



COMPARATIVE EFFICACY OF PYTHIUM OLIGANDRUM, THRUSH POWDER AND MAGNESIUM SULPHATE TO TREAT THRUSH IN HORSES

Dr. Ali Qaisar^{1*}, Dr. Amber Fatima², Dr. Naveed Hussain³, Dr. Mahnoor Khan Jamali⁴, Dr. Sohaib Hassan⁵, Dr. Abdul Aziz⁶, Dr. Aresha Arkan⁷, Dr. Hamad Bin Rashid^{8*}

^{1*, 2, 3, 4, 5, 7, 8*}Department of Veterinary Surgery, University of Veterinary and Animal Sciences, Lahore - Pakistan.

⁶Department of Veterinary Clinical Sciences, University of Poonch, Rawalakot - Pakistan.

***Corresponding Authors:** Dr. Hamad Bin Rashid, Dr. Ali Qaisar

*Associate Professor, Department of Veterinary Surgery, University of Veterinary and Animal Sciences, Lahore – Pakistan **Email:** hamad.chaudhry@uvas.edu.pk

*Department of Veterinary Surgery, University of Veterinary and Animal Sciences, Lahore – Pakistan **Email:** aliqaisar143@gmail.com

Abstract

Horse thrush is a frequent ailment that affects the frog of the foot and, if left untreated, can result in disability. Using eighteen afflicted horses, this study evaluated three treatments: Pythium oligandrum (Ecosin), magnesium sulfate and thrush powder. Complete blood count (CBC), lameness score, and hoof clinical examination were components of the evaluation. Treatment impact analysis was done using GLM and One-way ANOVA. When compared to magnesium sulfate and thrush powder, ecosin shown a significant ($P < 0.05$) improvement in healing duration and lameness score. With the exception of platelets ($P = 0.004$) that preferred Ecosin over magnesium sulfate, CBC values did not differ substantially ($P > 0.05$) between treatments. In comparison to the other therapies, Ecosin showed early infection removal and faster thrush healing overall.

Keywords: Thrush, Horse hoof, Treatment, Pythium oligandrum, Healing observations

1. INTRODUCTION

Livestock is a major sub-sector of agriculture in Pakistan being backbone of economy, contributes almost 56% in agriculture and about 11% to GDP. Livestock pays a major share to value added agriculture services. In order to highlight actual performance of livestock, study sight seen the relationship between agriculture and livestock product outcome. Pakistan is gifted with various livestock genetic resources. Pakistan has a huge population of livestock that is well adapted to our local environmental suitable conditions. Current population of horses in Pakistan accounts up-to 0.4 million numbers (Anonymous 2019).

“No hoof, no horse” is a common phrase that is widespread in equine industry. Equine hoof is an important structure when it comes to the anatomy of equine leg. A robust structure which makes it able to support travel of up to 70 km per day, dating back to 5000 – 6000 years ago, when the domestic use of horses started (Back & Clayton 2001). As the health of hoof is associated with general health and well-being of the animal, there are many understudied pathologies that affect the

hoof health in equines. Thrush, laminitis and navicular disease are of particular concern hoof pathologies associated with domestic horses (Stoltz 2018).

Among livestock, horses are mostly used for transportation as well as riding. They are also used for pulling carts, carrying things, to help in fields in agriculture. People have cast-off choosy breeding to make heavy horses to do work more efficiently (Aiello et al. 2016). There are many diseases of horses which are affecting riding and transport. Most of which includes thrush, salmonellosis, quitter, anthrax, brucellosis, ringworm, eastern equine encephalitis, St. Louis encephalitis, cryptosporidiosis and also tick-borne diseases.

The health of the equine hoof is closely tied to the animal's overall condition and well-being. Despite this, hoof pathology remains relatively understudied. Laminitis, thrush, and navicular disease are three common disorders which impact domestic horses and donkeys. Laminitis can present itself in three different forms: acute, mechanical, and endocrine. Acute and endocrine laminitis are related to hormone imbalances in the body, specifically those regulating sugar consumption and insulin production.

Thrush is the destruction of a portion of the horse's hoof called the frog, caused by *Spherophorus necrophorus*. It is the devastation of a portion of the hoof of horse, which is called the frog, may be caused by a fungi and anaerobic bacteria. There will be strong foul odour from the affected area of frog. It is common condition, if left unattended, then may penetrate to the sensitive part of hoof leading to temporary to permanent lameness (Azargoon et al. 2011). It is an ailment often left untreated as it does not appear to be a particularly concerning problem for affected horses. However, horses which go years without successful treatment often develop the more severe white line disease. With current practices encouraging the use of ineffective and drastic shoeing practices, many horses never find relief.

The most understudied of these maladies is navicular disease. Without a clear understanding as to how to diagnose the disease, many horses are left for years with mystery lameness. Even once the horse has been diagnosed with navicular disease, the causes are understudied and there is minimal action that owners can take to help their horse. This diseased condition is often attributed to muddy and dirty sand condition. It is not only caused by water but also mixed with moister along with dirty pens. If thrush is treated early or at initial stages, its cure rate is higher and economical. Thrush is an infective condition of the frog and its sulci, which results in degeneration of the horn (the protective frog callous) and the production of foul smelling grey/black discharge (Aiello and Mays 2011).

Fusobacterium necrophorum is a gram negative obligate anaerobe which normally thrives in hooves (collateral groove and central sulcus) and horse's environment, particularly if it is wet, insanitary such as dirty stable or muddy paddock. Neglected hygienic practices, poor hoof hygiene like standing in urine or manure soiled bedding are common contributing factors for thrush (Stashak 2013).

Thrush in horses can be treated with a germ-killing product, such as iodine or bleach and water mix, diluted 50-50. A biological product mixed with water and used as a water spray or may be used in a water bath. Promoting natural microbial growth on hoof, skin is prone to the chafing, treating sign and symptoms of thrush in horses. It can also be successfully treated with application of triple phosphate powder containing equal amount of ferrous sulphate, copper sulphate and magnesium sulphate. Total 8-10 applications of packed bandage were applied on the hoof. The frog grooves were clearly visible after treatment and normal without noticeable pain.

Magnesium sulphate in combination with amoxicillin may be used for the treatment of thrush in horses which have good treatment results. For prevention of thrush, best offense is a virtuous defense. We can say that proactive farm management and prevention is crucial. We can control thrush by proper sanitation. Damp and dark environment is beneficial for thrush causative bacteria to survive. In view of above statements about thrush, the present study will be designed to treat the thrush in horse.

With current practices that encourages ineffective and drastic shoeing, the problem remains unsolved and untreated for many horses. Shoeing (to hold the foot together) done with good intentions, unintentionally, seals the bacteria in the hoof often leads to progression of disease rather

than regression. As the bacteria can manifest on hoof capsule, when improper and inadequate trimming or shoeing is done it leads to long contracted heels and deep sulci, which renders the hoof to work incorrectly such as unable to pop off dirt and other particulate debris out of foot sole, bacteria began to thrive. Some other causes other than insanitary conditions involve lack of exercise as many domestic horses are kept in stalls for a longer time, thus preventing their movement which leads to accumulation of debris and dirt in the sole for longer period of time (Petrov 2013).

2. REVIEW OF LITERATURE

The hoof is a vital part of the horse's musculoskeletal system, as it is an integral weight-bearing digit of the limb. Horse hoof is composed of outer keratinous part, the hoof capsule and an inner living part, containing soft tissues and bone. Hoof problems usually lead to development of lameness and pain in horses & ponies. Among these thrush and canker are very common. Thrush is an infective condition of the frog and its sulci, which results in degeneration of the horn (the protective frog callous) and the production of foul-smelling gray/black discharge (Aiello et al. 2011). Here, we define traditional way of successful treatment of this condition in a clinically affected pony. After treatment the pony was put on to normal work. The clinical sign as observed in this case report were principally indicative of thrush condition. Horses reared on wet and dirty bedding mostly suffer from thrush (Stashak 2013).

