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Evaluation of Maggot Therapy Effects on the Progression of Equine Sarcoid

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Abstract

BACKGROUND: Skin is the largest organ of the body and is of particular importance in the beauty as well as economic value of the horse. Equine sarcoid disease (ESD) is the most common skin tumor in horses. So far, various methods have been used to treat equine sarcoidosis though no fully effective treatment has been proposed till now. Larval therapy has been used for the treatment of human diabetic ulcers as well as horse hoof lesions. Reports on the use of larval therapy to treat the complications of tumor lesions have been promising.

OBJECTIVES: This study aimed to evaluate the effect of larval therapy on equine sarcoid lesions.

METHODS: In this study, 4 horses were diagnosed with equine sarcoid through the clinical examinations and pathological tests, and larval therapy was used to treat the sarcoid lesions. To place the larvae on the lesion, the method of implantation in an artificial nest was used. The number of larvae used per square centimeter was about 10 larvae.

RESULTS: The result of treatment was satisfactory in 2 cases of horses and even in one case, the lesion was largely limited but the skin began to renew. By the way, in general no positive result was seen in the other two cases, which was probably due to the extent of the lesion and the instability of the larvae on it.

CONCLUSIONS: According to the obtained results, it seems that larval therapy is effective for the treatment of sarcoid necrotic lesions, however more studies are needed.

KEYWORDS: Complementary therapy, Horse, Larval therapy, Lucilia sericata, Sarcoid

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Introduction

The appearance of horse skin is of particular importance because of its impact on its economic value. The incidence of skin tumors in horses is relatively high (Knottenbelt, 2009, 2012), and sarcoid is known as the most common skin tumor in horses. This neoplasm is a fibroblastic tumor that is found in both males and females in different breeds of equine, including donkeys and mules. Sarcoid tumor has a great ability to invade the local tissue though it usually does not have a true nature of metastasis (Funiciello and Roccabianca, 2020). Tumors such as squamous cell carcinoma (SCC), papilloma, and fibroma are very similar in appearance to sarcoid and may be confused with it (Jaglan *et al.*, 2018). The onset of sarcoid tumors is often followed by trauma and physical injury and can manifest itself in the healing wounds of horses (Knottenbelt, 2009). In most parts of the world, equine sarcoids are associated with bovine papillomavirus 1 (BPV 1), except in Canada where it is associated with type 2 viruses (Wobeser *et al.*, 2010; Wobeser, 2017). Vascular changes that occur in sarcoid lesions are most likely due to the presence of BPV in the lesions (Martano *et al.*, 2020). One study has shown that there is a familial-genetic predisposition (up to 21%) in the sarcoid disease (Christen *et al.*, 2014). Genetic studies have shown duplication and deletion in some sarcoid cell chromosomes (Bugno-Poniewierska *et al.*, 2016). A recent study has found some evidence on the influence of miRNA on the pathogenesis and aggressive behavior of equine sarcoid cells (Bogedale *et al.*, 2019).

The epidemiology of equine sarcoid is not well understood due to the lack of demographic data (Wobeser, 2017). The prevalence of equine sarcoidosis in horses has been reported to be between 1-8%. Most sarcoid sites include the head, limbs, neck, and shoulders. Horses in the age range of 0.5 to 31 years are majorly affected with sarcoid lesions, and the disease is most common among Quarter, Thoroughbred, and Arab breeds. Moreover, growing mares are at more risk of equine sarcoidosis compared to others (Wobeser *et al.*, 2010).

the equine skin tumors have been studied in Tehran and East-Azarbaijan provinces of Iran, the most common of which are fibroma (38.4%) and sarcoid (34.8%) in horses (Tolouei Kaleibar *et al.*, 2015). There have also been case reports of sarcoid among Caspian horses, donkeys, and ponies from Iran. In most reports, the lesion was observed in the perineal area (Sakha *et al.*, 2011; Farjani Kish *et al.*, 2014; Saadi *et al.*, 2019).

