DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF CONTAGIOUS ECTHYMA IN GOATS

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CERTIFICATE

This is to certify that the thesis entitled **DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF CONTAGIOUS ECTHYMA IN GOATS** submitted by **Mr. PAVAN** ID No. **MVNK 2014** in partial fulfillment of the requirements for the award of **MASTER OF VETERINARY SCIENCE** in **VETERINARY MEDICINE** of the **Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar** is a record of bonafide research work carried out by his during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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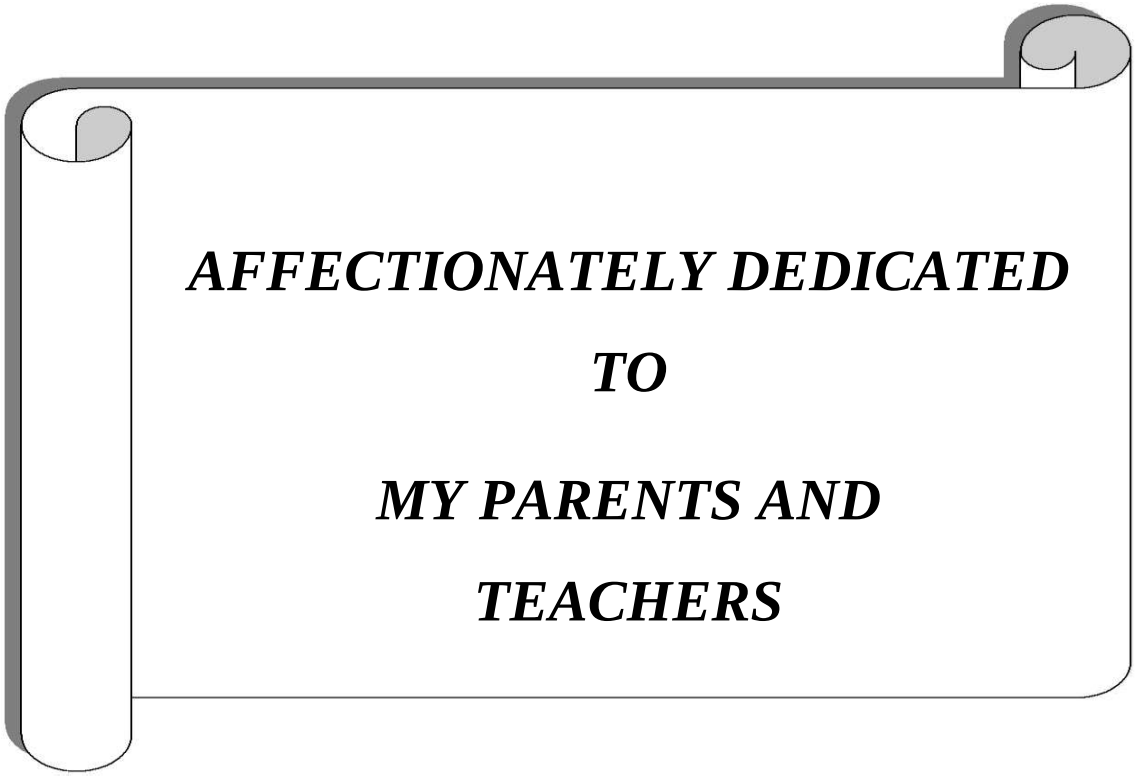
AFFECTIONATELY DEDICATED
TO
MY PARENTS AND
TEACHERS

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BIDAR
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CONTENTS

LISTS OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

Abbreviation	Description
%	Per cent
( )	Parentheses
/	Per
&	And
@	At the rate of
<	Less than
±	Plus, or minus
≤	Less than or equal to
®	Registered sign
µg	Micro gram
µL	Micro Liter
ALT	Alaline transaminase
AST	Aspartate transaminase
BLAST	Basic Local Alignment Search Tool
CE	Contagious ecthyma
cm	Centimeter
dL	Deciliter
DLC	Differential Leukocyte Count
EDTA	Ethylene diamine tetra acetate
et al.	Co workers
Fig.	Figure
g/dL	Grams per Deciliter

GTPV	Goatpox virus
Hb	Haemoglobin
i.e.,	That is
IU/L	International unit per liter
L	Liter
Ltd	Limited
mL	Milliliter
ºF	Degree Fahrenheit
ORFV	Orf virus
P	Level of significance
PCR	Polymerase Chain Reaction
PCV	Packed cell volume
PLT	Platelets
SE	Standard error
TEC	Total Erythrocyte Count
TLC	Total Leucocyte Count
TP	Total Protein
viz.	Namely

Introduction

I. INTRODUCTION

Goat is considered as poor man's cow and one of the most important economic source of income to the small and marginal farmers in India due to their high quality meat, milk and leather. Contagious diseases of livestock are the major causes of heavy economic losses to the Indian farmers amounting to billions of rupees annually. One of them is contagious ecthyma (CE), a highly contagious zoonotic viral disease that affects sheep and goats and causes significant financial loss to farmers. The disease is also known as sore mouth, contagious pustular dermatitis or scabby mouth (Thomas *et al*., 2003) and it is usually more severe in goats than in sheep. The Orf virus (ORFV), which belongs to the genus Parapoxvirus and the subfamily Chordopoxvirinae and family Poxviridae, is what causes contagious ecthyma, often known as Orf, sore mouth, or scabby mouth illness (Hosamani *et al*., 2009). According to the World Organization for Animal Health, Orf is a contagious disease that can infect humans as well as a number of other animal species. The disease is prevalent throughout the world and has various enzootic locations. The first human case of Orf was discovered in early 1920, and this infection is currently referred to as "human orf" (Kuhl *et al*., 2003; Georgiades *et al*., 2005; Uzel *et al*., 2005 and Pal *et al*., 2013).

Apart from sheep, goats, other domesticated and wild ruminants contagious ecthyma affects rein deer, musk ox, mule deer, white-tailed deer, pronghorn fawns and wapiti calves in natural, captive/ free ranging conditions as well as experimental infections (Lance *et al.*, 1983; Tryland *et al*., 2005 and Guo *et al*., 2004). The contagious Ecthyma virus is resilient in a dry environment, where it can persist for months or even

years, but in cold and wet settings, its life span may be reduced (McKeever and Reid, 1987).

Contagious ecthyma has worldwide distribution and is found particularly in sheep and goat farming countries throughout the year but is more common during spring and summer mainly among lambs and kids. The disease is common in young animals in 3-6 months of age, but few mature animals may also be affected (Gokce *et al.*, 2005).

On infected premises, outbreaks are possible every year, albeit older animals have some immunity. Clinical indications are more likely to occur when there are stressors. The disease has a very high morbidity rate that is close to 100% (Gokce *et al*., 2005), but mortality seldom cross 1%, especially in young animals younger than 1 year old. The mortality rate can go from 20 to 50%, because of secondary complications and combined infections with other viruses and bacteria (Bora *et al*., 2012).

Infected animals, animal byproducts, or accidentally when vaccinating are the most common ways for disease to spread to people (Lederman *et al*., 2007).

Steeb first identified Orf in sheep in 1787, and Hansen a Danish Veterinarian, first described it in goats in 1879 (Stephen *et al*., 2010). Goats typically experience it more severely than sheep do. Proliferative lesions on the mouth and muzzle are the disease's primary symptoms, and they resolve in 1-2 months (McKeever *et al*., 1988).

The morbidity of contagious ecthyma can reach 100%, whereas the fatality rate is less than 1%. In contrast, 15% of deaths may be caused by seconadary complications (Gumbrell and McGregor, 1997). Due to oral lesions, subsequent bacterial or fungal

infections or maggot infestations, which make it difficult for animals to eat and frequently cause anorexia and death in young animals, leading to high mortality rate (Haig and McInnes, 2002).

The farmers have known for a long time that ORFV affects tiny ruminants, creating "scabby mouth," although the etiological agent was only identified and described in 1787 (Robinson *et al*.,1982).

The Orf has unknown epidemiological information and is underreported in some nations due to its self-limiting nature and lower economic impact compared to other viral infections of sheep and goats (Kumar *et al*., 2015).

For the sensitive and specific detection of ORFV infections, nucleic acid-based techniques like PCR have been developed (Inoshima *et al*., 2000). To detect a number of target genes in Parapoxvirus which include GM-CSF/ interleukin-2 inhibitory factor (GIF) genes, B2L gene, Interferon resistant protein, ATPase (A32L), dsRNA-binding protein (E3L), VLTF and FIL, Polymerase chain reaction has been developed (Hosamani *et al*., 2007; Chan *et al*., 2009 and Mahmoud *et al*., 2010).