2.1. Hoof Thrush:

Hoof thrush is chronic bacterial disease and occurs at sole or horn of the hoof. In early stages, it is characterized by malodorous and black purulent discharge originating within central or collateral sulci of frog of the hoof (Reeves et al. 1989; Ross & Dyson, 2011). Although it causes minimal external damage and can be only visualized while trimming, yet if the horses suffering from it tend to go years without successful treatment often develop more severe white line disease, spreading to white line, sole and sensitive layers of foot, resulting in permanent lameness. Seedy toe or white line disease is a term which is used to highlight separation between hoof wall and sole at white line, which in later stages of disease gets filled with malodorous white chalky tar-like residual substance. The infection can spread to any part of the wall from toe to heel (Mansman 2007). Thrush is a degenerative infection affecting frog area of horse's hoof, leading to necrosis and damage of the soft tissues (Stashak 2013). *Fusobacterium necrophorum* is the most common anaerobic bacteria associated with thrush (Petrov and Dicks 2013). The secondary bacterial infection is common at the damaged area. Anaerobic bacterial infection is most predominant, and these bacteria are ubiquitous in horse faeces and soil (Sanjay et al. 2014).

2.2. Conventional treatment:

The living environment of a horse is the most important for perpetuation of these anaerobic bacteria responsible for this condition. This pony was being maintained within the premises of the house of the owner without proper hygienic and sanitary conditions. The floor on which this pony was being reared was totally muddy and soiled with faeces. All these management conditions might be responsible for thrush in this pony. The hooves of this affected pony were trimmed by the local person having little knowledge on the equine farriery. Regular and accurate hoof trimming is very important for prevention of this condition and helps in maintaining soundness of the hoof. The treatment protocol comprised of debridement of unnecessary tissue of frog in the central sulcus, of hoof and is topically treated with 7% of iodine or any other commercially available products. Central sulcus was flossed with iodine-soaked gauze make sure the debridement of deep central sulcus tissue. Depending on the severity of the thrush, it sometimes essential to apply a treatment-plate shoe to prevent contamination and recontamination off hoof and daily treatment is continued (Agne 2010). Laminitis that occurs secondary to the inflammatory processes has its severity related to the underlying disease and may lead to loss of hoof and death of the animal (Laskoski et al. 2015).

2.3. Hoof Hygiene:

Hindering oxygen flow to the frog tissue and nearby areas provide an environment for development of thrush. Packed debris and use of oil and grease at hoof tissue blocks the oxygen availability to hoof. Strong astringents such as copper sulfate, formaldehyde and chlorine are corrosive to live tissues. These drugs de-nature proteins in the external layer of hoof and reduce oxygen penetration to hoof tissue (Kumar et al. 2014). High humidity or wet environments pre-dispose horses to thrush. Once the organisms begin dividing in the frog sulci, the stage is set for a progressive invasion and subsequent infection of the frog tissue (Holzhauer et al. 2017).

Chronic thrush is intensely rooted and is impossible to kill with one strong topical agent application. Repeated usage of these chemicals can be destructive to adjacent healthy hoof tissues and delay healing. The researchers established a formula that aims fungi and bacteria without damaging sensitive part of hoof tissue. The product is not corrosive and can be used more often necessary to keep thrush under control conditions and also allow hoof tissue to prosper and thrive (Stoltz 2018).

Irrespective of thrush therapy that is mostly used, an important step for its control is to trim and clean the hoof. Debridement of dead tissues and exposing the frog flap by the farrier helps the horse in rapid recovery (Pollard et al. 2019). Improper trimming of hooves limits oxygen entry into the crevices. Timely cleaning prevents hooves from thrush and white line disease.

Four steps for treating deeply rooted thrush: wash the hoof, pick and brush, wipe and dry and apply Thrush Stop. Effective thrush prevention involves a combination of maintaining a clean, dry (but not too dry) environment. Cleaning of the hooves is done on routine basis. Proper nutrition and supplementation can create denser and healthier frog and sole tissue that is more resistant to infection. Regularly apply a non-caustic hoof topical or clay to maintain a healthy hoof (Al-Agele et al. 2019).

2.4. Thrush powder:

Thrush powder comprised of three different components which are ferric sulphate, zinc sulphate and copper sulphate. Copper sulphate is a deodorant and hence acts as fly repellent. Beside this, copper sulphate is a strong vascular endothelial growth factor (VEGF) and promotes angiogenic responses at wounded area (Sen et al. 2002 ; Voelker 2000). Ferric sulphate is an effective hemostatic agent, and also has strong astringent property (Azargoon et al. 2011). The normal treatment protocol includes removal of degenerated frog tissues, cleaning with disinfectants (iodine/tincture of iodine) or packing the sulci with cotton soaked in 10-15 percent sodium sulphapyridine solution, until infection is controlled (Stashak 2013). In this clinical case, we used indigenous method of treatment by using triple sulphate, which is easily available.

Probably all these properties of these triple sulphate worked together and synergistically helped in curing the thrush and regeneration of dead tissues and reshaping the frog soft tissue. This is the first report regarding the successful treatment of thrush by application of triple sulphate powder and will be useful to field veterinarian and equine owners (Shin et al. 2020).

2.5. Magnesium sulphate:

Wet and humid conditions provide suitable environment to *Fusobacterium necrophorum* bacteria to proliferate due to low oxygen availability and hoof hygiene. Risk of infection increase due to improper or inadequate trimming of hooves and shoeing, resulting in infection in deep sulci and long contracted heels (Abidina et al. 2013).

Magnesium sulphate is commonly known as Epsom salt and has been used in the treatment of war wound since a longtime (Morison 1918). Magnesium is involved in more than 300 essential metabolic reactions and magnesium sulphate is a strong hygroscopic salt, which helped in removal of degenerative tissues and debris and speeded up the healing process. In case of thrush, the necrosed area of corn and frog is debrided. The saturated solution of Epsom salts is used to sock the foot in it. Copper sulphate, chlorohexidine and also 2% Iodine are applied as topical agent on daily basis (O'Grady 2018).

2.6. *Pythium oligandrum*:

Pythium oligandrum is a strong biocontrol agent. It is a non-pathogenic soil-inhabiting oomycete that facilitates the root ecosystem of crop species. *P. oligandrum* distinguishes itself from the other species of *Pythium* by protecting the plants from biotic stresses and its ability to facilitate the plant growth (Benhamou et al. 2012). This microorganism shows tough myco-cellulases. *P. oligandrum* shows great anti-parasitic activity against dermatophytosis and it shows great results in the improvement of skin health and cure from disease (Načeradská et al. 2017). At the site of penetration, it acts by forming several papilla-like structures that goes deep human and veterinary medicine (Gabrielová et al. 2018). The results started to show in 2nd week in which there is a lot of improvement with no side effect to animal health or body condition as compared to other conventional topical antifungal products (Načeradská et al. 2021).

2.7. Lameness grading:

As hoof need to be trimmed on regular basis to prevent any irregular growth of hoof and to avoid any other complication like thrush which is caused by improper hoof trimming or late trimming as during this time dung or manure remain stuck in the hoof which is the main source of infection. In all major hoof ailments, thrush is one of the common problem which have higher prevalence of lameness (Kiros et al. 2016). Lameness depicts the soundness and unsoundness of horse. In case of thrush the lameness scoring was done and mainly its in higher percentage falls in Grade 2 lameness while it's also causes the lameness up-to Grade 3 (Fisahaye et al. 2018 ; Tadesse et al. 2019).

2.8. STATEMENT OF PROBLEM

Thrush is a common condition, if left untreated then it may lead to temporary to permanent lameness. The main objective of the study was: To compare the efficacy of *Pythium oligandrum*, thrush powder and magnesium sulphate for the treatment of thrush in horses.

3. MATERIALS AND METHODS

3.1. Experimental Animals and Station:

The study was conducted on horses at Indoor Surgery Clinic, Department of Veterinary Surgery and Pet Sciences, University of Veterinary and Animal Sciences, as well as in and around Lahore.

3.2. Experimental Design:

A total of eighteen (n=18) horses suffering from thrush were selected and categorized into three groups each having six horses. The groups were designated as A, B and C.

3.3. Inclusion Criteria:

Horses of various ages and breeds were included in the study which were suffering from thrush. These horses did not have any structural deformity.

