0 µg/mL or less. A reaction of 3+ or greater is considered **positive**.

2) Systemic Reaction

Any of the above systemic signs developed during the observation, we declared as positive, *ie.*, sensitive to bee venom, and had to be excluded from the treatment.

- **Administration and Dosage**

The standard single site injection is 0.05 - 0.1 mL, *intradermal*.

Recommended Dosing Schedule of Apitox

<i>Inj. No.</i>	<i>Dose</i>
1	0.2 mL (50%)
2	0.4 mL
3	0.6 mL
4	0.8 mL
5	0.6 mL (100%)
6	0.8 mL
7	1.0 mL
8	1.2 mL
9	1.4 mL
10	1.5 mL
11	1.5 - 1.7 mL
12	1.5 - 1.7 mL
13	1.5 - 1.7 mL
14	1.7 - 2.0 mL
15	1.7 - 2.0 mL
16	1.7 - 2.0 mL
17 and More:	2.0 mL (<i>Max.</i>)

1. Give injection twice every week for several weeks. Then once a week if the patient's condition markedly improved (therapeutic course takes average 16 - 20 sessions).

2. The amount of injection per shot should be 0.05 - 0.1 mL and give each injection to different part. In other words, when 0.6 mL and 1.5 mL of injection are given, give injection to parts 6 - 12 and 15 - 30 each other.

3. Give a half of total dose, and rest 10 - 15 minutes, then give remain half.

4. Always use the corresponding dermatomal points in the spine as well as local trigger or tender points to facilitate the efficacy.

5. Do not inject deep or intra-articular until you are very experienced and confident.

- **Course of Treatment**

The course of treatment usually last 16 to 20 sessions for chronic painful conditions or arthritis, and over 20 for autoimmune diseases such as rheumatism or multiple sclerosis. Usually when treatments are first started, there is a little or no swelling. Then as the treatment is continued, the areas will begin to swell much more, with redness and itching of the area treated. Sometimes during this stage, one may feel worse, more pain, and pain in areas never before affected. There may be slight nausea and feeling of discouragement. It is important to remember, this is a good sign that the treatment is working and treatments must be continued. The number of injections can be reduced if desired. It appears that the cause of this reaction is the purging of toxins from the body, the stimulation of the biotic processes of the body. It follows the classical *Hans Seyle Sign* of the “Stage of Reaction” and the “Stage of Resistance.” Soon after this, the injection is no longer swells and a person becomes “immune.” When on reaches this *“Immune Stage”* the first course of treatment can be terminated.

At this point a person will usually find their condition much improved. No more treatments are necessary for another month or two, and if there is no return of the symptoms, there is no longer any need for any further treatments for a period of several years or as long as 20 years or more. However, if after a “rest period” of a month or several months, there are still symptoms, another course of treatments can be followed, starting with one injection and building up as with the first course. However, with subsequent courses, the treatments are usually more effective and quicker results so that a shorter course of treatments will suffice. In very bad cases three or more courses of treatments may be necessary covering a period one year or more. However, in the usual cases where there is little or no joint damage, a few weeks of treatments will suffice.

4. Clinical Pharmacology & Human Studies

There are many patients seeking unproven remedies and questionable treatments because of dissatisfaction with conventional approaches. The venom of *Apis mellifera* (honeybee) has been used to treat chronic inflammatory painful illness for over 2,000 years as a folk medicine or alternative medicine (Broadman, 1962; Kim, 1989,1992, 1997; Kretschy,

1928; NIH Pub., 1992; Yorish, 1977); many of the components of bee venom that have been identified have strong anti-inflammatory properties and each component works in various ways to reduce inflammation (Artemov, 1959; Banks et al., 1980, 1990; Billingham et al., 1973; Chang and Bliven, 1979; Eiseman et al., 1982; Guyton, 1978; Habermann, 1972; Hadjipetrou-Kourounakis and Yiangou, 1988; Hyer and Smith, 1986; Lorenzetti et al., 1972; Sommerfield, 1986; Weissman et al., 1973; Zurier, 1973).