Contagious ecthyma is typically diagnosed symptomatically; however, it must be distinguished from conditions, such as Ulcerative dermatosis, FMD, Bluetongue, and Staphylococcal folliculitis. The main envelope gene, also known as the B2L gene or ORF011 gene, is frequently used in the molecular diagnosis of contagious ecthyma. The parapox infection has been identified using multiplex PCR, PCR targeting the B2L gene, or PCR targeting the virus interferon resistance [VIR] gene (Torfason and Gunadottir,

2002). Since the B2L gene is largely conserved among various ORFV isolates, its identification is the most essential and sensitive approach for molecular diagnosis (Abrahao *et al*., 2009). The B2L gene encodes a highly immunogenic main envelope glycoprotein that is 42 kDa in size and is a homolog of the vaccinia virus envelope protein antigen-p37K (Sullivan *et al*., 1994).

No doubt, the practice of veterinary medicine has been transformed due to availability and use of antimicrobial drugs but overuse/misuse of these drugs has dramatically accelerated the emergence of antimicrobial resistance. Therefore, a study on ayurvedic and homeopathic preparations in livestock sector has to be prioritized. Use of different thuja preparations and homeopathic preparations can be a novel approach. Thuja occidentalis possesses anti-inflammatory, antibacterial, antifungal, antiviral properties (Caruntu *et al.*, 2020). Aloe-vera has immune-boosting and anti-viral properties (Rajeswari *et al*., 2012). Therefore, thuja preparations and aloe-vera and turmeric mixture can be an affordable and a viable alternative for alleviating the severity of contagious ecthyma lesions and ensuring optimum productivity.

Contagious ecthyma in goats has been recorded from all across India and is an endemic condition. There have been an increasing number of reports of it internationally (Hosamani *et al*., 2009 and Nandi *et al*., 2011). Although the significance of contagious ecthyma is well known, only a few isolated reports are available on the occurrence and pathobiology of contagious ecthyma in goats in Karnataka. Therefore the present study was designed with the following objectives.

1. To study the prevalence of contagious ecthyma (ORF) in and around Bidar.
2. To study the clinical and haemato-biochemical changes in contagious ecthyma infected goats.
3. To confirm the contagious ecthyma virus by PCR technique.
4. To know the therapeutic efficacy of ayurvedic and homeopathic preparations against contagious ecthyma in goats.

Review of Literature

II. REVIEV OF LITERATURE

Contagious ecthyma is an acute, highly contagious, and economically important viral disease of small ruminants. The mortality rate is low for adults but is reported to be high for kids, although the incidence of morbidity is relatively high in goats of all ages. The virus causes localized persistent proliferative skin lesions, and because of its host immune evasion strategies, affected hosts become infected repeatedly. The disease is highly communicable and numerous cases have been reported in humans throughout the world which has increased the importance of the disease.

2.1 Prevalence

2.2 Clinical signs and symptoms

2.3 Hematology and serum biochemical changes in contagious ecthyma affected goat

2.4 Diagnosis

2.5 Treatment

2.1 Prevalence of contagious ecthyma

Murty and Singh (1971) revealed that CE lesions were seen in four of 587 Bikanery and Mandya sheep after keeping the animals in the same pen as Rajasthan sheep on their arrival.

Theil and Rudolph (1981) reported that in one year old sheep in Germany (GFR), 70 per cent out of total 300 in a farm suffered from Orf in a severe outbreak

Zamri-saad *et al*. (1989) revealed that 54% of 260 goats examined due to CE were kids less than 3 months old and the youngest kid with lesions was 20 days old.

Zamri-saad *et al*. (1992) reported CE cases from a herd of 186 goats, with 47 younger ones having a persistent history of CE in the herd.

Chandranaik *et al.* (2005) reported contagious ecthyma in a village in Bangalore rural district which occurred in a single flock with a mortality of two lambs and rampantly spread to the surrounding sheep flocks.

Nandi *et al.* (2011) mentioned that the disease is highly contagious, affects small domesticated and wild ruminants, and is more severe in goats as compared to sheep.

Maan *et al*. (2014) recorded the disease in five flocks of local breeds of goats (Jhakrana and Beetal) in Chokri village of Sikar district of Rajasthan State. In one flock of 100 goats, 60 animals were affected and 6 died.

Srinivasa Babu (2015) studied the outbreaks of contagious ecthyma in Karnataka and carried out isolation and molecular characterization of the Orf virus from sheep and goats in Karnataka.

Begum *et al.* (2016) stated that the overall prevalence of the orf virus was 68.04%. A relatively lower prevalence rate of infection was recorded in organized herds (45.35%) than in the animals from the unorganized herds (78.89%). A higher prevalence rate was recorded in kids below one year of age (78.68%) than in those above one year

(56.92%). The prevalence of orf in male and female animal groups was found to be 68.67% and 67.00% respectively.

Bora *et al.* (2016) mentioned that seroprevalence showed 76.62 percent of goats seropositive from a total of 231 serum samples collected from 12 districts of Assam from September 2013 to July 2014.

Gao *et al.* (2016) reported that out of 1,241 blood samples collected from goats without clinical signs of orf, 433 samples (34.89%) were positive for orf virus infection. Lambs under intensive management were more susceptible to orf virus infection.

Kumar *et al*. (2016) reported an outbreak of caprine contagious ecthyma (CE) in a flock of 12 adult Amritsari goats in Hisar district of Haryana (India) with some rare clinical lesions was reported. Of the 12 adult goats recently introduced, nine goats (75%) were severely affected showing characteristic signs.

Balakrishnan *et al*. (2017) reported an outbreak of contagious ecthyma in indigenous non-descript goats in high altitude (Ooty town in the Nilgiri hills) during the rainy season of the year (June-July, 2013) for the first time in Tamil Nadu in contrast to its usual outbreaks in late summer, and winter. A total of 174 out of 194 non-descript goats were clinically affected.

Bala *et al*. (2018) obtained a total of 180 serum samples from 90 sheep and 90 goats from 5 randomly selected farms It was revealed that prevalence rates of CE were 12.2% in sheep and 14.4% in goats.

Tedla *et al*. (2018) screened 400 animals for infection of the virus, out of which 48 animals were found positive to PCR and revealed an overall incidence of 12%. Also reported that the prevalence of Orf virus based on the species of the animal showed a higher prevalence (15.5%) in sheep than in goats (8.5%). Out of 190 male animals examined, 21 (11.05%) were positive for the Orf virus, and out of 210 female animals examined, 27 (12.85) were positive for the virus. The highest prevalence rate was recorded in the age group of 1–2 years (16.5%) than < 1 year (6.4%), 2-3 years (9.2%), and > 4 years (13.2%).

Bala *et al*. (2019) in his study found that out of the 504 animals sampled, 115 were positive for Orf virus which indicated an overall score of 22.8% among the animals sampled.

Anjum (2022) collected serum samples from 350 goats and 91 sheep on the 20th day post-infection for detection of antibodies. This study found an overall 13.2% seroprevalence of contagious ecthyma infection, indicating a higher percentage in goats (14.6%) than in sheep (7.7%).

Mohammed and Yousif (2022) mentioned that out of the 200 tested skin lips samples of both sheep and goats; 76 samples out of 100 tested (76%) from sheep and 71 samples out of 100 tested (71%) of goats.

2.2 Clinical signs and symptoms:

Samuel *et al*. (1975) stated that Orf infections in Boer and Boer cross goats are manifested by multifocal, severe proliferative dermatitis accompanied by chronic

pneumonia, arthritis, and moderate to severe lymphadenopathy. The disease usually runs a 3–4 weeks course. In the mouth of mountain goats, dry, brown, proliferative lesions are prominent on the markedly edematous hyperemic lips, but severity diminishes toward the commissures. Lesions of the oral cavity especially on the gum become moist, reddish-brown, and in certain sites, intensely hyperemic.

Murphy *et al.* (1999) observed the CE lesion within the mouth affecting the gums and tongue and also affecting the hairless part of the body like the eyelid, feet, and teats. Lesion of Orf progress from papules to pustules and then to thick crusts. The scabs are often friable and mild trauma causes the lesion to bleed.

