Effective Microbes Accelerate Granulation in Wound Healing Process of Strangles Affected Horses

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ABSTRACT: The study was carried out to explore the role of effective microbes in curing the septic wounds of Strangles disease affected horses. The submaxillary lymph nodes abscessation resulted during the course of disease and ultimately ended into formation of wounds which were subject to septic environment. Adopting conventional methods of treatment i.e. local use of antiseptic preparations and parenteral administration of antibiotics usually takes long time in healing of wounds. At times the horses do not respond to the treatment which results in loss of animal life as well as economic thrashing. Use of effective microbes in animal health is an area which needed exploration. We used effective microbes (EM Technology) orally / locally to cure the septic wounds of Strangle’s affected horses and also evaluated its sensitivity in vitro. We found that Bio Vet an EM Technology product as an adjuvant therapy is highly effective for the treatment of strangle wounds. It considerably reduces the healing time by quick granulation and S. equi (causative organism) was also found sensitive to Bio Vet in vitro.

Key Words; Strangles, Healing, EM Technology, Equines

INTRODUCTION

Strangles is a highly contagious and serious infection of horses and other equines caused by the bacterium, Streptococcus equi (Bazely 1942; Evers et al., 1968; Meyer et al., 1992; Vin et al., 2016). The disease is characterized by severe inflammation of the upper respiratory tract, with extensive swelling / rupture of the lymph nodes, which produces large amounts of thick, creamy pus. If untreated the animal may die (Bone et al., 1963; Anzai et al., 1999). Horses of all ages are susceptible, though strangles is most common in animals less than 5 years of age and especially in groups of weanling foals or yearlings. Foals under 4 months of age are usually protected by colostrum-derived passive immunity (Anzai et al., 2000). S. equi is maintained in the horse population by carrier horses but does not survive for more than 6–8 weeks in the environment. Although the organism is not very robust, the infection is highly contagious. Transmission is either by direct or indirect contact of susceptible animals with a diseased horse. Direct contact includes contact with a horse that is incubating strangles or has just recovered from the infection, or with an apparently clinically unaffected long-term carrier (Libardoni et al., 2016). Indirect contact occurs when an animal comes in contact with a contaminated stable (buckets, feed, walls, doors) or pasture environment (grass, fences, but almost always the water troughs), or through flies (Blunden et al., 1994). Since this is a contagious disease therefore prompt treatment and control is essential to save the other precious horses (Meyer et al., 1992). The mortality rate is more than 8 percent. Effective microbes can play an important role in curing this deadly disease. Susceptible horses develop strangles within 3–14 days of exposure (Meyer et al., 1992). Animals show typical signs of a generalized infectious process (depression, inappetence and fever of 39°C–39.5°C). More typically of strangles, horses develop a nasal discharge (initially mucoid, rapidly thickening and purulent), a soft cough and slight but painful swelling between the mandibles, with swelling of the submxillary lymph node (Boschwitz et al., 1994). This is thought to be the result of partial immunity although...
this may also result from infection by *S. equi* of relatively low virulence (Anzai et al., 1999). Classic strangles is a severe infection that can be fatal, usually because of a variety of complications that occur. The main and often fatal complications of strangles include dissemination of infection to unusual sites like abdominal or lung lymph nodes, inflammation of peripheral blood vessels, anaemia and inflammation of the subcutaneous tissue (Oikawa et al., 2006). The disease has also been reported communicable to humans (Piotr and Anca, 2016). Treatment of a horse in the early stages of strangles is usually effective and is not associated with untoward effects. The causative agent is highly susceptible to Penicillin G and combination of Streptomycin / Penicillin (Mukhtar et al., 1988; Bazely, 1992; Flanagan et al., 1998). Wound healing or wound repair is the body’s natural process of regenerating dermal and epidermal tissue (Adriana et al., 2015). Following wound, a set of complex biochemical events takes place in a closely orchestrated cascade to repair the damage (Lindmark et al., 1996; Garg, 2000; Midwood et al., 2004). In angiogenesis, new blood vessels grow from endothelial cells (Chantsavang and Watcharangkul 2004).