3.4. Therapeutic Trials:

Each horse suffering from thrush were administered anti-tetanus toxoid (ATT) prior to therapeutic trials. No other systemic drug was injected during the therapeutic trials as shown in table 3.1.

Table 3.1. Therapeutic trails of drugs in different groups

Group	Drug used	Duration of Medication	Route of Medication
A	<i>Pythium oligandrum</i> (Ecosin)	Thrice per week for three weeks	Topical
B	Thrush powder	Daily for three weeks	Topical
C	Magnesium sulphate	Daily for three weeks	Topical

3.5. *Pythium oligandrum* (Ecosin):

Pythium oligandrum is basically a fungus which acts as a myco-parasite for more than 50 different species which also include bacterial activity. It releases various hydrolytic enzymes to break the life cycle of bacteria and fungi. For the therapeutic trial, one (1) tablet of 3g was dissolved into two liters of Luke warm water in the bucket and let the ingredient to activate for 20-30 minutes. Meanwhile the horse was restrained and hooves were thoroughly cleaned and washed. The affected hoof was soaked in the bucket containing the dissolved tablet for 20 minutes. After completion of the time, the hoof was allowed to dry naturally and the procedure was repeated three times a week till the trail duration. Half of the hoof was soaked into the bucket.



Figure 3.1. Picture shows the Ecosin tablet



Figure 3.2. Dipping being done in Luke warm water in bucket containing Ecosin



Figure 3.3. Hoof placed in the water containing the Ecosin

3.6. Thrush powder:

Thrush powder comprises of copper sulphate, zinc sulphate and ferrous sulphate and is used as a conventional method for the treatment of thrush. Each ingredient has its own properties. Copper sulphate acts as a strong vascular growth factor and has angiogenic properties that promotes blood supply in the area and thus heals the tissue. It also acts as a deodorant and fly repellent. Zinc sulphate contains zinc which acts as a trace element in the body and helps in improving the health of hoof tissue by nourishing and healing it. Zinc sulphate acts as strong bactericidal as it degrades the cell structure of bacteria thus creating the hygienic environment in the hoof. Ferrous sulphate is used as an astringent agent that promotes the blood supply in the affected area and thus promotes healing. It also has hemostatic properties. Thrush powder used in the current study (Ferrous sulphate-Sigma Aldrich + Copper sulphate-Sigma Aldrich + Zinc sulphate-Sigma Aldrich) was purchased from the local medicine market and mixed by adding equal quantity of all the agents. Horse was restrained and hoof cleaning was performed. The mixture was applied on the ground surface of the hoof thoroughly. Gauzes were placed over the mixture and bandaging was done to keep the medicine in contact with hoof surface. It was done on daily basis during the therapeutic trial.



Figure 3.4. Picture shows Sigma-Aldrich Zinc Sulphate included in Thrush Powder



Figure 3.5. Picture shows Sigma-Aldrich Iron Sulphate included in Thrush Powder



Figure 3.6. Picture shows Sigma-Aldrich Copper Sulphate included in Thrush Powder



Figure 3.7. Application of thrush powder on the horse hoof.



Figure 3.8. Application of gauze for covering the thrush powder and for maintaining the contact with the skin

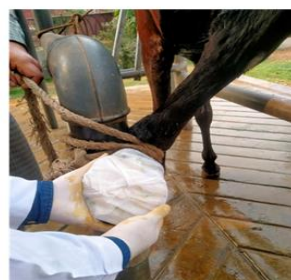


Figure 3.9. Bandaging the hoof for maintaining the contact time of the thrush powder

3.7. Magnesium sulphate:

Magnesium sulphate also known as Epsom salt. Magnesium helps in more than 300 vital metabolic reactions the body. Magnesium sulphate is a hygroscopic salt and helps in the debridement of dead tissue, promoting the circulation towards the hoof and thus decreases the healing duration. Magnesium sulphate (Magnesium Sulphate-Sigma Aldrich) which was used in the trial was purchased from the local medicine market. Horse was restrained and hoof cleaning was done. Magnesium sulphate was applied over the affected area and gauze was applied to keep it maintain to place. Bandaging was done to cover the hoof thoroughly and procedure was done on daily basis during the trial period.



Figure 3.10. Picture shows Sigma-Aldrich Magnesium Sulphate included in Thrush Powder



Figure 3.11. Application of magnesium sulphate on the frog area of hoof



Figure 3.12. Gauze application over the magnesium sulphate to keep it with the skin



Figure 3.13. Bandaging the hoof to maintain the contact time of drug with the frog surface

Table 3.2. Lameness Scoring for grading of gait abnormalities

Score	Description
0	Lameness is not detectable under any conditions
1	Lameness is hard to monitor and is not detectable under any conditions(i.e. circling, hard surfaces, inclines and under saddle)
2	Lameness is hard to monitor during walk or straight line trotting but constantly perceptible under various conditions(i.e. circling, inclines, weight bearing and hard surfaces)
3	Lameness is constantly observable at a trot under all conditions
4	Lameness is observable during walk
5	Lameness result in minimal weight bearing during motion or complete lack of ability to move

3.8. Statistical Analysis:

One-way ANOVA and General Linear Model were used to measure the effect of treatment. Analysis was performed using SPSS version 24 (Daniel and Cross 2018).

4. Results

The hoof is a vital part of the horse's musculoskeletal system, as it is an integral weight-bearing digit of the limb. Horse hoof is composed of outer keratinous part, the hoof capsule and an inner, living part, containing soft tissues and bone. Hoof problems usually lead to development of lameness and pain in horses & ponies. Among these thrush and canker are very common. Thrush is an infective condition of the frog and its sulci, which results in degeneration of the horn (the protective frog callous) and the production of foul smelling grey/black discharge (Aiello and Mays 2011). Horses reared on wet and dirty bedding mostly suffer from thrush (Satashak 2013). Here, we define traditional way of successful treatment of this condition in a clinically affected pony. After treatment the pony was put on to normal work. Table 4.1 shows the overview of treatment applied for thrush in hoof.

Table 4.1 Overview of the treatment applied for hoof thrush

Group	Drug used	Duration of Medication	Route of Medication
A	Pythium oligandrum (Ecosin)	Thrice per week for three weeks	Topical
B	Thrush powder	Daily for three weeks	Topical
C	Magnesium sulphate	Daily for three weeks	Topical

4.1. Evaluation parameters

The evaluation of hoof determines the effectiveness of trails and drugs used throughout the time in research. Various evaluation parameter was used to determine the effectiveness of treatment and best cure for the thrush in horse hoof.

4.1.1 Healing Duration:

Hoof recovery depends upon the time for the tissue to heal completely and it represent the healing duration during the treatment protocol and its effectiveness in healing the tissue. Table 4.2 shows the mean and standard deviation between different treatment groups. In the table, Group A is treated with Ecosin treatment, Group B treated with thrush powder and Group C in which Magnesium sulphate treatment was used. The mean and Standard deviation value of Healing Duration of group A was 17.50 ± 3.834 . While for Group B the healing duration was 32.67 ± 10.539 . In case of Group C, it was 31.50 ± 9.649 in the healing duration.

Table 4.2. The mean and standard deviation between different treatment groups in lameness scoring

Groups	No. of animals						Healing Duration
	1	2	3	4	5	6	Mean \pm S.D
A	14 days	21days	14 days	21 days	21 days	14 days	17.50 ± 3.834
B	35 days	28 days	42 days	35 days	14 days	42 days	32.67 ± 10.539
C	21 days	35 days	42 days	21 days	28 days	42 days	31.50 ± 9.649
P-Value (0.05)							

In the table 4.3, Group A was treated with Ecosin, Group B was treatment with Thrush powder and Group C that was treated with Magnesium sulphate. In the comparison between different treatment groups, Group A was found statistical significant ($P \leq 0.05$) to the Group B and Group C. While Group B was statistical significant ($P \leq 0.05$) to Group A but non-statistical significant with Group C ($P \geq 0.05$). The comparison of Group C was statistical significant ($P \leq 0.05$) to Group A but non-statistical significant with Group C ($P \geq 0.05$).