Housawi and Abu Elzein (2000) reported that the scabs were friable and bleed easily. Contagious ecthyma lesions are painful and may result in anorexia or even starvation. In some of the affected cases, the condition was highly aggravated by myiasis which appeared to have been favored by the abundance of flies during the hot season.

Inoshima *et al*. (2000) stated that Parapox viruses generally cause mild papular dermatitis around the mouth, teats and skin of infected animals and also occasionally infected humans. In severe cases, the papules progressed to ulcers on teats and caused necrosis.

Haig and McInnes (2002) reported that the infections were usually acute, but chronic infections have also been recorded. The clinical progression started with erythema, vesicle, pustule then scab formation. Virus was shed with the scab to seed the environmental pool. Infections were localized to the skin or buccal cavity. Systemic

infection did not occur. Primary infections could be severe with large often -contiguous lesions that usually resolved within 6 to 8 weeks. Reinfection lesions were smaller and resolved more rapidly, usually within 3weeks. For this reason, the disease was most serious in young lambs or kids.

Torfason and Guðnadottir (2002) noted that the lesions in sheep and goats commonly appear on the muzzle and lips (scabby mouth), sometimes also affecting the gums and tongue, especially in young lambs. Eyelids, feet and teats can also be affected. Severe facial and oral lesions in lambs may interfere with suckling. Suckling infected lambs may develop lesions on the udder of ewes.

De la Concha-Bermejillo *et al*. (2003) mentioned that skin lesions initially develop in the lips that disseminate to the skin of face, feet, flanks and scrotum. The disease is characterized by the appearance of vesicles, pustules, ulcers, and papillomatous proliferative lesions on the skin of lips and nostrils. In more severe cases, the skin of the eyes, feet, vulva, or udder also might be affected. Usually, lesions heal spontaneously within 3–4 weeks. Depending on the location, animals may be unwilling to nurse, eat, or walk.

Dal Pozzo *et al*. (2007) studied that the lesions appear after an incubation time of 3 to 10 days and progress through the stages of erythema, papule, pustule, and scab formation. It is commonly a self-limiting disease that resolves within 6 to 8 weeks; but complications can include bacterial infections, regional lymphadenopathy, lymphangitis, erythema multiforme, and bullous pemphigoid.

Guo *et al*. (2003) observed that in lambs or kids, after approximately a week of incubation period, an initial rise of temperature is accompanied by development of skin lesions at the area of the mouth, lips, and nose. Also, the lesions can be seen in the oesophagus or the abomassum of affected animals. Lesions are more severe in young (less than 2-months old) animals when they can extend to the skin of face, feet, flanks, scrotum and peri-anal area.

Chan *et al*. (2007) reported that affected lambs and kids suffer severely because of restricted suckling and grazing. The CE is characterized by proliferative lesions around the muzzle and lips (scabby mouth), and sometimes also affects the gums and tongue, especially in young lambs. Eyelids, feet, and teats can be affected occasionally. Rarely, the lesions may extend into the esophagus, stomach, intestines, or respiratory tract. The disease lasts for 3–4 weeks and usually resolves in 1–2 months, with mortality rates up to 10% and 93% in lambs and kids, respectively.

McElroy and Basset (2007) observed that ORF lesions can expand into the oral cavity (buccal mucosa, tongue and palate) in the form of papules that become ulcerated and covered with exudates.

Radostitis *et al*. (2007) mentioned that the ORF lesions appear 6-7 days post infection and are characterized by the formation of crusty vesiculo-proliferative lesions, papules, pustules, scabs covering ulceration and granulation tissue. Lesions can also be found in buccal cavity, esophagus and abomassum but can rarely be seen in rumen mucosa.

Billinis *et al*. (2012) mentioned that in affected adult animals, the lesions can be found in the genital organs, prepucial orifice leading to impotentiacoendi and loss of libido, and vulva and skin-vaginal junction.

Wilson and Mcfarlane (2012) studied that contagious ecthyma was not usually lethal, and lesions typically disappeared within 2 to 4 weeks, but death may result if secondary complications, such as bacterial infections or myiasis, develop. Additionally, lesions could be quite painful and hinder feeding in adults or nursing in lambs and kids, leading to emaciation and death, depending on the severity of the infection.

Bouznach *et al.* (2013) observed clinical signs of CE in goats with high body temperature and proliferative cauliflower-like crusty scab lesions on the lips, muzzle, mucocutaneous junctions and nostrils.

Ferede *et al.* (2014) observed clinical signs of CE in goats with slight rise in body temperature, pustular lesion around the muzzle and nostrils. Some of the goats were observed with foot lesions on interdigital space which led to lameness.

Zeedan *et al.* (2015) reported that, lesion of CE in goats were more severe proliferative dermatitis which became eventually developed into thick, brown, rapidly growing scabs over areas of granulation, inflammation and ulceration (papules, pustules and vesicles) on the lips, nose, ears and eyelids in young goat flocks.

Kumar *et al*. (2016) observed that of the 12 adult goats recently introduced, nine goats (75%) were severely affected showing characteristic signs. The lesions were mainly confined to the mouth and oral cavity which included swollen lips, gingivitis and in later

stages ulceration and scab formation on lips. Lesions on the dorsal surface of tongue were also observed in some cases which are relatively rare in occurrence.

Maganga *et al.* (2016) reported that CE is characterized by proliferative skin lesions on lips, muzzle, ears, eyelids, udder, foot, genital organs and also found around the mouth and nostrils.

Balakrishnan *et al.* (2017) recorded an outbreak that occurred over a range of 3 km radius in a hamlet near Ooty town in the Nilgiris hills. A total of 174 out of 194 non-descript goats were clinically affected with the symptoms of pyrexia (40°C), anorexia, depression, proliferative scab lesions on the skin around the mouth, oral commissures and nostrils.

Dalal *et al*. (2021) noticed that the affected animals showed signs of anorexia, hyper salivation, pain and fever around 104°F. There were typical skin lesions like erythema, papule, vesicle, pustule, and scabs on mouth, tongue, oral cavity, muzzle, lids of eye, perineum and scrotal area. Lesions were highly vascular and bleed easily.

2.3 Hemato-biochemical changes

Gameel *et al*. (1995) reported that the lambs had lower total serum protein values, haemoglobin concentration, erythrocyte counts and packed cell volume, but higher blood leukocyte counts and increased serum transaminase activity when compared to apparently healthy animals. Also reported hypoproteinemia and incresed serum aspartate transaminase values in Orf infected lambs.

Housawi (2000) reported that serum biochemistry after orf virus infection in sheep and goat shows hypoproteinemia, hyperglycemia and enhancement of hematocrit value, cortisol level, creatinine, aspartate aminotransferase, creatinine kinase but without any alteration in lactate dehydrogenase level.

Housawi (2002) reported that haematocrit values became elevated by day 2. The effects of orf infection on plasma concentrations of aspartate aminotransferase (ASAT), creatinine kinase (CK), lactate dehydrogenase (LD) and cortisol. The inoculated animal showed a significant increase in the activity of AST, CK and cortisol but not in LD concentration by day 2 control animals. Serum creatinine and glucose were significantly elevated.

Kazemi Asl *et al*. (2018) in his study found that serum BUN, cholesterol, triglyceride, glucose, HDL concentrations as well as CK, AST, GGT and catalase activities were significantly higher in Orf affected goats when compared with healthy goats. There was no significant difference in serum total protien, albumin, total and direct bilirubin and cholesterol between Orf affected and healthy goats. Higher levels of serum glucose may also confirm the effect of stress in goats with Orf. Also suggested that PCV value, WBC, Lymphocytes and neutrophils and serum iron concentration of goats with Orf were significantly higher than the healthy goats.

2.4 Diagnosis

Torfason and Gunadottir (2002) mentioned that the parapox virus can be diagnosed through Multiplex PCR and PCR targeting B2L gene or VIR gene.

Guo *et al*. (2003) carried out genetic characterization of ORF virus isolated from skin lesions of a goat kid using electron microscopy and amplification of B2L and VIR gene by PCR, restriction enzyme analysis of viral DNA and gene sequencing.

Abarhao *et al*. (2009) diagnosed the ORF outbreaks in South America (Brazil) using PCR based on the sequence of B2L and VIR genes.

According to Torfason and Guðnadóttir (2002) study twenty-two (95.7%) of 23 scab or swab specimens with suspected orf etiology were orf PCR positive. Electron microscopy demonstrated orf-like particles in orf-PCR positive specimens.