Effective microorganisms are a mixed culture of fermentative, soil based, beneficial microorganisms that can be employed to many environments to improve the health and vitality of water, soil, plants and animals. (Chantsavang et al., 2002; Chantsavang and Watcharangkul, 2004). EM is the fundamental mother culture of new products developed and produced within Australia for microbial balancing in soils and water. EM is a living entity containing active anaerobic and aerobic microbes. The most prominent organisms are photosynthetic bacteria, lactic acid bacteria and yeast (Daly and Stewart, 1999; Timmerman et al., 2006). EM also comprises fermentative fungi and Actinomycetes and has also been trialed on cash crops (Huang, 2016). The uniqueness of microorganisms and their often unpredictable nature and biosynthetic capabilities had made them likely candidate for solving particularly difficult problems in the life sciences and other fields as well. (Anjum, 1999) described a considerable increase in the production of eggs of layers offered with drinking water treated with EM. EM mixed in drinking water decreased mortality up to 35% in broiler chicken. Fecal and litter examination of EM treated layer chicken indicated lower parasitic oocyte count (Chantsavang and Watcharangkul, 2004). Most of the diarrhea cases caused by *E. coli*, typhoid cases by Staphylococcus and respiratory infections due to Pasteuralla can be treated successfully with EM culture. Bio Vet. an EM culture has positive effect on the growth rate of male Sahiwal calves (Maqbool et al., 1999). EM has a prophylactic efficacy against Avian Salmonellosis and average milk production in cattle is significantly increased and mortality is decreased by feeding hay treated with EM (Allaudin et al., 2009). Administration of EM culture orally reduced the incidence of Deg Nurrah disease in buffaloes (Maqbool et al., 1999).

The equine industry is a flourishing area demanding to produce and export world class horses free from diseases. A young horse recovering from strangles is required to be saved against exposure to secondary infections caused by septic sub-maxillary lymph node wounds in the least possible time to check the secondary infections and making them fit for work/export. Foregoing in view we postulate that EM can enhance wound healing process in a dynamic manner by releasing bio active metabolites and competing the septic pathogenic organisms in wounds of strangles affected horses (Fig. 3). Effective microbes can play an important role in curing this deadly disease. Our study is focused on the use of Effective Microbes Technology (EM Technology) as an adjuvant treatment for the horses suffering from strangles.

**Fig 1:** Ruptured and draining submaxillary lymph node

**Fig. 2:** A maturing abscess
MATERIALS AND METHODS

Subjects

Two to three years old thirty uncastrated, thoroughbred horses, suffering naturally from strangles with submaxillary lymph node wounds, and having body weight 280-300 kg without a lifetime history of any infectious/contagious disease are included in the study. The horses made study entry on the day of rupturing submaxillary lymph node abscesses. The horses were selected from Remount Depot Mona and Laboratory procedures were conducted at Biology Research Laboratory of Lahore Garrison University.

Materials

The EM preparation i.e Bio-Vet 20 lit was obtained from University Of Agriculture Faisalabad by the kind courtesy of Dr Azhar Maqbool Chairman Department of Parasitology, University of Veterinary and Animal Sciences, Lahore. The glass ware, autoclave, horse restraining hobbles and other lab facilities were used from Remont Depot Mona and Biology Research Laboratory of Lahore Garrison University.

Study Design

The horses were divided in two groups A and B comprising 15 animals each. Animals of group A were given available standard treatment i.e single I/M injection of Procaine Penicillin 40 lac IU mixed with 01gm Streptomycin given at the dose of 5 gm per animal for consecutive 7 days at 0900 hrs daily.

The wounds of these animals were cleaned daily with 1% copper sulphate solution thrice a day at 1000 hrs, 1800 hrs and 0200 hrs till scar formation of wound.

Animals of group B were given additional 100 ml of Bio-Vet (EM solution) orally with stomach tube and cleaning of wounds only by 40 ml of Bio-Vet Solution at the same timing as of group A.

Healing of wounds of both groups was monitored daily at 1800 hrs for scar formation by means of physical observation of growing granulation tissue.

For culture sensitivity tests nostrils discharge samples were taken aseptically from the wounds of all thirty horses of both groups by sterilized swabs during initial three days of study, which then were dipped in Nutrient broth and transported to laboratory at 25°C for sensitivity analysis and grown on culture media. The findings were recorded as very sensitive (++++) clear zone of inhibition more than 30 mm diameter, sensitive (+++) clear zone of inhibition 25-30 mm diameter, moderately sensitive (++ zone of inhibition 15-25 mm diameter with 1 or 2 colonies in the zone of inhibition, less sensitive (+) zone of inhibition 5-15 mm diameter with a few colonies of resistant microorganisms and not sensitive (-) zone of inhibition less than 5 mm diameter with many colonies of resistant microorganisms were recorded.