Table 4.3. The comparison in the healing duration between different treatment groups

Group	Comparison Groups	Mean Difference ±Std. Error	P-Value
A	B	-15.167±4.931	0.020
	C	-14.000±4.931	0.031
B	A	15.167±4.931	0.020
	C	1.167±4.931	0.970
C	A	14.000±4.931	0.031
	B	-1.167±4.931	0.970

4.1.2. Physical appearance:

Hoof is the vital organ of horse body and is the sensitive one. Any damage or ailment to hoof result in unsoundness of horse and horse will be unable to perform routine activities. Various treatment protocols were used for the treatment of hoof to see efficiency of treatment and healing duration in the hoof of the horse.

- i. Trial with *Pythium oligandrum* (Ecosin)
- ii. Trial with Thrush Powder
- iii. Trial with Magnesium sulphate



Figure 4.1. On Day 0, Picture shows degenerative frog having greyish discharge from the frog area and putrefied odour



Figure 4.2. On day 7, there was no greyish discharge from the frog area but frog area was soft and delicate ge from the frog area andputrefied odour



Figure 4.3. On day 14, the frog started to re-grow and no discharge or odour from the hoof



Figure 4.4. On day 21, picture shows regaining if frog shape and frog became healthy and is not soft as before



Figure 4.5. On day 28, frog is growing normally with no discharge or ailment



Figure 4.6. On day 35, picture shows normal health of hoof with frog gaining natural shape



Figure 4.7. On day 42, hoof is fully healthy with natural appearance and no signs of thrush

i. Trial with Thrush Powder'



Figure 4.8. Thrush in horse hoof resulting in softening of frog and discharge with putrefied smell at day 0



Figure 4.9. At day 7 Frog started dry and no discharge with putrefied odour



Figure 4.10. On day 14, Frog was dry and started to harden and there is no discharge or odour



Figure 4.11. On day 21, frog started to come in shape and was healthy



Figure 4.12. On day 28, there is no sign of thrush in the hoof and frog tissue was healthy and growing



Figure 4.13. On day 35, horse was normal is all sort of activity having healthy frog and normal tissue growth



Figure 4.14. On Day 42, Picture shows normal hoof structure with no discharge or odour

ii. Trail with Magnesium Sulphate



Figure 4.15. Frog of the hoof was degenerated having blackish discharge and foul smell on day 0



Figure 4.16. On day 7, there was slight improvement in frog as discharge reduced but the smell was present



Figure 4.17. On day 14, There is no foul smelling discharge from the frog area of hoof but frog was softer on palpation



Figure 4.18. On Day 21, Frog started to re-grow but soft on palpation and discharge was clear



Figure 4.19. Frog started to regain its natural shape and was harder in consistency with no discharge or foulsmell on Day 28



Figure 4.20. Hoof have got normal anatomy as frog was in natural appearance and consistency

4.2. Complete Blood Count:

Complete blood count (CBC) plays an important role in accessing the animal health status. It was performed to see effect of treatments during the trial period and improvement in values during the time period of treatment as shown in table 4.4.

In the table 4.4, Group A with treatment of Ecosin, Group B treated with thrush powder and Group C in which Magnesium sulphate was used. At Day 0, Mean and Standard deviation value of TLC of group A was 9.48±0.943. At Day 7, it slightly decreased to 7.80±1.175. At Day 14, it was noted 9.25±4.066. At Day 21, it was declined to 8.32±2.828. At Day 0, Mean and Standard deviation value of TLC of group B was 9.13±0.885. At Day 7, it slightly decreased to 7.50 ± 1.1094. At Day 14, it was noted 10.02±4.460. At Day 21, it was declined to 8.56 ± 2.542. At Day 0, Mean and Standard deviation value of TLC of group C was 8.75 ± 1.378. At Day 7, it slightly decreased to 6.57 ± 1.428. At Day 14, it was noted 7.00 ± 2.628. At Day 21, it was declined to 7.37 ± 2.542.

Table 4.4. The mean difference and standard deviation between treatment group in total leukocyte count(TLC)

Groups	Days	No. of animals						Total Leukocyte Count (TLC)
		1	2	3	4	5	6	Mean ± SD
A	0	8.6	9.8	9.6	9.9	10.8	8.2	9.48±.943
	7	8.2	8.8	8.3	8.5	7.4	5.6	7.80±1.175
	14	8	16.5	11.2	7.9	5.2	6.7	9.25±4.066
	21	8.13	11.7	10.3	9.3	3.7	6.8	8.32±2.828
B	0	8.8	8.3	9.3	10.8	9	8.6	9.13±.885
	7	5.4	7.6	8	7.4	8.4	8.2	7.50±1.094
	14	10	10.8	18.2	5.2	7.7	8.2	10.02±4.460
	21	8.3	9.6	11.7	3.7	9.9	8.13	8.56±2.706
C	0	7.2	10.8	7.5	8.9	8.3	9.8	8.75±1.378
	7	5.2	7.4	4.6	8.2	7.6	6.4	6.57±1.428
	14	4.7	5.2	4.4	7.9	10.8	9	7.00±2.628
	21	6.1	3.7	5.8	10.1	9.6	8.9	7.37±2.542
P-Value (0.05)								

In the table 4.5, Group A was treatment with Ecosin, Group B was treated with Thrush powder and Group C that was treatment with Magnesium sulphate. In the comparison between different treatment groups, Group A was found non-statistical significant (P≥0.05) with Group B and Group C. While Group B was also non-statistical significant (P≥0.05) with Group A and Group C. The comparison of Group C with both two groups was found non-statistical significant (P≥0.05). So there was non-statistical significance (P≥0.05) between all treatment groups in case of total leukocyte count (TLC).

Table 4.5. The comparison between total leukocytes count between different treatmentgroups

Groups	Comparison groups	Total Leukocyte Count(TLC)	P-Value
		Mean Difference ± Std. Error	
A	B	-0.9±.713	0.992
	C	1.29±.713	0.174
B	A	0.09±.713	0.992
	C	1.38±.713	0.138
C	A	-1.29±.713	0.174
	B	-1.38±.713	0.138

In the table 4.6, A represent the Group A with treatment of Ecosin, B represents the Group B treated with thrush powder and C represent the Group C in which Magnesium sulphate was used. At Day 0,

Mean and Standard deviation value of RBCs of group A was 6.43 ± 0.527 . At Day 7, it slightly increased to 6.50 ± 1.105 . At Day 14, it was noted 7.61 ± 0.811 . At Day 21, it was declined to 7.23 ± 0.741 . At Day 0, Mean and Standard deviation value of RBCs of group B was 6.31 ± 0.845 . At Day 7, it was noted 6.31 ± 1.151 . At Day 14, it was increased to 7.01 ± 1.287 . At Day 21, it was declined to 6.74 ± 0.471 . At Day 0, Mean and Standard deviation value of RBCs of group C was 6.26 ± 0.764 . At Day 7, it slightly increased to 6.36 ± 1.030 . At Day 14, it was noted 7.13 ± 1.300 . At Day 21, it was declined to 7.06 ± 0.479 .

Table 4.6. The mean difference and standard deviation between treatment group in Red Blood Cell (RBC)

Groups	Days	No. of animals						Red Blood Cells(RBC)
		1	2	3	4	5	6	Mean \pm SD
A	0	5.96	6.7	5.92	7.31	6.5	6.2	6.43 ± 0.527
	7	6.85	6	4.68	6.64	8.02	6.8	6.50 ± 1.105
	14	6.81	7.64	6.8	7.54	8.99	7.9	7.61 ± 0.811
	21	7.2	7.13	8.5	6.4	6.63	7.5	7.23 ± 0.741
B	0	6.97	4.91	6.2	6.5	7.31	5.96	6.31 ± 0.845
	7	5.23	4.85	6.3	8.02	6.64	6.85	6.31 ± 1.151
	14	6.04	5.28	7.36	8.99	7.54	6.85	7.01 ± 1.287
	21	6.02	7.1	7.09	6.63	6.4	7.2	6.74 ± 0.471
C	0	6.3	6.5	6.77	7.1	4.91	6	6.26 ± 0.764
	7	6.58	8.02	6.43	6.4	4.85	5.9	6.36 ± 1.030
	14	7.94	8.99	6.36	7.5	5.28	6.7	7.13 ± 1.300
	21	7.44	6.63	6.61	6.8	7.1	7.8	7.06 ± 0.479
P-Value (0.05)								

In the table 4.7, A represent the Group A was treatment with Ecosin, B represents the Group B was treated with Thrush powder and C represent the Group C that was treatment with Magnesium sulphate. In the comparison between different treatment groups, Group A was found non-statistical significant ($P \geq 0.05$) with Group B and Group C. While Group B was also non- statistical significant ($P \geq 0.05$) with Group A and Group C. The comparison of Group C with both two groups was found non-statistical significant ($P \geq 0.05$). So there was non-statistical significance ($P \geq 0.05$) between all treatment groups in case of Red Blood Cells (RBCs).