Venkatesan *et al*. (2012) reported that Contagious ecthyma disease is enzootic in India, causing high morbidity in sheep and goats. Though it is easy to diagnose, the lesions and symptoms confounding with 29 other skin diseases require laboratory confirmation, which includes serological and nucleic acid-based techniques.

Bouznach *et al*. (2013) observed no significant differences among the different ORF isolates throughout the world based on viral B2L gene.

Dalal *et al.* (2015) reported that the Orf viral gene specific PCR amplicons were visualized in 1% agarose gel electrophoresis by ethidium bromide staining. The appropriate size or correct band was cut and gel purified using Qiaquick\gel extraction kit (Qiagen) as per manufacturer's protocol.

Kumar *et al*. (2016) collected samples (swab and scab samples) from severely affected goats were processed for confirmatory diagnosis by semi nested polymerase

chain reaction (PCR) assay based on CE virus specific primers for B2L gene which give a characteristic band of 235 bp. All the representative samples were found positive confirming a CE outbreak.

Karki *et al*. (2019) suggested that for the detection of parapoxvirus DNA in clinical samples, PCR methods detecting a number of target genes have been developed.

2.4.1 Polymerase chain reaction (PCR) of B2L gene of ORFV

Inoshima *et al.* (2000) carried out semi-nested PCR for detection of CEV. They described that the semi-nested PCR could detect low copy numbers of viral DNA and was effificient for diagnosis of Parapox virus infection in clinical samples. The results suggested that the PCR method described would be specifific for detection of viruses belonging to the genus Parapox virus and applicable for rapid and easy diagnosis of Parapox virus infections in both domestic and wild animals.

De la Concha-Bermejillo *et al.* (2003) evaluated 16 goats and Boer crossed kids for persistent, multifocal areas of severe papillomatous proliferation of the skin. They identified CEV in skin lesions by amplification of viral DNA using polymerase chain reaction. They carried out a semi-nested PCR (Sn PCR) for viral DNA amplification by using primers that targeted a portion of CEV enveloped gene which resulted in amplification of the 235 and 594 bp fragments from DNA extracted from skin lesions of 15 affected goat kids.

Gallina *et al*. (2005) developed a real time quantitative PCR assay based on TaqMan® technology or CEV DNA quantification in clinical samples, infected cells and

organotypic cultures. This method was based on the amplification of a 70 bp fragment from the CEV B2Lgene that encodes the major envelope protein. The TaqMan® PCR was subsequently used to determine the titer of several batches of the CEV strain NZ-2, with it was possible to quantify virus solutions in the range of 1×101 to 1×106 TCID50/ml. A good correlation between the titer determined by the TaqMan® PCR and by conventional end point dilution was found. The PCR assay was reproducible and could be used for a rapid quantification of CEV in vitro and ex vivo, being readily achievable within 1 hr. The TaqMan® assay was performed on the virus samples. It produced a positive result in 84 of the 86 CEV DNA samples giving a sensitivity of 97.7%. The amplification products were also checked on a 3 % (w/v) agarose gel stained with ethidium bromide. A 70 bp specific band was visualized for all the positive samples.

Klein and Tryland (2005) reported that the vIL-10 PCR and GIF PCR were able to amplify DNA from all 25 isolates, whereas the B2L PCR did only amplify DNA from 17 isolates from Norwegian semi-domesticated reindeer (Rangifer tarandus tarandus).

Hosamani *et al.* (2006) amplified full length B2L gene by using PCR. They collected scab samples of goats from the outbreaks that occurred in Mukteswar in Uttaranchal state of India. The amplified full length B2L gene PCR product size was 1206 bp.

Kottaridi *et al.* (2006) developed a new polymerase chain reaction (PCR) assay for rapid diagnosis of contagious ecthyma and applied it to 21 clinical samples from Greece. The assay detected a highly conserved gene from the parapox genome. A comparative study with two published PCR protocols one using primers PPP1–PPP3,

PPP1–PPP4 which targeted putative virion envelope gene B2L and the other using VIR1–VIR2 primers which amplifified CE virus interferon resistant (VIR) gene. All samples tested were amplifified successfully with the newly developed PCR protocol.

Chan *et al.* (2007) carried out Polymerase chain reaction (PCR) to amplify B2L gene from tissue specimens and described PCR as a powerful tool for 20molecular diagnosis. This method successfully detected DNA of CE virus from field specimens of CE affected animals. The disease was confirmed by amplifying a partial region of B2L gene using PCR. To confirm the causative agent, the first goal was to amplify a partial region of B2L gene by PCR. A specific product of expected size (594 bp) representing the region (157–750 nt) of B2L gene was amplified from the sample extracted from lip lesions of affected goat.

Ramesh *et al.* (2008) reported that, polymerase Chain reaction (PCR) assay as a rapid method for diagnosis of contagious ecthyma. They used fifty five number of scab materials collected from contagious ecthyma suspected outbreaks among the sheep and goat population reported during the period 2004 to 2008 with the history of papular lesions in oral commissure, muzzle and lower jaw regions. Out of 55 samples tested for CEV, 48 scab samples were found positive by PCR showing the predicted PCR amplicon size of 408 bp.

Oem *et al.* (2009) conducted the study on five suspected cases of contagious ecthyma in Korean black goats that were reported in different geographic areas of Korea. The outbreaks of contagious ecthyma were diagnosed and confirmed by PCR and the

sizes of the amplified PCR products were 708 bp, 1206 bp for full length of B2L gene and 552 bp for the partial VIR gene.

Zhang *et al.* (2010) used PCR for confirmation of the CE in field specimen of skin sample from affected animals. They carried out PCR of the complete B2L gene and obtained 1137 bp amplified PCR fragments. They reported PCR technique as a powerful and widely used tool in molecular diagnosis of desired genomic fragments from tissue specimens.

Chu *et al.* (2011) investigated an outbreak of mixed infection with Goatpox virus (GTPV), CEV and *Mycoplasma capricolum subsp. Capripneumoniae* (MCCP) that occurred on a chinese goat farm, with a case fatality rate of 60.2%. They observed clinical signs were ecthyma and accelerated respiration with frequent coughing. They amplified synchronously specific fragments of the p32 gene of GTPV, B2L gene of CEV and 16S ribosomal RNA gene of MCCP by PCR from the tissues of 12 dead goats. They obtained specific CEV B2L gene fragment of 507 bp from the tongue of 12 goats.

Bora *et al.* (2012) reported two outbreaks of CEV infected in two goat flocks at two different districts of Assam state. The disease was confirmed by using full length B2L gene-based PCR revealed specific amplifications, of 1206 bp products from viral DNA extracted from scab samples/cell culture isolates, which confirmed the etiology of the outbreaks as CEV.

Li *et al.* (2012) carried out PCR of DNA template that was prepared from tissue suspensions, infected cell culture supernatant and purified viral particles. The sizes of PCR products were 1137,1017, 615, 628 and 721 bp respectively.

Ali *et al.* (2013) performed conventional PCR for the diagnosis of CEV infection. The PCR technique proved to be more rapid and efficient for diagnosis of CEV infection than other test. The amplification and running of characteristic 708 (bp) fragments of CE viral DNA were obtained.

Davari *et al.* (2013) reported that it is difficult to differentiate contagious ecthyma from different diseases like Sheeppox, Goatpox and PPR. They carried out PCR for molecular detection of contagious ecthyma from 50 muzzle crust from sheep and goats of Shiraz suburb. They carried out PCR of the scab samples and amplified 045 gene. They observed that 25 (50%) samples were contagious ecthyma positive and the product size of the amplicon was 393 bp.

Maan *et al*. (2014) confirmed the presence of ORFV in clinical samples using GIF/IL-2 gene specific PCR which yielded 408 bp amplicon.

Mwanandota *et al*. (2016) confirmed the presence of ORFV in tissue scrapings from the lips by GIF/IL-2gene polymerase chain reaction (PCR), DNA sequencing and phylogenetic analysis of the GIF/IL-2 gene from Goats in Tanzania.

Ferede *et al.* (2014) investigated an outbreak of CE by using PCR. In this study B2L gene was amplified by using specific primers and the amplicon product size was 1206 bp.

Abdullah *et al.* (2015) detected CEV by conventional PCR using specific primers of B2L and F1L. They collected samples from infected goats with clinical signs of Orf in the Ladang Angkat program, University Putra Malaysia in 2014. Approximately, 10 % of goats in the farm were infected and two goats (age 6–10 months) died. A total of three samples were collected from the farm. All three samples were positive for CEV with all four sets of primers used. The three samples also produced a single positive band with sizes corresponding to each gene; B2L and F1L, respectively.