Clinical sarcoid lesions are divided into six groups in terms of clinical form, including: 1, occult form, 2. wart form, 3. nodular form, 4. fibroblastic form, 5. mixed form, and 6. malignant form (tumor cords in the lymphatic vessels). Milder forms of the tumor, especially if traumatized, can quickly become more invasive (Knottenbelt, 2005). The definitive diagnosis of sarcoid is made by histopathological examination (Knottenbelt, 2009). Although many efforts have been made for many years to find a standard treatment for sarcoid, no effective and reliable method has yet been recorded (Taylor and Haldorson, 2013). Hence, it can be worthwhile to try to find new ways to treat ESD.

Larval therapy is a treatment that uses sterile larvae of fly to treat superficial lesions in humans as well as animals. This method has been used for many years to heal superficial wounds such as war wounds (Fleischmann *et al.*, 2004). In 2003, the US Food and Drug Administration (FDA) approved the method proposed by Dr. Sherman (Sherman, 2003). Today, the most used larvae in larval therapy are *Lucilia (Phoenicia) sericata*. This fly belongs to the Calliphoridae family and is more popular because it does not have a destructive attachment style to the living tissue (Kenawy and Abdel-Hamid, 2020). Other therapeutic properties have also been mentioned for larval therapy such as antibiotic and anti-biofilm properties, synergy with other antibacterial, antifungal, and anti-inflammatory agents, immune system modulator, angiogenesis stimulator, fibroblast

growth regulator, coagulation enhancer, nerve repair, anti-atherosclerosis, and anti-tumor properties (Yan *et al.*, 2018; Sherman *et al.*, 2000; Malekian *et al.*, 2019). The use of larvae in the treatment of infectious and diabetic wounds is much faster and sometimes more effective than conventional treatments. Larval therapy has been started by Dr. Mirabzadeh and his colleagues in Iran for several years and is currently being performed in certain centers in Iran (Mirabzadeh *et al.*, 2017).

Maggot therapy in veterinary medicine is not as well-known as that in human medicine, however many studies have been done. Larval therapy significantly accelerates the rate and extent of recovery in osteomyelitis, secondary infections due to chronic laminitis, navicular bursitis, chronic joint infection, equine canker, collateral cartilage necrosis, panniculitis, ulcers, and abscesses (Bell and Thomas, 2001; Morrison, 2005, 2010; Kočišová *et al.*, 2006; Sherman *et al.*, 2007; Jones and Wall, 2008; Choudhary *et al.*, 2016; Fontenot *et al.*, 2018; Durán *et al.*, 2020).

All in all, there are no reliable studies on the treatment of superficial neoplasms with larval therapy, however hopes have been expressed by various authors (Sherman and Shimoda, 2004; Whitaker *et al.*, 2007). In some reports, larval therapy has been used to treat cancerous lesions (Jones *et al.*, 1998; Lin *et al.*, 2015).

Given that these larvae feed on skin lesions and are known as small surgeons of nature, it seemed that there could be the possibility of a positive effect of this type of treatment as a primary or adjunct treatment to sarcoid. Therefore, the present study was designed and performed to investigate the effect of larval therapy on equine sarcoid disease (ESD).

Materials and Methods

Preparation of Larvae

The larvae used in this study were *L. sericata*. The larvae were prepared in sterile packages of 200 pieces in special packages from the Larval Therapy Center of Tehran University of Medical Sciences. Air transport was used to transport the larvae to reduce the time it took for the larvae to reach the patient's bed. During the transport of larvae, the package should be kept at 4°C.

Animals

To find horses with ESD, periodic 6-month examinations were performed for skin lesions in the East-Azarbaijan province of Iran. By clinical examination, follow-up of the appearance of lesions, and performing histopathological tests, four horses were diagnosed with equine sarcoidosis which were selected for this study.

Application of Maggots

Larval therapy was performed for each horse at different times, after attracting the attention of horse owners. The lesion was first flushed with normal saline to remove wound exudates and to delineate the tumor border. There are two ways to place the larvae on the wound: the open method and the basket-enclosed method. Depending on the condition and characteristics of the wound, one of these two methods can be used for maggot therapy (Zubir *et al.*, 2020). In this study, direct implantation of larvae at the lesion site (open method) was used.