Table 4.7. The comparison between Red Blood Cells between different treatment groups

Groups	Comparison Groups	Red Blood Cells (RBCs)	P-Value
		Mean Difference \pm Std. Error	
A	B	0.35 ± 0.266	0.394
	C	0.24 ± 0.266	0.646
B	A	-0.35 ± 0.266	0.394
	C	-0.11 ± 0.266	0.908
C	A	-0.24 ± 0.266	0.646
	B	0.11 ± 0.266	0.908

In the table 4.8, A represent the Group A with treatment of Ecosin, B represents the Group B treated with thrush powder and C represent the Group C in which Magnesium sulphate was used. At Day 0, Mean and Standard deviation value of Hb of group A was 11.87 ± 1.226 . At Day 7, it slightly decreased to 11.37 ± 1.597 . At Day 14, it was noted 11.72 ± 0.084 . At Day 21, it was slightly declined to 11.66 ± 1.239 . At Day 0, Mean and Standard deviation value of Hb of group B was 11.68 ± 1.461 .

At Day 7, it decreased to 10.55 ± 2.249 . At Day 14, it was noted 11.75 ± 2.151 . At Day 21, it was decreased to 11.08 ± 1.121 . At Day 0, Mean and Standard deviation value of Hb of group C was 12.18 ± 1.541 . At Day 7, it decreased to 11.35 ± 2.008 . At Day 14, it was increased to 11.67 ± 2.568 . At Day 21, it was noted 11.53 ± 0.937 .

Table 4.8. The mean difference and standard deviation between treatment group in Haemoglobin (HB)

Groups	Days	No. of animals						Haemoglobin (HB)
		1	2	3	4	5	6	Mean \pm SD
A	0	10.9	12.2	9.9	13.2	12.4	12.6	11.87 ± 1.226
	7	12.8	10.9	8.7	11.3	13.2	11.3	11.37 ± 1.597
	14	12	11.9	7.8	12.3	14.1	12.2	11.72 ± 0.84
	21	13.3	12.36	10.7	10.5	10.5	12.6	11.66 ± 1.239
B	0	12.9	9.3	11.4	12.4	13.2	10.9	11.68 ± 1.461
	7	8.3	7.9	9.8	13.2	11.3	12.8	10.55 ± 2.249
	14	11.2	7.8	12.3	14.1	12.3	12.8	11.75 ± 2.151
	21	10.3	10.8	11.1	10.5	10.5	13.3	11.08 ± 1.121
C	0	12.6	12.4	12.8	12.1	9.3	13.9	12.18 ± 1.541
	7	11.3	13.2	10.9	13.5	7.9	11.3	11.35 ± 2.008
	14	12.2	14.1	9.5	14.3	7.8	12.1	11.67 ± 2.568
	21	12.6	10.5	11	12.7	10.8	11.6	11.53 ± 0.937
P-Value (0.05)								

In the table 4.9, A represent the Group A was treatment with Ecosin, B represents the Group B was treated with Thrush powder and C represent the Group C that was treatment with Magnesium sulphate. In the comparison between different treatment groups, Group A was found non-statistical significant ($P \geq 0.05$) with Group B and Group C. While Group B was also non- statistical significant ($P \geq 0.05$) with Group A and Group C. The comparison of Group C with both two groups was found non-statistical significant ($P \geq 0.05$). So there was non-statistical significance ($P \geq 0.05$) between all treatment groups in case of Haemoglobin (Hb).

Table 4.9. The comparison between Haemoglobin (HB) between different treatment groups

Groups	Comparison Groups	Haemoglobin (Hb)	P-Value
		Mean Difference \pm Std. Error	
A	B	$0.39 \pm .506$	0.727
	C	$-0.03 \pm .506$	0.998
B	A	$-0.39 \pm .506$	0.727
	C	$-0.42 \pm .506$	0.690
C	A	$0.03 \pm .506$	0.998
	B	$0.42 \pm .506$	0.690

In the table 4.10, A represent the Group A with treatment of Ecosin, B represents the Group B treated with thrush powder and C represent the Group C in which Magnesium sulphate was used. At Day 0, Mean and Standard deviation value of PCV of group A was 40.55 ± 4.796 . At Day 7, it increased to 48.02 ± 7.099 . At Day 14, it was noted 54.13 ± 10.908 . At Day 21, it was decreased to 51.62 ± 3.209 . At Day 0, Mean and Standard deviation value of PCV of group B was 39.88 ± 5.233 . At Day 7, it increased to 47.45 ± 7.508 . At Day 14, it was noted 52.20 ± 11.081 . At Day 21, it was noted 50.82 ± 3.408 . At Day 0, Mean and Standard deviation value of PCV of group C was 40.22 ± 4.738 . At Day 7, it was recorded 45.92 ± 6.699 . At Day 14, it was noted 52.08 ± 11.369 . At Day 21, it was declined to 50.33 ± 2.787 .

Table 4.10. The mean difference and standard deviation between treatment group in Packed Cell Volume (PCV)

Groups	Days	No. of animals						Packed Cell Volume (PCV)
		1	2	3	4	5	6	Mean \pm SD
A	0	45.6	38.5	32.1	44	41.6	41.5	40.55 \pm 4.796
	7	53.9	46.3	36.1	49.1	56.3	46.4	48.02 \pm 7.099
	14	52.9	51.2	35.1	58.7	67.2	59.7	54.13 \pm 10.908
	21	53.2	55.2	51	48.7	47.2	54.4	51.62 \pm 3.209
B	0	39.8	31.1	37.2	41.6	44	45.6	39.88 \pm 5.233
	7	39.6	37.6	48.2	56.3	49.1	53.9	47.45 \pm 7.508
	14	45.1	35.1	53.2	67.2	58.7	53.9	52.20 \pm 11.081
	21	47.7	52.6	55.5	47.2	48.7	53.2	50.82 \pm 3.408
C	0	41.5	41.6	42.7	44.6	31.1	39.8	40.22 \pm 4.738
	7	46.4	56.3	47.5	48.1	37.6	39.6	45.92 \pm 6.699
	14	59.7	67.2	49.4	56	35.1	45.1	52.08 \pm 11.369
	21	54.4	47.2	50.4	49.7	52.6	47.7	50.33 \pm 2.787
P-Value (0.05)								

In the table 4.11, A represent the Group A was treatment with Ecosin, B represents the Group B was treated with Thrush powder and C represent the Group C that was treatment with Magnesium sulphate. In the comparison between different treatment groups, Group A was found non-statistical significant ($P \geq 0.05$) with Group B and Group C. While Group B was also non- statistical significant ($P \geq 0.05$) with Group A and Group C. The comparison of Group C with both two groups was found non-statistical significant ($P \geq 0.05$). So there was non-statistical significance ($P \geq 0.05$) between all treatment groups in case of Packed Cell Volume (PCV).