Bora e*t al*. (2015) investigated CE outbreak that occurred in goats of six district of Assam. They collected scab samples (n= 52) from the affected animal for confirmative diagnosis of CE. Scab samples were initially screened by Countercurrent immunoelectrohoresis (CIE) and semi-nested PCR. They amplified full length B2L gene by conventional PCR from the DNA of CEV isolates extracted from cell culture harvest. The initial screening by counter current immune electrophoresis confirmed seven samples positive. Further confirmation was done using semi-nested PCR and fourty (76.92%) samples were found positive in semi-nested PCR.

Dalal *et al.* (2015) employed three different polymerase chain reaction (PCR) assays and nucleotide sequence analysis for the identification and characterisation of contagious ecthyma virus from nine [scabs (n = 8) and cell culture adapted (n = 1)] field samples. The B2L specific assays yielded amplicons of 1,137 and 235 bp, whereas the IL-2 PCR yielded 408 bp products.

Kumar *et al*. (2015) studied that the disease can be diagnosed by PCR/ quantitative real-time PCR, the multiplex PCR and PCR targeting B2L or virus interferon

resistance (VIR) gene has been employed to diagnose the parapox virus infections. They described that a real-time PCR has developed based on B2L gene to detect and differentiate from Pseudocowpox virus, Bovine popular stomatitis virus and Seal parapox virus in clinical samples.

Zeedan *et al.* (2015) carried out molecular characterization of CEV using PCR from the scab lesion affected sheep, goat and biopsy samples from human. With partial B2L gene they obtained PCR product approximately 592 bp for B2L gene fragments of CEV DNA that were typical to reference CEV strain in human and animal virus.

Alam *et al.* (2016) collected 13 scab samples from contagious ecthyma suspected goat (11) and sheep (2) from three different farms of Sylhet and Dhaka divisions. They processed the scab samples for virus detection by PCR and isolated the virus in Vero cell. Four different sets of primers used such as GIF5 and GIF6, vIL-10-3 and vIL-10-4, OVA32LF1 and OVA32LR1, VIR1 and VIR2 and were targeted against Granulocyte-macrophage-colony-stimulating factor (GM-CSF) and Interleukin-2 inhibition factor (GIF), viral interferon resistance, A32L, interlukin-10 genes of CEV.Out of 13 samples 10 were found positive for CEV by PCR. The amplification of about 400 base pair DNA from ten samples.

Gelaye *et al.* (2016) collected 24 samples from an outbreak and found 18 samples were positive by using CEV specific primers CE 1 and CE 2 which produced the expected fragment size of 140 bp.

Maganga *et al.* (2016) reported eight suspected clinical cases of CE in goats in the rural area of Tebe, in south eastern Gabon, in January 2013. The CEV was confirmed in six out of eight sick goats by using specific PCR that targeted the major envelope protein (B2L) and the CEV interferon resistance (VIR) genes.

Balakrishnan *et al.* (2017) confirmed CEV using various diagnostic test such as agar gel immunodiffusion test, counter immunoelectrophoresis, and by polymerase chain reaction. It was confirmed by PCR using specific primer which flanks a 140bp sequence gene of CEV.

2.4.2 Phylogenetic analysis

Chan *et al.* (2007) carried out phylogenetic analysis by neighbor joining and boot strap analysis. They demonstrated that the amino acid sequences of B2L gene of the Nantou isolates was closer to the ORFV-Mukteswar 59/05 isolated from India. The sequences of Nantou isolate and the ORFV Mukteswar 59/05 showed 98.2 and 98.9% similarities at the nucleotide and amino acid level, respectively.

Abrahão *et al.* (2009) reported that, the phylogenetic analysis demonstrated a high degree of identity with CEV strains, and the isolate was closest to the ORFV-India 82/04 isolate. Another Brazilian CEV isolate, NE1, was sequenced for comparative analysis and also showed a high degree of identity with an Asian CEV strain.

Zhao *et al.* (2010) studied the phylogenetic analysis of the ORFV 011(B2L) and ORFV 059 (F1L) genes and showed that the ORFV-Jilin province isolate from sheep clustered in different branches and was closer to the ORFV Mukteswar 67/04 isolate and

the ORFV-OV/C2 isolate respectively. They observed that the Jilin isolates shared close relationship with other CEV isolates from different regions (96.5–98.9% and 95.3–97.4%), and shared the highest homology with ORFV-Mukteshwar 67/04 isolate from India (98.9% and 97.4%) based on the sequence analysis of the nucleotide and deduced amino acid of ORFV011 (B2L) gene.

Chu *et al.* (2011) constructed phylogenetic tree based on the related sequences with the neighbor-joining method with 1,000 bootstrap replicates using MEGA version 4.0. The results showed that Gpv_Chq09 cluster consisted of strains from Vietnam (EU625263), and 2 Chinese provinces of Guangxi (EF522180, AY773088) and Guizhou (EF514890). It was consistent due to a fact that Chongqing, Guizhou, and Guangxi are geographically adjacent to each other in the southwest region of China, and Guangxi shared a border with Vietnam. Orfv_chq09 was genetically closer to the 2 CEV stains from Taiwan (EU935106, DQ904351) compared to other strains from the mainland of China (GU903501, GU320351).

Venkatesan *et al.* (2011) performed phylogenetic analyses of sheep isolates based on the B2L gene which revealed that the isolates were closely related to goat isolates retrieved from an outbreak at the same geographic location. It also showed close genetic similarities with other Indian isolates reported earlier.

Billinis *et al.* (2012) performed phylogenetic analysis on a part (498bp) of B2L gene of Parapox virus strains. They analyzed that the maximum nucleotide and amino-acid variation amongst CEV strains isolates worldwide (n= 33) was 8.1% and 9.6%, respectively. The homology of the nucleotide and amino-acid sequences between the two

Greek isolates was 99.0% and 98.8%, respectively. The two Greek isolates clustered only with CEV strains.

Bora *et al.* (2012) carried out phylogenetic analysis based on B2L amino acid sequences that showed the CEVs identifified in these outbreaks were closely related to each other and both were closer to ORFV-Shahjahanpur 82/04 isolate from north India. They also reported that the precise characterization of the genomic region (B2L gene) might provide evidence for the genetic variation and movement of circulating CEV strains in India.

Oem *et al.* (2009) performed phylogenetic analyses revealed that CEVs from Korean black goats were most closely related to an isolate (ORF/09/Korea) from dairy goats in Korea. They indicated that the CEVs have been introduced from dairy goats into the Korean black goat population.

Chi *et al.* (2013) determined the phylogenetic analysis relationship between ORFV 011 and ORFV 059 gene sequences from a total of 11 CEV isolates at Fujian Province in China and other sources world-wide using MEGA 5.0 software with boot strap values calculated from 1000 replicates. The phylogenetic algorithm used for the building of tree was the maximum likelihood method based on ORFV 059. They observed that the 11 different CEV isolates clustered together and showed 100% identity at the nucleotide level among the isolates from the two flock that were located one kilometer away from each other. The phylogenetic analysis based on ORFV 011 of the 11 CEV isolated sequences did not clustered. The 8 isolates of ORFV obtained from goats in

northern Fujian Province, China were highly divergent and indicated considerable heterogeneity among themselves.

Abdullah *et al.* (2015) carried out phylogenetic analysis using both CEV B2L and F1L gene sequences. The phylogenetic tree was constructed using MEGA 6 software program. Phylogenetic analysis showed that the Malaysian strain had close homology to the Chinese and Indian CEV isolates. The study gave more insight into the existing CEV strains in Malaysia and their relationship with other strains globally.

Bora *et al.* (2015) performed molecular analysis of major envelop protein (B2L) of CEV. The sequencing and phylogenetic analysis of the selected sequences at nucleotide level revealed that the CEV isolates were closely related to each other (97.6–100 %) and showed highest similarity (98.4 %) to the CEV isolate 82/04 reported from Shahjahanpur, India.

Dalal *et al.* (2015) performed phylogenetic analysis of full length B2L gene of CEV using MEGA 5 software that revealed high conservation in B2L gene. CEV strains showed 84–86% nt identities with BPSV, 93–96% with PCPV and 43–46% with PVNZ. The study showed lowest nucleotide identities (95.4%) with few Chinese strains while highest nucleotide identities (99.1%) with some other Chinese and Indian strains (CPDV/IND2013/01 and PDV/IND2013/02) of the CEV isolates.