More than 30 different substances have been characterized in bee venom. The main pharmacological components that reduce inflammation are peptides, including the following: mellitin, apamin, peptide 401, adolapin and protease inhibitors. Mellitin stimulates the hypophyseal-adrenal system and releases cortisone that is 100 times more potent than hydrocortisone (Couch, 1972; Knepel et al., 1987; Vick et al., 1972, 1976). Mellitin also stabilizes the lysosomal cell membrane to protect against inflammation (Shkenderov et al., 1986). Apamin works like mellitin to release cortisol (Vick and Shipman, 1972), and inhibits the complement (C₃) system which is involved in the inflammatory process (Gencheva et al., 1986). Peptide 401 or MCD peptide blocks arachidonic acid and inhibits prostaglandin synthesis (Hanson et al., 1974; Neubould, 1963; Surfer et al., 1973). Adolapin inhibits the microsomal cyclo-oxygenase system and is 70 times stronger than indomethacin in animal models (Shkenderov et al., 1986). It also inhibits platelet lipoxygenase which is involved in the production of hydroperoxy-eicotetranonic acid (HPETE) and leukotrienes (Koburova et al., 1985). Furthermore, adolapin inhibits thromboxane (TXA₂) and prostacycline (PGI₂) which are activated during inflammation (Shkenderov et al., 1986). Protease inhibitors inhibit carrageenin, prostaglandin E₁, bradykinin and histamine-induced inflammations, as well as chymotrypsin and leucineaminopeptidase (Shkenderov, 1986). Schmidt-Lange (1941), Ortel (1955) and Fennell et al. (1968) have reported that bee venom has strong anti-bacterial, anti-fungal, and radioprotective effects (Ginsberg et al., 1968; Kanno et al., 1970; Shipman et al., 1967, 1968). These properties are consistent with the strong anti-inflammatory effects and other benefits that are observed after bee venom injections. In addition to its anti-inflammatory properties, bee venom is a strong immunologic agent that stimulates the body's protective mechanisms

against disease (Artemov, 1959; Hyre et al., 1986; Yunginger et al., 1978).

The followings are the summary of the properties of HBV.

I. Modifying Immune System

- HBV stimulates the vital system of the organism.
- HBV increases the body's defense mechanisms against disease.

II. Anti-inflammatory Effects

1. Melittin

- Melittin stimulates hypophyseal-adrenal system and release cortisol and catecholamine. It was 100 times potent than hydrocortisone in animal models.
- Melittin stabilize the lysosome cell membrane. It is one of the specific mechanism against the inflammation.

2. Apamin

- Apamin stimulates hypophyseal-adrenal system and release cortisol and catecholamine.
- Apamin reduces the inflammation caused by Dextran and serotonin induced inflammation.
- Apamin inhibits the complement system, C₃, which is involving in the inflammation.

3. MCD-Peptide (Peptide 401)

MCD-Peptide blocks the arachidonic acid and inhibits prostaglandin synthesis.

4. Adolapin

- Adolapin inhibits the microsomal cyclooxygenase. It is 70 times stronger than Indomethacin in animal models.
- Adolapin has a property of analgesic effect. This analgesic effect is partially blocked by Naloxone.
- Adolapin inhibits platelet lipoxygenase which is involving hydroperoxyeicosotetranonic acid (HPETE) and leukotriens.
- Adolapin inhibits the thromboxane (TXA₂) and prostacycline (PGI₂) which are activated during the inflammation.

5. Protease Inhibitor

- Protease inhibitor inhibits carrageenin, prostaglandin E₁, bradykinin and histamine-induced inflammation.
- Protease inhibitor inhibits chymotrypsin and leucineaminopeptidase.

III. Neurotoxic Effects

1. Efferent System – Postsynaptic Effects

- Apamin blocks inhibition by α not by β adrenoreceptors. It is also antagonizes the neurotensin induced relaxation.
- In vertebrate smooth muscle, apamin blocks most of the hyperpolarizing inhibitory effects, including α -adrenergic, cholinergic, purinergic and neurotensin-induced relaxations, but not β -adrenergic relaxation.
- Apamin also block either the Ca-dependent K⁺ channels present on the cell membrane or the mechanism that controls the channels.

2. Central Effects

- Adolapin has an analgesic effect in which central mechanism may also be involved. This is suggested by the fact that Naloxone, a blocker of the opiate receptors partly eliminates the analgesic effect of adolapin.
- Injection of apamin into the spinal cord caused an increase in the monosynaptic extensor reflex potentials and the polysynaptic potentials from flexor afferents.

3. Cardiovascular Effects

- MCD-Peptide and phospholipase A₂ has properties of blood pressure depression.
- Cardiopep (Apamin) has a cardiac anti-arrhythmic effect.