Table 4.11. The comparison between Packed Cell Volume (PCV) between different treatment groups

Groups	Comparison Groups	Packed Cell Volume (PCV)	P-Value
		Mean Difference \pm Std. Error	
A	B	0.99 \pm 2.084	0.883
	C	1.44 \pm 2.084	0.769
B	A	-0.99 \pm 2.084	0.883
	C	0.45 \pm 2.084	0.975
C	A	-1.44 \pm 2.084	0.769
	B	-0.45 \pm 2.084	0.975

In the table 4.12, A represent the Group A with treatment of Ecosin, B represents the Group B treated with thrush powder and C represent the Group C in which Magnesium sulphate was used. At Day 0, Mean and Standard deviation value of MCV of group A was 64.90 \pm 6.066. At Day 7, it increased to 74.32 \pm 3.389. At Day 14, it was noted 74.10 \pm 3.798. At Day 21, it was 74.77 \pm 2.087. At Day 0, Mean and Standard deviation value of MCV of group B was 63.60 \pm 6.831. At Day 7, it increased to 75.53 \pm 3.024. At Day 14, it was noted 74.15 \pm 4.414. At Day 21, it was inclined to 75.93 \pm 2.886. At Day 0, Mean and Standard deviation value of MCV of group C was 61.58 \pm 2.555. At Day 7, it inclined to 73.73 \pm 2.903. At Day 14, it was noted 74.48 \pm 4.173. At Day 21, it was 75.07 \pm 2.790.

Table 4.12. The mean difference and standard deviation between treatment group in Mean Corpuscle Volume (MCV)

Groups	Days	No. of animals						Mean Corpuscle Volume (MCV)
		1	2	3	4	5	6	Mean \pm SD
A	0	76.6	61.4	65.7	60.3	64	61.4	64.90 \pm 6.066
	7	78.7	75.3	77	74	70.3	70.6	74.32 \pm 3.389
	14	77.7	70.1	68.9	77.9	74.8	75.2	74.10 \pm 3.798
	21	76.4	75.2	76.4	76.1	71.3	73.2	74.77 \pm 2.087
B	0	57.2	63.4	60.1	64	60.3	76.6	63.60 \pm 6.831
	7	75.9	77.7	76.6	70.3	74	78.7	75.53 \pm 3.024
	14	74.7	66.5	72.3	74.8	77.9	78.7	74.15 \pm 4.414
	21	79.3	74.2	78.3	71.3	76.1	76.4	75.93 \pm 2.886
C	0	61.4	64	63.2	60.3	63.4	57.2	61.58 \pm 2.555
	7	70.6	70.3	73.9	74	77.7	75.9	73.73 \pm 2.903
	14	75.2	74.8	77.8	77.9	66.5	74.7	74.48 \pm 4.173
	21	73.2	71.3	76.3	76.1	74.2	79.3	75.07 \pm 2.790
P-Value (0.05)								

In the table 4.13, A represent the Group A was treatment with Ecosin, B represents the Group B was treated with Thrush powder and C represent the Group C that was treatment with Magnesium sulphate. In the comparison between different treatment groups, Group A was found non-statistical significant ($P \geq 0.05$) with Group B and Group C. While Group B was also non- statistical significant ($P \geq 0.05$) with Group A and Group C. The comparison of Group C with both two groups was found non-statistical significant ($P \geq 0.05$). So there was non-statistical significance ($P \geq 0.05$) between all treatment groups in case of Mean Corpuscle Volume (MCV).

Table 4.13. The comparison between Mean Corpuscle Volume (MCV) between different treatment groups

Groups	Comparison Groups	Mean Corpuscle Volume (MCV)	P-Value
		Mean Difference \pm Std. Error	
A	B	-0.28 \pm 1.151	0.967
	C	0.80 \pm 1.151	0.765
B	A	0.28 \pm 1.151	0.967
	C	1.09 \pm 1.151	0.614
C	A	-0.80 \pm 1.151	0.765
	B	-1.09 \pm 1.151	0.614

In the table 4.14, A represent the Group A with treatment of Ecosin, B represents the Group B treated with thrush powder and C represent the Group C in which Magnesium sulphate was used. At Day 0, Mean and Standard deviation value of MCH of group A was 18.55 \pm .589. At Day 7, it slightly decreased to 16.92 \pm 1.111. At Day 14, it was noted 16.33 \pm .841. At Day 21, it was increased to 17.15 \pm 1.596. At 0 Day, Mean and Standard deviation value of MCH of group B was 18.48 \pm .397. At Day 7, it decreased to 16.58 \pm 1.114. At Day 14, it was noted 16.73 \pm 1.563. At Day 21, it was 16.37 \pm 1.078. At Day 0, Mean and Standard deviation value of MCH of group C was 18.63 \pm .378. At Day 7, it decreased to 16.57 \pm .516. At Day 14, it was noted 15.88 \pm 1.401. At Day 21, it increased to 16.33 \pm .715.

Table 4.14. The mean difference and standard deviation between treatment group in Mean Corpuscle Haemoglobin (MCH)

Groups	Days	No. of animals						Mean Corpuscle Haemoglobin (MCH)
		1	2	3	4	5	6	Mean \pm SD
A	0	18.2	18.1	19.5	18	19	18.5	18.55 \pm .589
	7	18.6	15.2	17.2	17	16.4	17.1	16.92 \pm 1.111
	14	17.6	16.9	16.3	16.3	15.6	15.3	16.33 \pm .841
	21	18.1	15.8	19.9	16.4	15.8	16.9	17.15 \pm 1.596
B	0	18.5	18.9	18.3	19	18	18.2	18.48 \pm .397
	7	15.8	16.2	15.5	16.4	17	18.6	16.58 \pm 1.114
	14	18.5	14.7	16.7	15.6	16.3	18.6	16.73 \pm 1.563
	21	17.1	15.2	15.6	15.8	16.4	18.1	16.37 \pm 1.078
C	0	18.5	19	18.9	18	18.9	18.5	18.63 \pm .378
	7	17.1	16.4	16.9	17	16.2	15.8	16.57 \pm .516
	14	15.3	15.6	14.9	16.3	14.7	18.5	15.88 \pm 1.401
	21	16.9	15.8	16.6	16.4	15.2	17.1	16.33 \pm .715
P-Value (0.05)								

In the table 4.15, A represent the Group A was treatment with Ecosin, B represents the Group B was treated with Thrush powder and C represent the Group C that was treatment with Magnesium sulphate. In the comparison between different treatment groups, Group A was found non-statistical significant ($P \geq 0.05$) with Group B and Group C. While Group B was also non- statistical significant ($P \geq 0.05$) with Group A and Group C. The comparison of Group C with both two groups was found non-statistical significant ($P \geq 0.05$). So there was non-statistical significance ($P \geq 0.05$) between all treatment groups in case of Mean Corpuscle Haemoglobin (MCH).

Table 4.15. The comparison between Mean Corpuscle Haemoglobin (MCH) between different treatment groups

Groups	Comparison Groups	Mean Corpuscle Haemoglobin (MCH)	P-Value
		Mean Difference \pm Std. Error	
A	B	0.20 \pm .297	0.788
	C	0.38 \pm .297	0.406
B	A	-0.20 \pm .297	0.788
	C	0.19 \pm .297	0.804
C	A	-0.38 \pm .297	0.406
	B	-0.19 \pm .297	0.804

In the table 4.16, A represent the Group A with treatment of Ecosin, B represents the Group B treated with thrush powder and C represent the Group C in which Magnesium sulphate was used. At Day 0, Mean and Standard deviation value of MCHC of group A was 27.23 \pm 3.516. At Day 7, it slightly decreased to 24.28 \pm 3.571. At Day 14, it was noted 25.18 \pm 5.827. At Day 21, it was 25.77 \pm 4.937. At Day 0, Mean and Standard deviation value of MCHC of group B was 30.60 \pm 4.248. At Day 7, it declined to 22.05 \pm 1.479. At Day 14, it was noted 23.35 \pm 1.662. At Day 21, it was recorded 23.13 \pm 3.886. At Day 0, Mean and Standard deviation value of MCHC of group C was 31.05 \pm 1.746. At Day 7, it decreased to 26.58 \pm 5.292. At Day 14, it was noted 23.92 \pm 7.045. At Day 21, it was inclined to 28.03 \pm 7.001.