Zhang *et al.* (2015) analyzed the genetic evolution of three strains such as SDLC, SDTA and SDJN isolated from goats at different areas of China. The phylogenetic analysis showed that ORFV 011 of SDLC and SDTA strains clustered together with the

Gansu, Liaoning, Shanxi, Nantou, Hoping and FJ-YX strains, whereas SDJN clustered with the FJ-GS and FJ-GO strains.

Alam *et al.* (2016) performed Phylogenic analysis by analyzing the nucleotide sequences of Bangladeshi isolates with those of CEV obtained from the GenBank database by neighbor-joining trees using MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Phylogenetically Bangladeshi viruses were clustered with viruses from New Zealand and India.

Azad *et al.* (2016) carried out phylogenetic analysis using neighbor joining tree inference analysis with the 1000 bootstrap replications. Nucleotide sequences of viral interlukin-10 (vIL10) gene were used in the phylogenetic analysis. The phylogenetic study indicated that the CEV isolated from sheep and goat were closely related to each other but clustered separately. Sheep virus was found to have 95.20-97.20% homology with goat viruses. On the other hand goat isolates were found very closely related to each other and the identity among these viruses ranged from 97.20-98.60%.

Gelaye *et al.* (2016) studied phylogenetic and the comparative analysis of the deduced amino acid profile and suggested that there were two main clusters of CEV isolates i.e. B2L and A32L which were responsible for the investigated outbreaks. Additionally the analysis of these two genes showed limited variability to CEVs encountered elsewhere.

Maganga *et al.* (2016) performed phylogenetic analysis based on B2L and partial VIR gene sequences of Gabonese CEV strains and were aligned with homologous

sequences of Parapox virus reference strains from GenBank, using the Clustal W algorithm of MEGA program version 5. Phylogenetic analysis based on the partial B2L and VIR genes indicated that the Gabonese strain was closely related to the Asian strains, especially the South Korean strains, with which it has evolved from a common ancestor

2.4.3 Histopathology of Contagious Ecthyma

Guo *et al*. (2004) reported that characteristic intra-cytoplasmic poxvirus eosinophillic inclusion bodies of varying size were found in keratinocytes of the stratum spinosum in Shetland sheep and Sichuan takin lip lesions.

Barraviera (2005) mentioned that Orf virus affected skin tissues reveal epidermal hyperplasia with hyperkeratosis, ballooning and degeneration of keratinocytes. Hyperkeratosis, parakeratosis and acanthosis of the epidermis, degenerative changes in stratum spinosum and infiltration of mononuclear cells including macrophages, lymphocytes and neutrophils are evident. Eosinophilic inclusion bodies are demonstrable in the cytoplasm of the infected cells but may not be a consistent feature.

Radostits *et al*. (2007) reported that the significant pathological changes observed on necropsy revealed proliferative lesions in buccal cavity which could be due to replication of ORF virus in the cells of underlying replacement epidermal layer.

Mahmud *et al.* (2014) observed that the skin lesions caused by CE virus infection usually begin from oral commissures and later spread to the muzzle and other parts of the oral cavity.

Hematian *et al*. (2016) suggested that the morphologic changes (cytopathic effect, CPE) in the cell monolayer, such as shrinking, granulation, rounding, ballooning, and degeneration of the cells, show the presence of viruses.

Mohammed and Yousif (2022) mentioned that histopathological study in goat showed prolifration of epidermis layer and papillae projection, Highly proliferation structures spinosum, sebaceous gland, sweet gland, and proliferation hair follicles with thickening it wall, hyperkeratosis of epidermis, infiltration of inflammatory cells and ulceration with necrotizing of epidermis.

Hussain *et al*. (2023) examination revealed histopathological changes such as hyperplasia, anastomosing rete ridges formation and degenerative changes, including spongiosis and vacuolation of epidermal cells. Keratinocytes exhibited eosinophilic intracytoplasmic inclusion bodies with pyknotic and karyorrhexis nuclei.

2.5 Treatment

Shuttleworth (1988) made preliminary studies on a small group of infected lambs, using Thuja occ. for three days, established that the treatment was effective. Scabs dropped off in about eight days, accompanied by an observable improvement in condition.

Nandi *et al*. (1999) suggested that special treatment should also be performed regarding secondary bacterial infections that commonly coexist with the disease. Use of antibiotics can help and should be applied for this purpose. Locally, an antibiotic ointment can be applied.

Wilson *et al*. (2002) suggested occasional use of immunostimulant Levamisole in ORF infection.

De la Concha-Bermijillo *et al*. (2003) reported that ORF lesions can be treated with application of 3% iodine solution. Antibiotics may be used in secondary bacterial infections.

McCabe *et al*. (2003) observed that application of 1% Cidofovir cream prepared in Beeler basis for 4 consecutive days resulted in milder lesions that resolved more quickly than untreated lesions.

Sonvico *et al*. (2009) observed better results with an experimental preparation containing Cidofovir and Sucralfate in ORF affected lambs.

Nandi *et al*. (2011) described therapeutic management of contagious ecthyma using 1:10 boric acid, topical and systemic antibiotics, immunostimulants like Levamisole and anti-viral drug like 1% Cidofovir.

Bharathy and Akila (2015) reported that in an organized farm goats affected with contagious ecthyma were treated with ayurvedic agents like neem oil and turmeric paste or glycerin and boric acid paste on external lesions and got completely recovered after two weeks of continuous treatment.

Spyrou and Valiakos (2015) reported that treatment costs involved prohibits use of anti-viral drug like Cidofovir in treating ORF affected lambs though the same had shown satisfactory results in human ORF virus infections.

Reddy *et al*. (2016) reported use of boroglycerine ointment topically and enrofloxacin @ 3 mg/kg B.Wt as therapeutic management of contagious ecthyma in sheep. Improvement in clinical signs was noticed after 5 days except for the lesion on lips.

Balakrishnan *et al*. (2017) investigated an ORF outbreak in goats over a range of 3 Km radius in a hamlet near Ooty town in the Nilgiri hills. Further, goats treated with herbal topical spray (Topicure) and/or neem and turmeric paste recovered in 4-5 weeks.

Brahma *et al.* (2020) treated lesions externally by application of herbal paste prepared from turmeric powder and aloe-vera gel in 1:1 ratio. After each application fresh paste was prepared and applied twice daily. There was complete recovery within one week of treatment.

Kumar *et al*. (2020) clinically managed the case with use of anti-inflammatory agent (Meloxicam at the dose rate of 0.5 mg/kg body weight), herbal antidiarrhoeal, neblon. The paste of boroglycerine, turmeric powder and boric acid used topically to treat the oral lesions.

Iqbal *et al*. (2020) treated group A by Trisym (Symans Pharmaceuticals Private Limited) having trimethoprim 8% w/v and sulphadiazine 14% w/v and Melonac (ICI Pakistan Limited) having meloxicam 7.5mg/ml intramuscular for 5 days depicts better results than Group B has been treated by Trisym (Symans Pharmaceuticals Private Limited) having trimethoprim 8% w/v and sulphadiazine 14% w/v and Phlogen (ICI Pakistan Limited) having Flunixine meglumine 1mg/ml intramuscular for 5 days.

Topically Somogel (Abbot Laboratories) having Lignocaine, eucalyptus, alcohol, menthol was applied in both groups over the lesions for 10 days after washing with potassium permanganate solution as an astringent.

Dalal *et al*. (2021) stated that affected animals were treated with enrofloxacin antibiotic (2.5 mg/kg) to combat secondary bacterial infections along with other supportive treatment of analgine and ascorbic acid. Lesions were cleaned by potassium permanganate (0.1%) and topical antiseptic fly repellent cream was applied.

Materials and Methods

III. MATERIALS AND METHODS

The present study was conducted in the Department of Veterinary Clinical Complex, Veterinary College Bidar to record the prevalence and outbreaks of contagious ecthyma (CE) in goats in and around Bidar. The occurrence of CE in goats, by hematological and biochemical analysis of blood and serum samples, and confirmed by polymerase chain reaction followed by histopathological evaluation of scab and skin biopsies, and evaluation of therapeutic efficacy of ayurvedic and homeopathic preparations against contagious ecthyma.

3.1 Place of work

The present study entitled was undertaken at Veterinary Clinical Complex, Veterinary College, Bidar. The study was approved by Institutional Animal Ethics Committee (IAEC) with number 20/2022/VCB/VMD.