RESULTS

Wound Healing Duration

Average healing time of wounds among group A animals was found to be 14.4 days whereas in group B it was observed to be 8.26 days. The group B administered with Bio-Vet indicated decreased healing time which is the result of accelerated healing process as shown in Fig. 4.
Sensitivity Effect

*S. equi* was found equally sensitive to Bio-Vet compared with combination of Penicillin/Streptomycin (Combiotic) because there was no significant difference in sensitivity results, however Tetracyclin and Ampicillin were found having minimum antibacterial activity against *S. equi* as indicated in Fig. 5.

**Fig 5:** Sensitivity comparison of *S. equi* to Tetracycline, Combiotic, Ampicillin and Bio-Vet.

DISCUSSION

Up till now no adequate research work has been carried out for the reason that more interest for the use of beneficial microorganisms has been shown in agriculture sector. In the present study the period of healing of submaxillary lymph node wounds of strangles affected horses was reduced by treating with combiotic (Penicillin+Streptomycin) alongwith oral and local administration of Bio-Vet an EM product.

In livestock the use of Bio-Vet is successfully done in poultry and farm animals. Maqbool et al., (2001) studied the effect of effective microorganisms in controlling the most important disease in cattle i.e. "Deg Nala" and experienced that the disease was very common in areas where rice straw was being used as hay for animals to feed. They concluded that in a therapeutic trial effective microorganism were given orally (2% solution) and a vasodilator (Nitroglycerine ointment) applied locally on the lesions affected the highest percentage (95%) cure rate. However in present study 100 ml Bio-Vet orally and 40 ml for the local treatment were used that gave 100% cure rate within average period of 8.26 days. Our study commensurate with these findings.

Gut flora and EM have potential to affect health and disease far beyond the gut (Takai et al., 2000). There is increasing evidence that EM have beneficial effects in preventing a wide range of conditions and improving health. We observed that in a combination with combiotic the cure rate was 100%. This study suggests that the oral administration of EM improves the gut flora and their substrates play an important role in animal health most probably through boosting the immune system or direct antibacterial activity, for which a separate study is awaited.

In the field of animal production the beneficial effects of EM on many aspects of animal production systems has been widely demonstrated in China. However, the exact mechanisms of how EM, once ingested, elicits beneficial effects on animal health, growth and metabolism is not known. To study the effect of EM without Combiotic was not found feasible keeping in view of the uncertain fate of precious horses, however the same may now be done in other animals. Improvement of microflora of the gut is also suggestive that EM can be used in preventing various infectious diseases in animals and probably in humans too.

The examination of the slides made from culture revealed Gram positive cocci and arranged in drain of various lengths. Colonies were small, smooth glistening drop like growth on nutrient agar as also observed by (Walker et al., 2002; Wizeman et al., 2001). Bio-Vet also showed excellent sensitivity against *S. equi*. Rehman et al., (2012) studied 5 different effective microorganisms against various pathogenic bacteria in-vitro and it showed good antibacterial activity against all the bacteria under study. Overall results of EM culture which are biologically active against the microorganisms suggest that skin wound infection due to *Staphylococcus* spp; and respiratory infection due to *Pasteurella* spp; can be treated with beneficial microorganisms.

The antibacterial activity of Bio-Vet against *S. equi* in early healing of strangles affected submaxillary lymph node wounds is suggestive of the sterilizing role of lactic acid produced by EM thus accelerating the granulation process and inhibiting the proliferation of pathogenic bacteria to
which the wound is exposed.

**CONCLUSION**

Based on the results of this study, it is concluded that Bio-Vet (EM) has a positive role in curing the strangles affected submaxillary lymph node wounds. The improvement of gut microflora resulting into beneficial metabolites and bio active substances like vitamins etc and release of lactic acid by EM are the plausible mechanisms of speedy granulation by which the wounds are healed in less than 10 days. Bio-Vet and Combiotic have synergistic activity against *S. equi*. The use of Bio-Vet in horses is quite safe and free of side effects, therefore can be used for improving their health condition. Wounds can easily be treated by oral administration of Bio-Vet with local treatment, thus reducing the expenditure incurred on antibiotics. The market price of Bio-Vet is only rupees 500 per gallon. Present study also justifies that Bio-Vet may be utilized and introduced in the country on a wide scale for the improvement of animals health.

**REFERENCES**