Table 4.16. The mean difference and standard deviation between treatment group in Mean Corpuscle Haemoglobin Concentration (MCHC)

Groups	Days	No. of animals						Mean Corpuscle Haemoglobin Concentration (MCHC)
		1	2	3	4	5	6	Mean \pm SD
A	0	23.9	21.8	28.5	30	29	30.2	27.23 \pm 3.516
	7	23.7	20.3	31	23	23.4	24.3	24.28 \pm 3.571
	14	22.6	24.1	36	20.9	27.1	20.4	25.18 \pm 5.827
	21	24.1	22	33.2	21.5	30.7	23.1	25.77 \pm 4.937
B	0	32.4	29.9	30.6	29.8	37	23.9	30.60 \pm 4.248
	7	20.9	21	20.3	23.4	23	23.7	22.05 \pm 1.479
	14	24.8	22.2	23.1	20.9	25.4	23.7	23.35 \pm 1.662
	21	21.5	20.5	20	22.2	30.5	24.1	23.13 \pm 3.886
C	0	30.2	29.8	29.9	30	34	32.4	31.05 \pm 1.746
	7	24.3	23.4	22.9	25	37	26.9	26.58 \pm 5.292
	14	20.4	20.9	13.2	27	32	30	23.92 \pm 7.045
	21	23.1	22.2	21.8	35	38	28.1	28.03 \pm 7.001
P-Value (0.05)								

In the table 4.17, A represent the Group A was treatment with Ecosin, B represents the Group B was treated with Thrush powder and C represent the Group C that was treatment with Magnesium sulphate. In the comparison between different treatment groups, Group A was found non-statistical significant ($P \geq 0.05$) with Group B and Group C. While Group B was also non- statistical significant ($P \geq 0.05$) with Group A and Group C. The comparison of Group C with both two groups was found non-statistical significant ($P \geq 0.05$). So there was non-statistical significance ($P \geq 0.05$) between all treatment groups in case of Mean Corpuscle Haemoglobin Concentration (MCHC).

Table 4.17. The comparison between Mean Corpuscle Haemoglobin Concentration (MCHC) between different treatment groups

Groups	Comparison Groups	Mean Corpuscle Haemoglobin Concentration (MCHC)	P-Value
		Mean Difference \pm Std. Error	
A	B	0.83 \pm 1.321	0.804
	C	-1.78 \pm 1.321	0.375
B	A	-0.83 \pm 1.321	0.804
	C	-2.61 \pm 1.321	0.126
C	A	1.78 \pm 1.321	0.375
	B	2.61 \pm 1.321	0.126

In the table 4.18, A represent the Group A with treatment of Ecosin, B represents the Group B treated with thrush powder and C represent the Group C in which Magnesium sulphate was used. At Day 0, Mean and Standard deviation value of Plt of group A was 121.83 \pm 36.717. At Day 7, it slightly decreased to 120.83 \pm 30.779. At Day 14, it was noted 131.67 \pm 42.420. At Day 21, it was inclined to 139.33 \pm 27.990. At Day 0, Mean and Standard deviation value of Plt of group B was 106.00 \pm 28.643. At Day 7, it decreased to 97.83 \pm 16.412. At Day 14, it was noted 109.00 \pm 16.935. At Day 21, it was inclined to 119.33 \pm 13.967. At Day 0, Mean and Standard deviation value of Plt of group C was 112.67 \pm 35.427. At Day 7, it declined to 89.83 \pm 36.690. At Day 14, it was noted 102.17 \pm 30.162. At Day 21, it was declined to 90.33 \pm 37.766.

Table 4.18. The mean difference and standard deviation between treatment group in Platelets (Plt)

Groups	Days	No. of animals						Platelets (Plt)
		1	2	3	4	5	6	Mean \pm SD
A	0	98	191	100	116	132	94	121.83 \pm 36.717
	7	76	152	112	111	160	114	120.83 \pm 30.779
	14	65	164	132	142	183	104	131.67 \pm 42.420
	21	88	168	146	130	150	154	139.33 \pm 27.990
B	0	83	100	84	94	116	159	106.00 \pm 28.643
	7	87	80	113	86	100	121	97.83 \pm 16.412
	14	99	79	120	114	121	121	109.00 \pm 16.935
	21	101	110	131	134	130	110	119.33 \pm 13.967
C	0	94	94	91	114	100	183	112.67 \pm 35.427
	7	85	86	77	51	80	160	89.83 \pm 36.690
	14	104	114	92	81	68	154	102.17 \pm 30.162
	21	64	82	100	62	72	162	90.33 \pm 37.766
P-Value (0.05)								

In the table 4.19, A represent the Group A was treatment with Ecosin, B represents the Group B was treated with Thrush powder and C represent the Group C that was treatment with Magnesium sulphate. In the comparison between different treatment groups, Group A was found non-statistical significant ($P \geq 0.05$) with Group B but statistically significant ($P \geq 0.05$) with Group C. While Group B was non-statistical significant ($P \geq 0.05$) with Group A and Group C. Group C was statistical significant ($P \geq 0.05$) with Group A but non-statistical significant ($P \geq 0.05$) with Group B.

Table 4.19. The comparison between Platelets (Plt) between different treatment groups

Groups	Comparison Groups	Platelets (Plt)	P-Value
		Mean Difference \pm Std. Error	
A	B	20.38 \pm 8.893	0.065
	C	29.67 \pm 8.893	0.004
B	A	-20.38 \pm 8.893	0.065
	C	9.29 \pm 8.893	0.552
C	A	-29.67 \pm 8.893	0.004
	B	-9.29 \pm 8.893	0.552

4.1. Lameness Scoring:

Lameness scoring play an important role to access the soundness of horse. Thrush is detrimental disease in which horse become lame and unsound. Gait scoring was done to access the improvement in gait during the course of treatment in case of different treatment protocols. Table 4.6 shows the gait analysis scoring.

In the table 4.20, A represent the Group A, B represents the Group B and C represent the Group C. At Day 0, mean gait score of group A was 2.33 \pm 1.033. At Day 14, it slightly decreased to 1.00 \pm 1.169. At Day 28, it was noted 0.33 \pm 0.516. At Day 42, it was declined to 0.17 \pm 0.408. At Day 0, mean gait score of group B was 3.00 \pm 0.894. At Day 14, it slightly decreased to 1.83 \pm 1.169. At Day 28, it was noted 0.83 \pm 0.983. At Day 42, it was declined to 0.50 \pm 0.837. At Day 0, mean gait score of group C was 2.33 \pm 1.211. At Day 14, it slightly decreased to 1.00 \pm 0.894. At Day 28, it was noted 0.83 \pm 0.753. At Day 42, it was declined to 0.33 \pm 0.516.

Table 4.20. Mean difference and standard deviation between different treatment groups on gait scoring

Groups	Days	No. of animals						Gait score
		1	2	3	4	5	6	Mean \pm SD
A	0	2	4	3	1	2	2	2.33 \pm 1.033
	7	1	4	2	1	2	1	1.83 \pm 1.169
	14	0	3	1	1	1	0	1.00 \pm 1.095
	21	0	2	0	0	1	0	0.50 \pm .837
	28	0	1	0	0	1	0	0.33 \pm .516
	35	0	1	0	0	0	0	0.17 \pm .408
	42	0	1	0	0	0	0	0.17 \pm .408
B	0	3	2	4	3	2	4	3.00 \pm .894
	7	3	2	4	2	1	3	2.50 \pm 1.049
	14	2	2	3	1	0	3	1.83 \pm 1.169
	21	2	1	3	0	0	2	1.33 \pm 1.211
	28	1	0	2	0	0	2	0.83 \pm .983
	35	1	0	2	0	0	2	0.83 \pm .983
	42	0	0	2	0	0	1	0.50 \pm .837
C	0	1	3	4	1	2	1	2.33 \pm 1.211
	7	1	3	4	1	2	1	2.33 \pm 1.211
	14	1	2	3	1	1	1	1.67 \pm .816
	21	0	1	2	0	1	0	1.00 \pm .894
	28	0	1	2	0	1	0	0.83 \pm .753
	35	0	1	1	0	0	0	0.50 \pm .548
	42	0	0	1	0	0	0	0.33 \pm .516
P-Value (0.05)								

In the table 4.21, A represent the Group A treated with Ecosin, B represents the Group B treated with Thrush powder and C represent the Group C that was treated with Magnesium sulphate. In the comparison between different treatment groups, Group A was found statistical significant ($P \leq 0.05$) to the Group B while with Group C it was non-statistical significant ($P \geq 0.05$). While Group B was statistical significant ($P \leq 0.05$) to Group A but non-statistical significant with Group C ($P \geq 0.05$). The comparison of Group C with both two groups was found non-statistical significant ($P \geq 0.05$).