3.1.1 Study of clinical cases

Suspected cases of Contagious ecthyma presented to Veterinary Clinical Complex, Veterinary College, Bidar with history of scabby lesions on the mouth, reduced feed and water intake, were subjected to physical examination. In clinical evaluation rectal temperature, conjunctival mucus membrane, palpation of superficial lymph nodes and other symptoms suggestive of characteristic symptoms of contagious ecthyma i.e., scabby lesions typically developed on the mouth and muzzle and various sites of body were recorded. Based on history and clinical signs goats were utilized for study. Blood

sample from goats were collected for complete blood count and serum biochemical analysis. Tissue samples collected were subjected to PCR reaction and histopathology.

3.1.2 Study of healthy goats

Six apparently healthy goats which were maintained in good managemental condition were taken as healthy control group (group I) to study the normal haematological and biochemical parameters.

3.1.3 Retrospective study of contagious ecthyma in goats

The retrospective study on contagious ecthyma was studied in goats from Veterinary Clinical Complex, Veterinary College Bidar during January – December 2022. Point prevalence study was conducted in and around Bidar. Goats suspected for Contagious ecthyma having characteristic scabby lesions were selected and detailed signalment of age, number of animals in the herd, gender, history of scabby lesions appeared on the mouth, and other part of body were recorded in every case.

3.2.1 Collection of clinical samples from outbreaks

Samples were collected from 25 goats with presence of characteristic scabby lesions on the mouth. Scabs around the mouth, eyes, ears, nostrils and teat regions were collected aseptically in 10 % neutral buffered formalin for histopathology. Representative scab samples were also collected in sterile zip lock plastic covers and stored in ice pack thermacol containers for PCR which were stored under deep refrigeration (-20°C) until further use. In addition in some cases biopsy samples of lesions were also collected

aseptically in 10% NBF for conducting histopathology. Blood and serum samples were also collected for analyzing hematology and serum biochemistry.

3.2.2 Haematological analysis

3.2.2.1 Collection of Blood

Whole blood was collected aseptically in vacutainers from suspected goats with CE disease for hematological and biochemical evaluation. 2 ml of blood was collected aseptically from jugular vein from goat into vacutainers containing anticoagulant ethylene diamine tetra acetic acid (EDTA) vials (QUANTUM BIOMEDICALS INDIA, CB-PLUS)) and subjected to haematology analysis using a fully automatic haematology analyser (ERMA PCE 210® fully automatic blood cell counter, manufactured by ERMA Inc., Tokyo, Japan) was used for analysis of above blood parameters (Plate:1).

3.2.2.2 Hematological parameters

Hemoglobin [Hb (g/dL)], Total erythrocyte count [TEC ($\times 10^6$/µL)], Total leucocyte count [TLC ($\times 10^3$/µL)], Differential leukocyte count [DLC (%)] and Platelets count [PLT ($\times 10^3$/µL)] were carried out using fully automatic blood cell counter.

Differential Leucocyte Count (DLC) was done using Giemsa stain as per standard protocol (Coles, 1986).

3.2.3 Serum Biochemistry

For serum biochemical evaluation 4 ml of blood sample was collected in vacutainers without anticoagulant from 18 animals. The samples were allowed to clot,

serum was separated from blood clot by centrifuging at 3500 rpm for 10 minutes. The serum was collected and stored at -20^{0}C until further use. Biochemical parameters such as Aspartate Transaminase (AST), Alanine Transaminase (ALT), Total Protein (TP) and Albumin were estimated using semi-automatic Microlab 300® (semi-automated clinical chemistry analyser, manufactured by ELITechGroup Biomedical systems, France) (Plate:2).

3.2.3.1 Aspartate transaminase (IU/L)

Serum AST was estimated by using ERBA diagnostic reagent kits, manufactured by TransAsia Bio-medicals limited, Nalagarh, village Malapur, Baddi, district Solan, (HP)- 173205. The estimation was carried out and values were expressed as IU/L. The detailed procedure was described in Appendix-A.

3.2.3.2 Alanine transaminase (IU/L)

Serum ALT was estimated by using ERBA diagnostic reagent kits, manufactured by TransAsia Bio-medicals limited, Nalagarh, village Malapur, Baddi, district Solan, (HP)- 173205. The estimation was carried out and values were expressed as IU/L. The detailed procedure was described in Appendix-B.

3.2.3.3 Serum total protein (g/dL)

Serum total protein was estimated by using ERBA diagnostic reagent kits, manufactured by TransAsia Bio-medicals limited, Nalagarh, village Malapur, Baddi, district Solan, (HP)- 173205. The estimation was carried out and values were expressed as serum total protein (g/dL). The detailed procedure was described in Appendix-C.

3.2.3.4 Serum albumin (g/dL)

Serum albumin level was estimated by using the commercial ERBA diagnostic kit manufactured by TransAsia bio-medicals limited, Nalagarh, village Malapur, Baddi, district Solan, (HP)- 173205. The estimation was carried out values were expressed as Albumin (g/dL). The detailed procedure was described in Appendix-D.

3.2.4 Gross pathology

During collection of scab or biopsy materials from CE suspected goats in outbreaks the lesion on mouth, muzzle, lips, skin around the mouth and mammary gland were examined in detail and description of the lesions were recorded.

3.3.1 Molecular diagnosis by Polymerase Chain Reaction

Detection of Orf virus (ORFV) by polymerase chain reaction (PCR) using scabs/nodules.

3.3.1.1 General Requirements

The glass wares used in this study were of neutral glass of Corning or Borosil make. The buffers and other biochemical reagents were prepared in quartz glass doubled distilled water. The chemicals of analar, excellar or molecular biology grade were used for the preparation of various solutions and reagents.

3.3.1.2 Preparation of glass wares

The glass wares used in this study were prepared by soaking them in detergent solution overnight. Next day, they were washed thoroughly in running tap water ten

times, followed by rinsing in distilled water two times. Then the glass wares were immersed in distilled water overnight. The air dried glass wares were packed and sterilized in hot air oven for one and half hour at 160°C.

3.3.1.3 Preparation of plastic wares

The plastic wares including micro centrifuge tubes and micropipette tips were sterilized by autoclaving at 15 psi at 121°C for 15 min.

3.3.1.4 Rubber corks and other rubber-ware

The rubber corks and screw caps used in study were cleaned in teepol, rinsed in running water and finally soaked in triple distilled water. After drying, the different sized caps and corks were assorted and placed in petri plates, wrapped in aluminium foil and sterilized by autoclaving at 121°C for 15 min.

3.3.1.5 Pipettes

The RNAse DNAse free non-pyrogenic 1 ml sterile pipette procured from the M/s AXYGEN® Scientific was used for the PCR work.

3.3.1.6 Buffers and reagents

The chemicals, buffers and molecular reagents used were of molecular grade and were procured from M/s Sigma, SRL, Hi-Media and Merck. All reagents were prepared in Milli-Q water using Analytical reagent (AR) / Guaranteed reagent (GR) grade of laboratory chemicals.

3.3.1.7 Phosphate buffered saline (PBS), pH 7.2 (1X)

Sodium chloride	8.00 gm
Disodium hydrogen phosphate, 2H2O	1.44 gm
Potassium chloride	0.20 gm
Potassium dihydrogen phosphate	0.20 gm
Milli-Q water	1000 ml

The pH of the buffer was adjusted to 7.2 with 1N NaOH and autoclaved at 121°C for 15 min then buffer was stored at 4°C until further use.

3.3.1.8 Ethanol (70%)

Absolute Ethanol	70 ml
Milli-Q water	30 ml

3.3.1.9 Processing of tissue for PCR

About 30-50 mg of scab sample was collected from each case in sterile zip lock covers and stored at -20°C. The scabs or tissue samples were minced with a disposable blade or sterile scissor. Samples were then transferred into a centrifuge tube and vortexed while thawing.