IV. Anti-bacterial, Anti-fungal and Anti-viral Effects

Melittin has anti-bacterial, anti-fungal and anti-viral properties.

V. Radioprotection Effects

Melittin increases the resistance against X-irradiation.

VI. Anti-alkylating Activity

HBV is effective against poisoning with bismethylamine-HCl when given prophylactically.

Human Studies in Korea

Twenty healthy (10 male, 10 female) subjects between the ages of 23 and 45 years of age were enrolled and 15 completed the study. Five subjects dropped out of study because of failure to keep study appointments, not following study directions, or due to consumption of alcohol during the study. Each subject received an initial test dose of 0.05 mL and then 12 doses of Apitox, starting with 0.1 mL with the first intradermal injection and increasing to 0.2 mL (second injection), 0.25 mL (third injection), 0.3 to 0.7 mL (fourth through twelfth injections). Injections were administered 2 to 3 times per week over a period of 4 to 6 weeks. Physical examination, blood and urine laboratory evaluations, and vital sign measurements were performed. There were no significant changes from baseline noted. Localized itching was the most common adverse experience (11/15). Edema (5/15), pain at injection site (2/15), and blister at injection site (1/15) were also reported, but no serious adverse experiences were reported. Thus, it was concluded that Apitox can be safely administered to humans when applied in therapeutic doses.

This was a randomized, active-controlled (nabumetone) study in which 101 subjects with osteoarthritis of the knee or spine were given twice weekly injections of Apitox (maximum doses of 0.7 mg [Group A], 1.5 mg [Group B], or 2.0 mg [Group C]) or an oral once daily dose of the control (1000 mg nabumetone, Group D) over a period of 6 weeks. A 4-point Likert-like symptom severity rating scale was used to assess pain, disability, and physical signs. A 5-point scale was used for subject self-evaluation. Safety was assessed through observation of adverse experiences and blood and urine laboratory measurements.

A total of 81 completed the study. Those subjects assigned to an Apitox treatment group demonstrated a statistically significant greater improvement than those in the nabumetone group ($p < 0.01$). Within the Apitox groups, Groups B and C demonstrated greater improvements than Group A ($p < 0.01$). The most common adverse experiences reported were injection site itching and generalized body aches.

The bee venom therapy (BVT) is exceptionally safe and effective in the treatment of osteoarthritis. A 4 week comparison trial with 60 people of the effectiveness of BV acupuncture (bee venom injection at acupuncture points) versus traditional acupuncture found that bee venom acupuncture

produced even more pain relief than acupuncture alone. Both were found effective. 82.5% of BV acupuncture patients rated the effectiveness of their treatment as either excellent or good. All patients reported pain relief and they improved significantly in a number of areas including infrared thermograph (IRT) readings; 18 of 26 patients' IRTs were normal after treatment.

Following approval of Apitoxin in Korea, a post-marketing survey was conducted in 2004 - 2009. Included in this survey were 3,194 patients who received Apitox therapy. Patients, without any compensation, voluntarily filled out the survey forms. The information collected included personal information, present illness, past history, and present medications. The physicians recorded the treatment records, including the diagnosis, treatment dates for 12+ sessions, doses, and adverse experiences (including a detailed description). A complete blood count was performed before treatment and after the last treatment. The physician immediately notified the pharmaceutical company and Korean FDA in the event of a serious adverse experience. According to the survey, no major adverse experiences were reported. In addition to this survey, The Pain Center, PC University Medical Center located in Korea has documented the use of Apitoxin in 6,132 intractable medical condition and autoimmune disease patients between 2003-2009. No major adverse experiences were reported. Minor adverse experiences included itching (injection site), swelling (injection site), pain, low-grade fever, flushing, headache, and diarrhea.

Human Studies in the US

1) Healthy Subjects

Fourteen healthy male (10) and female (4) subjects between the ages of 26 and 52 years of age were enrolled in and completed this study. Among the male subjects, 3 were beekeepers who were included in order to obtain data from the maximum end of the spectrum without having to administer venom in high dosages (they received 10-350 stings per week during the course of their normal work). Injections were administered subcutaneously twice weekly, with each dose being dependent upon tolerance to the previous dose. All subjects were administered a maximum dose of 0.07 of Apitox and maintained on that dose for a week before continuing with an arthritic injection schedule. Each subject received 13 doses of Apitox according to the following schedule:

Injection #	Concentration per Injection	Total Dose(mg)
1	1 × 0.07	0.07
2	1 × 0.07 + 0.05	0.12
3	2 × 0.07	0.14
4	2 × 0.07 + 0.05	0.19
5	3 × 0.07	0.21
6	3 × 0.07 + 0.05	0.26
7	4 × 0.07	0.28
8	4 × 0.07 + 0.05	0.33
9	5 × 0.07	0.35
10	5 × 0.07	0.35
11	5 × 0.07	0.35
12	5 × 0.07	0.35
13	5 × 0.07	0.35

Skin tests, vital sign measurements, and blood and urine laboratory evaluations were performed. One subject who became ill and was subsequently hospitalized was shown to have contracted an adenovirus infection. A female subject had a delayed local reaction: 18 hours after injection, she developed a 4+ wheal with a very large flare. A repeat injection of the same concentration and all subsequent injections were normal. None of the subjects had a systemic reaction and there were no changes noted in vital sign measurements. None of the subjects reported significant pain.

2) Chronic Pain and Inflammation Study

In the chronic pain and inflammation study, 180 subjects were randomized to receive, over a period of 6 weeks, twice weekly injections of either Apitox (1 mg/mL) or histamine phosphate (0.275 mg/mL). The number of injections increased with each subsequent visit (e.g. 3, 6, 9, 12, 15, 18, 20, 20, 20, 20, 20, and 20 injections). The injections were administered to the area of pain first and then beginning with the fifth session, as the number of injections increased, to the corresponding dermatomal area of the spine. The visual analogue scale (VAS) and McGill Pain Questionnaire (MPQ) were used to assess the level of pain.

Thermographic evaluations and physical examinations (to evaluate swelling, tenderness, and range of motion limitations) also were performed.

Both groups had reductions in pain scores following treatment, with the Apitoxin treatment group demonstrating a greater improvement (pain scores: control 57 vs Apitox 18). In addition, there were significant differences between control and the Apitox treatment group at the 6-month follow-up visit (pain scores: control 83 vs Apitox 29). The Apitoxin treatment group also demonstrated greater improvement in the physical examination and thermographic findings.

3) Lyme's Disease

Tests of melittin's inhibitory actions against Lyme organisms were carried out at the U.S. government's Rocky Mountain Laboratories Microscopy Branch, National Institute of Allergy and Infectious Diseases in Hamilton, Montana.

Borrelia burgdorferi has demonstrated a capacity to resist the in vitro effects of powerful eukaryotic and prokaryotic metabolic inhibitors. However, treatment of laboratory cultures on Narbour-Stoenner, Kelly medium with melittin, a 26-amino acid peptide contained in honeybee venom, showed immediate and profound inhibitory effects when they were monitored by dark-field microscopy and optical density measurements. Furthermore, at melittin concentrations as low as 100 microg/mL, virtually all spirochete motility ceased within seconds of inhibitor addition. Ultrastructural examination of these spirochetes by scanning electron microscopy revealed obvious alterations in the surface envelope of the spirochetes. The extraordinary sensitivity of *B. burgdorferi* to melittin may provide both a research reagent useful in the study of selective permeability in microorganisms and important clues to the development of effective new drugs against Lyme disease.

4) Multiple Sclerosis

The study was a scientific attempt to evaluate the efficacy of bee venom injections on stopping or reversing the course of multiple sclerosis. Fifty-one patients with clinically documented MS received anaphylactic testing to ensure safety of participation before being titrated to tolerable, yet effective doses of venom. Therapy was administered 1 – 3 times per week, consisting of an average of 11 intradermal injections (0.1 mL) per

session. Patients' clinical response was evaluated every three months for one year. A positive correlation between BVT and improvement in symptoms related to MS was found. Fifty-eight percent of the participants experienced positive results, 29.8% experienced no benefits, and only one patient reported a worsening of her condition. The first symptoms to respond to BVT were fatigue and endurance, improving by 44 and 42%. Significant improvements were also made in balance and bowel control (32.2%) and coordination (31.4%). Average scores for the ROSS survey went from 36.1 upon enrollment to 48.6 after 12 months.

BVT in patients with multiple sclerosis seem to be effective in decreasing a patient's functional debilitation due to the disease. With over 68% of patients enrolled in the study experiencing some kind of positive effects from the venom. These results indicate that BVT may be a legitimate alternative therapy in the treatment of MS.