Table 4.21. Table shows the comparison between treatment groups A, B and C in gait scoring

Groups	Comparison Groups	Mean Difference \pm Std. Error	P-Value
A	A	0.64 \pm .201	0.005
	B	-0.38 \pm .201	0.145
B	A	0.64 \pm .201	0.005
	C	0.26 \pm .201	0.397
C	A	0.38 \pm .201	0.145
	B	-0.26 \pm .201	0.397

DISCUSSION

Hoof infections in horses are very common problem. Most of the infections are mild and can be treated without any high cost treatment, although some cases result in chronic lameness and can be life threatening. The hoof is argumentatively the most important component of a horse's anatomy. It protects the internal structures of the foot such as the coffin bone and other soft structures. The major components of the hoof are the wall, coronary band, frog, white line and sole.

5.1. Hoof Thrush:

In this study, the occurrences of the most common hoof disorders of horses were evaluated and identified potential risk factors associated with their occurrence. The occurrence of equine hoof thrush at the time of regular hoof maintenance was 85%. However, the majority of the lesions observed were mild and the horses were clinically healthy. The information was only available for horses that were regularly trimmed by farriers as compared to horses of which the hooves are rarely trimmed. Various factors like improper hoof trim, placing the horses at dirty stables and no proper cleaning of hooves result in the development of the problem. Holzhauser (2017) stated that hoof condition started improving with proper barn cleaning and regular cleaning and trimming of hoof. The secondary bacterial infection is common at the damaged area in which anaerobic bacterial infection is most predominant, and these bacteria are ubiquitous in horse faeces and soil as stated by (Sanjay et al. 2014). In the study by Petrov and Dicks (2013), *Fusobacterium necrophorum* is the most common anaerobic bacteria associated with thrush in horses resulted by dirty stables. The current study was in support as by keeping the place clean during the treatment trial the healing duration was reduced and resulted in early recovery of hoof tissue as compared to dirty pens. It helped in maintaining the hoof hygiene because thrush is a disease caused by poor hygienic condition of hoof due to wet bedding and manure. Hoof hygiene, regular trimming and stall cleaning is the basic key to prevent thrush occurrence in horses.

Pythium oligandrum (Ecosin) showed good result on skin and in topical application but there were no particular studies available on the treatment of hoof thrush by this medicine. Gabrielová (2018) stated that it acts by forming several papilla-like structures that goes deep to the fungal hyphae and initiate the enzymatic degradation activity which facilitate in active growth by providing carbon sources and antagonism development. It is basically parasitic fungus which result in release of certain enzymes that result in degradation of bacteria, fungi and other microbes life cycles. It is frequently used for the treatment of dermatophytosis in animals and shows good result and rapid recovery can be seen in 2 weeks of application stated by (Načeradská et. al. 2017 ; Načeradská et. al. 2021) and the current study is also in agreement, as it resulted in early recovery as compared to other two therapeutic trials and also other conventional methods. It resulted in the elimination of putrefied smell and discharge from the frog area in 3-4 treatments and promoted the frog area to become dry and gain its shape portraying the hoof health. The enzymatic activity of the *Pythium oligandrum* helped in maintaining good hoof hygiene. In most of the cases complete recovery was seen in 21days post-treatment on alternative days irrespective of the depth of infection.

In the study by (Kumar et al. 2014) triple sulphate including ferrous sulphate, zinc sulphate and copper sulphate were used in equal portions and after proper hoof trim and removing of dead tissue, the thrush powder applications were performed. After 8-10 bandaging the recovery was seen and horse became sound. Shin (2020) stated that all these properties of these triple sulphates worked together and synergistically helped in curing the thrush and regeneration of dead tissues and reshaping the frog soft tissue. The current study was in support of the mentioned study. Using pure salts in thrush powder and doing 8-10 applications on daily basis by maintaining the premises clean and maintain hoof hygiene the healing is good with thrush powder and it accelerate with time. Hoof clean and trimming was thoroughly done before it to remove dead part of frog area that helped to improve the contact of thrush powder with the hoof surface thus promoting the healing. But if the problem is old and infection is deeply imbedded then it takes more time to heal even up to 34-42 days.

O'Grady (2018) in his study used the Epsom salt and its effect on the hoof for treatment of thrush. The necrosed area was debrided from the frog surface and hoof is thoroughly cleaned by placing in the saturated solution of Epsom salts. Morison (1918) stated that it was used in the war wounds and resulted in the debridement and healing of the frog tissue. In the current study, the hoof was trimmed and cleaned thoroughly. Then application of magnesium sulphate was done on the frog surface of daily basis and then bandaging was done to maintain the contact time with the frog surface for absorption and healing. It appeared less affective as compared to thrush powder.

Lameness due to thrush is one of the main issue and Kiros (2016) stated that thrush is the most

common problem having higher occurrence of lameness. In the study conducted by (Fisahaye et. al. 2018), Grade 1 lameness mainly caused by hoof overgrowth and hoof crack. Grade 2 was mostly linked with hoof crack, hoof over growth and thrush. Grade 3 and 4 lameness was mostly due to thrush and trauma. In case of thrush, it was most likely to linked with poor hoof hygiene and improper hoof trim that resulted in anaerobic environment in the hoof and thus proliferation of the organism. In the current study most of the horses during the grading fell in Grade 2 and Grade 3 at the time of start of therapeutic trials. The animals treated with Ecosin showed early recovery from lameness as compared to thrush powder and magnesium sulphate. Group A was treated with Ecosin, Group B was treatment with Thrush powder while Group C that was treated with Magnesium sulphate. Group A was found statistical significant ($P \leq 0.05$) to the Group B while with Group C it was non-statistical significant ($P \geq 0.05$). While Group B was statistical significant ($P \leq 0.05$) to Group A but non-statistical significant with Group C ($P \geq 0.05$). The comparison of Group C with both two groups was found non-statistical significant ($P \geq 0.05$).

Complete blood count depicts the status of infection or any change in blood parameters during the disease and during treatments. There was no previous study available on thrush that included complete blood count as a parameter. In the current study, CBC showed no significance in the hoof infection and healing of tissue. All the three groups were found non-statistically significant ($P \geq 0.05$) during the 4 weeks of sampling and treatment trails.

Lundeberg et. al. (2017) reported that healing duration or the recovery from lameness depended upon various factors including trimming and shoeing, cleaning of hoof, moisture content and diet. Proper hoof trimming and maintaining hoof hygiene by daily cleaning and keeping relatively low moisture level in the hoof results in early healing of tissue and restricts the growth of harmful pathogens that affect the hoof health as stated by Popescu (2013). Healing duration in the current study was depended on the factor duration of infection and initiation of the treatment trial. During the current study, animals treated with Ecosin showed early recovery as compared to thrush powder and magnesium sulphate alone, as it promoted the frog growth after 4-5 treatments. Thrush powder worked better than magnesium sulphate in the healing time. In the comparison between different treatment groups, Group A was found to be statistical significant ($P \leq 0.05$) than Group B and Group C. While Group B was statistical significant ($P \leq 0.05$) to Group A, but non- statistical significant with Group C ($P \geq 0.05$). The comparison of Group C was statistical significant ($P \leq 0.05$) to Group A but non-statistical significant with Group C ($P \geq 0.05$) showing the better results with Ecosin then the rest of the treatment protocols.

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Conclusion:

Most of the horse suffering from hoof thrush included in this study were mild and showed good results after treatment protocols. Therefore, horse owners need to improve their management practices to reduce the risk of a specific disorder especially the thrush by doing regular hoof trimming and good stable management. Furthermore, continuous education and preventive measures depend on early diagnosis and advice given by the farrier and equine veterinarians. The owners should follow and adhere to the advice by the farriers/veterinarians.

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