About 30 minutes before the beginning of maggot-therapy, the larvae were released out of the refrigerator to adapt to the environment. To limit the larval mission to the desired location, a nest-like environment was made for the larvae using tampons and Locoplast adhesive. The nest was formed for small lesions by cutting the middle of several tampons and for large lesions by stacking tampons together. After making the artificial nest wall, the larvae were washed with some physiological normal saline from a special container and passed through a sterile grid to be placed on the nest.

The larvae were gently taken with surgical forceps and placed on the lesion. Next, a piece of sterile mesh was glued to the tampons using the Leucoplast glue. Using a two-meter bandage, a light bandage was applied to the entire nest to absorb both secretions and as a fall that allowed oxygen to the larvae to be used (Figure 1). It should be noted that closing the larval therapy site should not be too tight or too loose since if it is too tight it will put too much pressure on the larvae and will kill them. And if the bandage is too loose, it will cause instability of the artificial nest of larvae and the nest will break with the slightest movement of the animal.

After 2-4 days from the beginning of larval therapy, depending on the secretions absorbed by the bandage and the condition of the animal to go along with the larval therapy, the bandage was opened and after washing, the lesion was examined. Based on the results and conditions of the animal, it was decided whether re-larval therapy was necessary or the process should be stopped. If the expected improvement was not achieved in one round of larval therapy, it was repeated with the same actions as for the first time. In this study, clinical observation of the lesion was used to evaluate the effects of larval therapy.

Figure 1. Maggot therapy in one of the horses with sarcoid lesions. A: Primary lesion. B: Wash the lesion. C: Rub the zinc oxide cream around of lesion. D: Put a tampon around the lesion. E: Tighten the tampon to the limb. F: Put the larvae on the lesion. G: Grid attaching on the structure. H: Apply a light dressing on the structure of the artificial nest

Results

Out of 136 horses examined in 12 equestrian farms, 5 horses were suspected of having sarcoid lesions, in four of which ESD was confirmed by histopathological examination (Figure 2). All ESDs confirmed in the present study were in fibroblastic form. The percentage of ESD diagnosed in the horses in this study was about 3%.

Larval therapy was completed for two ESD cases. Horse number 1 was a 5-year-old stallion of *Kurd* breed and was white. This horse was kept in the *Shabestar* area and the conditions of the stables were not good. The lesion was located in the distal part of the right hindlimb. Three days after larval therapy, the dressing was removed and it was observed that

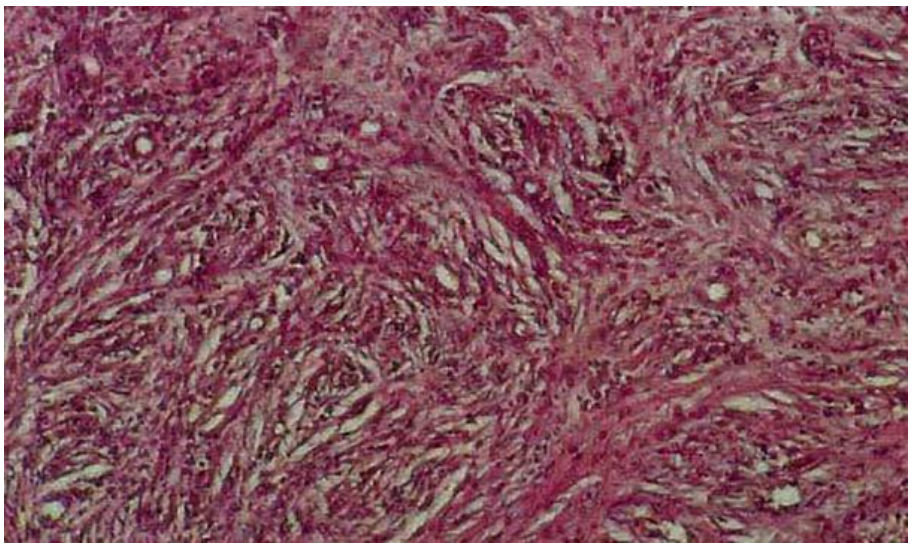

Figure 2. One of the histopathology pictures of ESD horses. The proliferation of dermal fibroblast cells and proliferation of collagen fibers are observed in curvilinear and eddy bands. Epithelial components are rarely seen in this image.

the larvae had grown only slightly larger and had died in their secretions. Two more larval therapies were performed for this horse, which resulted in a great improvement after the third round (Figure 3).