5. Indication, Contra-Indication and Pre-Caution

INDICATIONS (***bold: main***)

1. Musculo-Skeletal Disorders

- Degenerative Arthritis (Osteoarthritis), Gouty Arthritis
- Ankylosing Spondylitis, Spondylosis
- Bursitis, Tendonitis, Tennis & Golfer's Elbow, Frozen Shoulder
- Myalgia, Fibromyositis, Myofascial Dysfunction Pain Syndrome

2. Neurological Disorders

- Peripheral Neuropathy, Neuralgia

3. Autoimmune Disorders

- Rheumatoid Arthritis, Polyarthritis Rheumatica
- Multiple Sclerosis (MS)
- Lupus (SLE), Behcet's Syndrome, Sjogren's Syndrome

4. Others

- Vascular: Migraine
- Dermal: Psoriasis, Eczema
- Ophthalmic: Iritis, Iridocyclitis Rheumatica
- Infectious: Acute Rheumatic Fever
- Chronic Surgical Inflammation of the Soft & Bony Tissues

CONTRAINDICATIONS

- Acute Infections
- Advanced Complicated Cardiovascular Complications
- Unstable Diabetes Mellitus (*Insulin pump*)
- Syphilis (*Active*)
- Tuberculosis (*Active*)
- Gonorrhea (*Active*)

PRECAUTIONS

- Complicated Diabetes Mellitus
- Menstruation Period
- Pregnancy - The safe use of Apitox in pregnancy has not been established. Because of the risk of systemic reactions, the benefit-to-risk ratio must be carefully evaluated before therapy program is being initiated.
- In both testing and treatment, a separate sterilized needle and syringe should be used for each individual patient to prevent transmission of homologous serum hepatitis and other infectious agents from one person to another. All other aspects of good sterile technique should be observed. Care should be taken to avoid injecting into a blood vessel. The precaution of withdrawing the plunger slightly after inserting the needle is advisable to determine if a blood vessel has been entered.
- A patient should be kept under direct observation for at least half an hour after skin testing and after each treatment. The patient should be advised to contact the physician promptly at first sign of systemic allergic reaction.
- The patient should also be advised to report all types of reactions after the previous injections. Such information should be used to determine the next dose.

6. Warning and Patient Information

WARNING

Apitox (*Purified honeybee toxin*) should be used only by physicians experienced in administering venom to the maximum tolerated dose.

Because of the possibility of severe systemic reactions, the patient should be fully informed by the physician of the risk involved and should be under his constant supervision. The Apitox should be used only when adequate means for treating such severe systemic reactions are immediately available.

Patients should be confirmed by skin testing for negative allergic reaction before treatment with the solution is initiated.

All patients receiving the Apitox should be instructed in the procedure for emergency self-injection of subcutaneous epinephrine (Adrenalin). These patients should be advised to carry an emergency epinephrine kit while receiving Apitox therapy.

Before administering *honeybee toxin* solution physicians should be thoroughly familiar with the information concerning precautions, adverse reactions, and treatment of overdose.

Patient Information

- Alcohol is strictly forbidden during the treatment.
- If the local reaction is very annoying due to swelling and itching, ice packs and Rhuli gel, or mentholated ointment may be applied.
- In case of developing generalized itching, take Benadryl 75 mg at bedtime and may take Benadryl 50 mg during the day. It may cause drowsiness, so be careful about driving.
- When one develops a low grade fever and chills, take Tylenol 650mg (2 Tab) every 3 - 4 hours as needed.
- Any serious reaction occurs, contact your physician immediately.

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Chapter 24. Apitherapy (Bee Venom Therapy)

Potentiating Health and the Crisis of the Immune System.

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ASSESSMENT OF LARVAL THERAPY IN FOUR CLINICAL CASES OF ANIMALS IN BOGOTA, COLOMBIA

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Larval therapy is a natural, simple, safe and highly successful method for treating necrotic wounds when conventional treatments fail (1, 2). Also, this therapy is now used as an adjunct to conventional modalities of treatments (3). The larvae's action in the wounds may be categorized into three main areas: debridement, disinfection and bacterial death, and stimulation of wound granulation and repair (4-5). The larval therapy has been less used in veterinary medicine and there are only a few cases of animals treated with this technology. The objective of this work was to evaluate the larval therapy treatments applied to four cases of animals with infected wounds that were brought to the veterinary Clinic of the University of La Salle in Bogotá, Colombia.