Figure 3. Horse number one before and after maggot therapy

Horse number 2 was an 8-year-old crossbred chestnut horse that was kept in *Gogan* city. The location of the lesion was the end of the horse's right forelimb. When the mares were transported, a wound was created at the site for traction, which later became sarcoid. The dressing was removed three days after larval therapy. Even in this case, the larvae had died after a small increase in the secretions. The secretions, in this case, were more than that in the

previous case and the lesion itself was completely cleared of necrotic and dead tissues. After larval therapy, signs of healthy skin growth were observed around the lesion. After removing the dressing, larval therapy was performed for the second time, in which no significant changes were observed compared to the first round (Figure 4).

Figure 4. Horse number two before and after maggot therapy

In the other two horses with equine sarcoid, there were also lesions in the limbs. In these two horses, due to the greater extent of lesions than the previous two cases, the expected results were not obtained.

Discussion

A field study of 136 horses suspected of having skin lesions in the East-Azarbaijan province of Iran revealed that about 3% of the horses examined for skin lesions had sarcoid lesions. The prevalence of sarcoid in the world has been reported 1-8% in a previous study (Wobeser *et al.*, 2010). In one study, the proportion of equine sarcoid cases from equine tumor lesions was about 34.8% (Tolouei Kaleibar *et al.*, 2015).

The results of larval therapy on equine sarcoid lesions showed that *L. sericata* larvae could help limit ESD complications by superficial debarment of tumor lesions. Necrotic tissues were well cleared and, in some cases, there was a noticeable change in the size and nature of the lesion. The growth of healthy tissue around the lesion appeared to have been increased.

Different treatments and various surgical procedures including conventional surgical resection, laser, and CO2 surgery, cryotherapy, thermotherapy, radiation therapy, chemotherapy, immunotherapy and local immunomodulation, and antiviral drugs have had relative success in ESD treatment (Taylor and Haldorson, 2013). Expensive and not available methods such as radiation therapy have shown good results in research, however they can be used in a limited number of tumors. Moreover, cryosurgery often requires general anesthesia (Knottenbelt, 2009). However, there is no definitive cure for equine sarcoid (Taylor and Haldorson, 2013).

One of the problems with research on equine sarcoid tumors is that horses are large and expensive animals, therefore research studies on large populations of horses are very rare. Hence, a lot of studies are retrospective (Wobeser, 2017). Some experiments are performed in a laboratory environment

(in-vitro) on samples taken from the lesions (Weber *et al.*, 2020).

There are limited studies on the effect of larval therapy on neoplastic lesions. In previous reports, however, good results were not obtained for larval therapy of two equine skin tumors (melanoma and SCC), while a proliferative lesion in horses was completely healed with larval therapy (Lepage *et al.*, 2012).

There are few limited reports on the use of larval therapy for human tumors. In one case, larval therapy was performed for an SCC wound in a human chest. In this case, the odor and appearance of the wound improved after two rounds of larval therapy, but due to pain in the area, larvae therapy was not repeated (Jones *et al.*, 1998). In one report, the use of *L. sericata* for the treatment of a type of tumor called Kaposi's Sarcoma reduced odor and infection, improved blood flow, and prevented amputation. The author considers the use of maggot-therapy beneficial for malignant wounds and recommends that more studies be done in this regard (Lin *et al.*, 2015). In some cases of cutaneous neoplasms such as SCC, associated with myiasis, the tumor has grown slowly. Fly larvae may have positive effects in controlling the growth of tumor lesions (Hawayek and Mutasim, 2006; Wollina, 2010; Biswas and McNerney, 2016). Our findings are in line with previous human medical findings on some of the cancers.

In one study, the anti-tumor and anti-leukemic properties of fatty acids isolated from larval extract were demonstrated. w-6 PUFA was the most abundant fatty acid in larval extract (Jun-Qing, 2008). In another study, larval extract was shown to inhibit hepatoma H22 through the activated mitogenic protein kinase p38 (p38MAPK) pathway in mice (Zhang *et al.*, 2017). Two recent studies carried out on the extracts from the larvae of another Calliphoridae fly (*Chrysomya megacephala*) confirmed the antitumor property of larvae.

According to some scientists, studies on horse skin can be considered a model for human skin lesions (Harman *et al.*, 2019). Therefore, our findings can be the basis for the use of maggot-therapy in the treatment of human skin cancers.

One of the challenges in this study was to place the larvae in restless horses. Manipulation of the dressing by the animal, which was performed even after larval therapy, required constant care of the horse. The slight irritation and itching caused by the larvae can also increase the horse's tendency to manipulate the dressing. In previous reports as well, some horses were discomfort during the maggot-therapy (Sherman *et al.*, 2000; Choudhary *et al.*, 2016). In large lesions, as in the case of horse number 4, the dressing was not tightened well and the instability of the artificial nest affected the performance of the larvae on the lesion. In modern larval therapy, the risk of transmitting infectious agents from the larvae to the patient is very unlikely (Ahmadnejad and Kaboudari, 2020). Another concern about larval therapy is how to deal with flies that produce maggots and the larvae used in wounds, which must be compatible with human and environmental standards (Stadler, 2020).

According to the results obtained in the present study, larval therapy seems to be a good modality to remove necrotic tissue of fibroblastic sarcoid lesions. This therapy can also be facilitated by stimulating the regeneration of healthy skin around the sarcoid lesion. However, it is suggested that further studies be performed on the use of larval therapy for treating various forms of sarcoids and even other neoplastic lesions in horses.

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Conflict of Interest

The authors declared no conflict of interest.

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بررسی تاثیر لارودرمانی بر روند بیماری سارکوئید تکسمیان

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چکیده

زمینه مطالعه: پوست بزرگترین ارگان بدن است و در زیبایی و ارزش اقتصادی اسب اهمیت ویژه ای دارد. سارکوئید شایعترین تومور پوستی در اسب می باشد. تاکنون از روش های مختلفی برای درمان سارکوئید استفاده شده ولی هنوز روش درمانی کاملاً موثری برای آن ارائه نشده است. استفاده از لارودرمانی به عنوان یک روش درمانی جایگزین، در پزشکی و دامپزشکی در حال گسترش است و موفقیت های قابل توجهی در درمان ضایعات و بیماری های پوستی مثل زخم های دیابتیک انسان، ضایعات سُم اسب داشته است. گزارش های استفاده از لارودرمانی برای درمان عوارض ضایعات توموری در انسان امیدوارکننده است.

هدف: در مطالعه حاظر هدف اصلی ما بررسی اثرات لارو درمانی بر ضایعات سارکوئید در اسب ها بود.

روش کار: در این مطالعه از میان اسب های مورد بررسی ۴ مورد با معاینات بالینی و آزمایشات آسیب شناسی، مبتلا به سارکوئید تشخیص داده شدند و از روش لارودرمانی برای درمان ضایعات سارکوئیدی بهره گرفته شد. برای این مطالعه از لاروهای استریل استفاده شد. برای استقرار لاروها بر روی ضایعه، از روش لانه گزینی در لانه مصنوعی استفاده شد. تعداد لاروهای به کار برده شده برای هر سانتیمتر مربع حدوداً ۱۰ لارو بود.

نتایج: نتیجه درمان در ۲ مورد از اسب ها رضایت بخش بود و حتی در یک مورد ضایعه تا حدود زیادی محدود شد و پیدایش پوست سالم شروع شد. در دو مورد نتیجه مثبتی دیده نشد که احتمالا به علت وسعت ضایعه و ناپایداری لاور ها بر روی آن بود.

نتیجه‌گیری نهایی: با توجه به نتایج به دست آمده به نظر میرسد لارودرمانی برای درمان ضایعات نکروتیک سارکوئید موثر می باشد ولی لازم است مطالعات بیشتری در این زمینه انجام گیرد.

واژه‌های کلیدی: اسب، سارکوئید، لارو درمانی، لوسیلیا سریکاتا، درمان مکمل

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