Materials and methods

Disinfected larvae from a laboratory colony of *L. sericata* were prepared, using a previously standardized protocol. Sterilized eggs were transferred to Petri dishes, containing a thin layer of blood agar, in which the larvae hatched at 27°C. The sterile larvae were refrigerated at 4°C, before being used in each treatment.

Cases evaluated: four animals (two bovines, one equine and one canine) were treated with larval therapy at the Veterinary Clinic of La Salle University, Bogotá, Colombia, during the years 2007 and 2008. A physical clinical examination was conducted on each animal before, during and after the treatment.

The variables evaluated were the following: edema, exudates, odor, swelling, and presence of granulation tissue, according to the method of Wollina et al. 2002 (6).

Results

There was a significant improvement in each of the animal cases, and the lesions reacted favorably throughout the time of the treatments with larval therapy.

The table shows the values of qualitative variables, according to the parameters of Wollina et al. (2002). The established scale from 3 to 0 records the variable values, which correspond to the worst and the best condition of animals' wounds respectively, during the process of healing.

Day	edema					exudates					odor					swelling					granulation tissue				
	0	3	6	9	12	0	3	6	9	12	0	3	6	9	12	0	3	6	9	12	0	3	6	9	12
Case 1	3	2	2	1	1	3	2	1	0	0	3	3	2	1	0	3	2	1	1	0	3	3	2	0	0
Case 2	3	2	1			3	2	1			3	3	2			3	3	2			3	3	2		
Case 3	2	2	1	1	0	3	2	2	1	0	3	2	2	0	0	3	2	2	1	0	3	2	1	0	0
Case 4	2	2	1			2	1	1			3	2	1			3	2	2			3	2	1		

Conclusions

- The treated animals showed adequate repair of different lesions without adverse effects.
- The action of these treatments was demonstrated by a better quality of tissue healing and in relatively short times.
- Larval therapy is a potentially effective alternative for the treatment of wounds in different animal species.

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Medical Detection Dogs

Registered Charity Number 1124533



1

Cancer and Bio-Detection Dogs

Claire Guest MSc
www.cancerdogs.co.uk
www.hypoalertdogs.co.uk



2

Formation of new Charity

- Formed in 2008 to train dogs to detect human disease and medical conditions, through the detection and recognition of specific odours
- In addition to the training of dogs the charity is committed to publishing peer reviewed research papers



3

Introduction

- Varying roles of Detection Dogs
- Detection dogs, drugs, fruit, fire, etc
- Seizure alert dogs, Medical assistance dogs
- All protect human health
- Cancer detection dogs



4

Seizure alert dogs

- Alert to imminent epileptic seizures
- Dalziel et al (2003)
- Strong et al (1999, 2003)
- Exactly how is unknown - scent or body changes.



5

Canine Olfactory Detection of Cancer

- 1st Lancet letter, Williams & Pembroke (1989)
- Series of anecdotal stories
- 2nd Lancet letter, Church & Williams (2001)
- Claims of successful dog training
- Buckinghamshire partnership - late 2002



6

Skin samples

- Pickel et al (2004)
- Evidence for canine olfactory detection of melanoma
- Majority of work used the same melanoma



7

Aim of study published in the BMJ in 2004

- A "Proof of Principle" to prove that dogs can be trained to identify people with bladder cancer on the basis of urine odour more successfully than would be expected by chance alone
- To train dogs to recognise an odour, or combination of odours (an "odour signature") characteristic of bladder cancer but distinct from those associated with secondary effects of the tumour such as bleeding, inflammation, infection and necrosis



8

Methods

- Six dogs of varying breeds completed a seven month training period and were taught to indicate the appropriate sample by lying beside it
- None of the dogs had been previously trained for search or scent discrimination tasks



9

The Alert



10

Results

- Published in the BMJ September 2004
- Dogs correct 41% - compared to 14% by chance
- There were individual differences between dogs, the success rate ranged from 56% in the best performing dogs to 11% in the worst performing dog



11

Conclusions

- Further work is in progress to improve accuracy rates for detection and train dogs to detect other types of human cancer



12

- Dogs are now specifically bred and selected for this role - current dogs in training are young, working strains with proven search and discrimination ability
- Working with scientists to assist the development of an electronic nose.
- Training and research has begun on other forms of disease



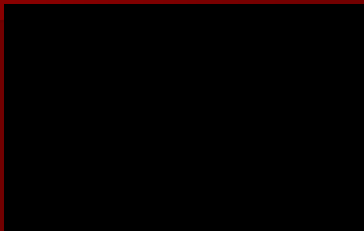
13

- We now have updated equipment and we recently completed a new clinical trials with the Buckinghamshire Hospitals Trust



14

ITN NEWS



15

Medical Assistance Dogs

- In addition to our specialist dogs trained to recognise and detect the distinctive odour of human disease from samples, we also train medical assistance dogs to alert clients who are managing life threatening conditions on a daily basis



16

Diabetes and Hypoglycaemia

- Known as one of the most common chronic health conditions affecting all age groups, diabetes is reaching epidemic proportions, affecting more than 260 million people worldwide



17

Diabetes: a worldwide problem

- Today almost 395,000 children worldwide live with Type 1 diabetes
- Diabetes has become one of the major causes of premature illness and death in most countries impacting heavily on work and productivity in people of working age



18

- The normal metabolic process of releasing glucose from the food intake ensures that the amount of sugar in our blood remains within the region of 5.0-6.0 mmol/l (90-108 mg/dl)
- Although cases are rare it is possible that if blood glucose levels drop low and long enough brain damage can occur. There is also concern about the long term effect of recurrent hypos on the brain

UK reading = mmol/L: Millimoles per litre USA reading = mg/dl: Milligrams per 100 millilitres



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- It has been suggested that repeated severe hypos may be linked to permanent changes in mental functions and cognitive behaviour including mood or personality changes, reduced IQ, memory loss and even dementia
- Many individuals suffer from poor or no hypoglycaemic awareness and therefore are unaware when their blood sugars are dropping dangerously low.



20

Hyperglycaemia

- When individuals become hyperglycaemic (high blood sugars) this is not normally a medical emergency
- However over a longer period this causes significant health problems including sight loss, amputation, heart attack and kidney damage
- Dogs can successfully identify high blood sugars early



21

Diabetes Blood Sugar Detection Dogs



- The charity trained it's first hypo/hyperglycaemic detection dog for a gentleman with poorly controlled Type 1 Diabetes and poor hypoglycaemic awareness in 2008



22

- The dogs are trained to reliably identify and alert when the owner's blood sugar drops to a level that would result in a hypoglycaemic episode or hyperglycaemic episode
(below UK - 4.5mmol/l USA - 81mg/dl)

- The dog accompanies his owner everywhere in order that he can live an independent life



23

Training protocol

- Blood sugar detection dogs can be successfully trained without the need for access to blood
- The dogs are trained following the collection of breath and sweat samples
- Dogs that are trained to alert on an individual will also identify the odour on an unknown person



24

Maintaining healthy blood sugars

- Trained dogs assist in prevention of acute hypoglycaemia episode and paramedic call out and also assist client in reducing blood sugar levels and hyperglycaemic symptoms
- Experts report that severe damage occurs from high blood sugars in the first five to ten years
- Huge impact can be made placing trained dogs with children with brittle diabetes



25

Assisting children with diabetes

- We have now successfully placed two alert dogs with young children and their families
- Noah is a four year old boy who suffers hypoglycaemic episodes regularly and as a result has already experienced two brain seizures



26

Rebecca and Shirley

First assistance dog placed in UK primary school



27

Current placements

- We currently have 14 trained medical-alert dogs and have 4 dogs in training
- We also have a growing waiting list



28

Saving lives on a daily basis



29

Implications of Our Work

- Our data indicates high accuracy in hypo-alert detection and reduction in paramedic attention,
- Assists individual in reducing hyperglycaemic associated conditions
- There is growing evidence of improved stability of client's HbA1C



30

Current work in Progress

- We are now working with endocrinological specialists and researchers at Bristol University to assess sensitivity and reliability of our dogs
- Current indications are that dogs are accurate over 98% of the time and sensitive to a one milimole per litre blood sugar change.



31

Addison's Disease Alert Dog

- We have also successfully trained the first Medical Assistance Dog to alert a client to an oncoming Addison's crisis (life threatening due to dangerously low levels of cortisol)



32

BBC INSIDE OUT



33

New Projects

- We are currently training a dog to assist an individual with severe narcolepsy
- Plans for the future include the training of dogs to detect substances that result in severe allergic response in atopic clients



34



35



"He's a working dog, but I don't know what he does."



36

ICB-2010

Abstracts and Handouts

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