3.3.1.10 Polymerase chain reaction (PCR) for B2L gene

Components of PCR

The reaction mixture include

Component	Amount
Master mix 2X (Thermo scientific Dream Taq PCR Master mix)	12.5µl
Orf B2L Forward primer	0.5µl
Orf B2L Reverse primer	0.5µl
Nuclease free water	9.5µl
Template (sample DNA)	2.0µl
Total reaction volume	25.0µl

Table 1: List of published primers used for polymerase chain reaction for B2L gene amplicons

Name	Primers	Amplicon size (bp)
OVB2LF1 OVB2LR1	5’ TCCCTGAAGCCCTATTATTTTTGTG 3’ 5’ GCTTGCGGGCGTTCGGACCTTC 3’	1206

3.3.1.11 PCR assay was carried out in thermocycler (Plate:3) with following cyclic Conditions

Amplification conditions

Steps	Temperature (^{0}C)	Time (min)	Number of cycles
Initial denaturation	95^{0}C	4 min	1
Denaturation	95^{0}C	1 min	35
Annealing	52^{0}C	1 min	
Extension	72^{0}C	1 min	
Final extension	72^{0}C	10 min	1

3.3.1.12 Reconstitution of primers for B2L gene PCR

Protocol

Primer mixture was prepared in 1:30 concentration.

· Mixed 336 µl of nuclease free water to primers.

· Vortexed for 5 min and spun under 2000 rpm for 2 min (repeated 3 times).

· Labelled the working tube and added 30 µl NFW+ 10 µl primer mixture.

· Vortexed for 5 min and spun under 2000 rpm for 2 min.

· Ready to use.

3.3.1.13 DNA extraction from scab samples

DNA extraction from the scab samples was carried out as per the manufacturer's protocol. DNA was isolated using QIAamp DNA Mini Kit (catalog no. 51306, Qiagen

Pvt. Ltd) and final elution of DNA was done in 30 µl of elution buffer and stored at -20°C for long term use.

The contents of the kit are as listed below

Contents	Quantity
QIAamp Mini Spin Columns	250 no's
Collection tubes (2ml)	750 no's
Buffer AL	54 ml
Buffer ATL	50 ml
Buffer AW1 (concentration)	95 ml
Buffer AW2 (concentration)	66 ml
Buffer AE	110 ml
Proteinase K	6 ml

DNA isolation from scab samples (DNeasy Blood & Tissue Kit, Qiagen)

Protocol

1. Cut up to 25 mg tissue into small pieces and tissue was triturated with pestle and morter and 1 ml of PBS was added. Centrifuged at 13000 rpm for 15 min.
2. Discard the supernatant without disturbing the pellet or concentrate.
3. To the pellet add 180µl of Buffer ATL and 20 µl of Proteinase K, mix by vortexing and incubate at 56°C for 20 minutes (until the pellet is completely lysed).
4. Briefly centrifuge the tube to remove the drops from the inside of the lid.
5. Add 200 µl of Buffer AL to the sample, mix by pulse-vortexing for 15 seconds

6. Add 200 µl ethanol (96-100%) to the sample and mix by pulse-vortexing for 15 seconds. After mixing, briefly centrifuge the tube to remove the drops from inside the lid.

7. Carefully apply the mixture (including the precipitate if any) to the QIAamp spin column (in a 2 ml collection tube) without wetting the rim. Centrifuge at 6000 x g (8000 rpm) for 1 min. Discard flow-through and collection tube.

8. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AWI, and centrifuge for 1 min at 6000 x g (8000 rpm). Discard flow-through and collection tube.

9. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.

10. Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube, and pipet 50 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 3-5 min, and then centrifuge for min at 6000 x g (8000 rpm) to elute.

11. For maximum DNA yield, Repeat elution with 50 µl of Buffer AE in same tube and incubate at room temperature for 3-5 min and centrifuge at 6000 x g (8000 rpm) for 2 min and store the DNA at-20°C for further use.

3.3.1.14 Agarose gel electrophoresis

Analysis of amplified products by agarose gel electrophoresis

Reagents

a. Agarose (Himedia)

b. TAE (Tris Acetate EDTA) Buffer

Stock-50X-TAE (Himedia)

Prepare working IX-TAE by addition of 20 ml of 50X TAE stock to 980 ml of distilled water.

c. Molecular weight marker-1000 bp plus DNA ladder (Fermentas, MD, USA)

d. Ethidium bromide (10 mg/ml) (Sigma)

Ethidium bromide (Biogene. USA)	100 mg
Double distilled water	10 ml

The suspension was stirred to ensure that the dye was completely dissolved. The container was then wrapped in aluminum foil and stored at 4 °C until use.

The concentration of DNA was determined by electrophoresis on one per cent agarose as described below.

1. Required quantity of agarose was dissolved in Ix TAE buffer and melted in a microwave oven for one min.
2. Once the molten gel cooled, ethidium bromide was added @ 0.5µg/ml and mixed thoroughly by gentle swirling.
3. Warm agarose solution was then poured into the gel casting tray with comb by avoiding the formation of air bubbles and allowed to solidify.

4. Once agarose solidified, a small amount of electrophoresis buffer was poured on the top of the gel to remove the comb. Then the buffer was poured off and the tape was removed.
5. The gel casting tray was mounted on the electrophoresis tank and the electrophoresis buffer was added just enough to cover the gel to a depth of one mm.
6. Twenty five microlitre of amplified product was slowly loaded into the slots of submerged gel using a micropipette.
7. The gel tank was closed with the lid and electrical leads were attached so that the DNA would migrate towards the anode.
8. The electrophoresis was carried out at 80 volt for 45 min at RT until the bromophenol blue dye migrated to an appropriate distance through the gel.
9. Following electrophoresis, the gel was visualized using a UV trans-illuminator and recorded in a gel documentation unit.

3.3.2 Histopathology

3.3.2.1 Collection of tissue samples

The representative pieces of or incisional biopsies scab from the affected animals were collected and examined grossly, trimmed and were fixed in 10% neutral buffered formalin in sterilized containers for histopathological examination.

3.3.2.2 Tissue processing and staining

The tissues were processed by the routine paraffin technique and sections of 5 µm thickness were cut using microtome then mounted on microscopic slides. The mounted

slides were subjected to routine haematoxylin and eosin staining (Luna, 1968) and finally sections covered with coverslip using DPX mounting medium.

3.3.2.3 Tissue examination and photomicrography

The stained slides were examined and screened for the presence of microscopic lesions using light microscope. The selected microscopic lesions were photomicrographed at different magnifications using photographic trinocular microscope (Olympus CX 43, Japan).

3.3.3 DNA sequencing and phylogenetic analysis

The positive PCR DNA samples were send to commercial firm (eurofins, India) for sequencing using both forward and reverse primers. The raw sequence data was edited for comparison based on standard pasture virus strain sequence (M13215.1) by using sequence of DNASTAR software. To determine the species of *Orf virus* each sequence was compared to GenBank sequences of Orf virus at NCBI and the per cent identity / homology was determined.

3.3.4 Phylogenetic analysis

The sequences obtained were subjected to phylogenetic study to determine the comparative differences in each species of Orf virus. Allignment of all positive Orf virus B2L gene sequence were done by clastal W method. Phylogenetic reference was done by the Neighbor-Joining method in Mega 11.0 software. The branch reliability was assessed by bootstrap method with 100 replications. The evolutionary distances were computed

using p-distance method and all the gaps were completely removed and phylogenetic tree was constructed.

3.4 Therapeutic study:

For studying the therapeutic efficacy of topical application of Thuja ointment, aloevera and turmeric combination(1:1 ratio) and Thuja occidentalis mother tincture orally in reducing the clinical severity of ORF lesions, 18 affected goats from outbreak were enrolled for clinical study and were divided randomly into 3 groups of 6 each (Fig. 1). The different therapeutic protocols/regimens tested were given in Table 2. The comparative efficacy of different interventions was assessed on number of days taken for resolution of clinical signs, complication(s) developed during the course of treatment. The following protocol were followed for therapeutic evaluation.

Table 2: Evaluation of therapeutic efficacy

Sl. No.		**Group 1**	**Group 2**	**Group 3**
01	No. of animals	06	06	06
02	Treatment protocol	Thuja occidentalis ointment bid for 1 week application to lesions	Aloe-vera gel with turmeric in 1:1 ratio bid for 1 week application to lesions	Thuja occidentalis tincture 10 drops bid for 1 week orally
03	Common Supportive therapy: Antibiotic Anti-inflammatory Anti-histamine	Enrofloxacin @ 5mg/kg b. wt. im OD Meloxicam @ 0.3 mg/kg b. wt. im OD Chlorpheniramine maleate @ 0.4mg/kg b. wt. im OD The above treatment was continued for three days.		

3.5 Statical analysis

Statistical analysis was performed using the statistical software GraphPad Prism, version 10 for windows. Mean values and standard error were calculated and all values were expressed as Mean ±SE The data obtained were analyzed by student t-test.

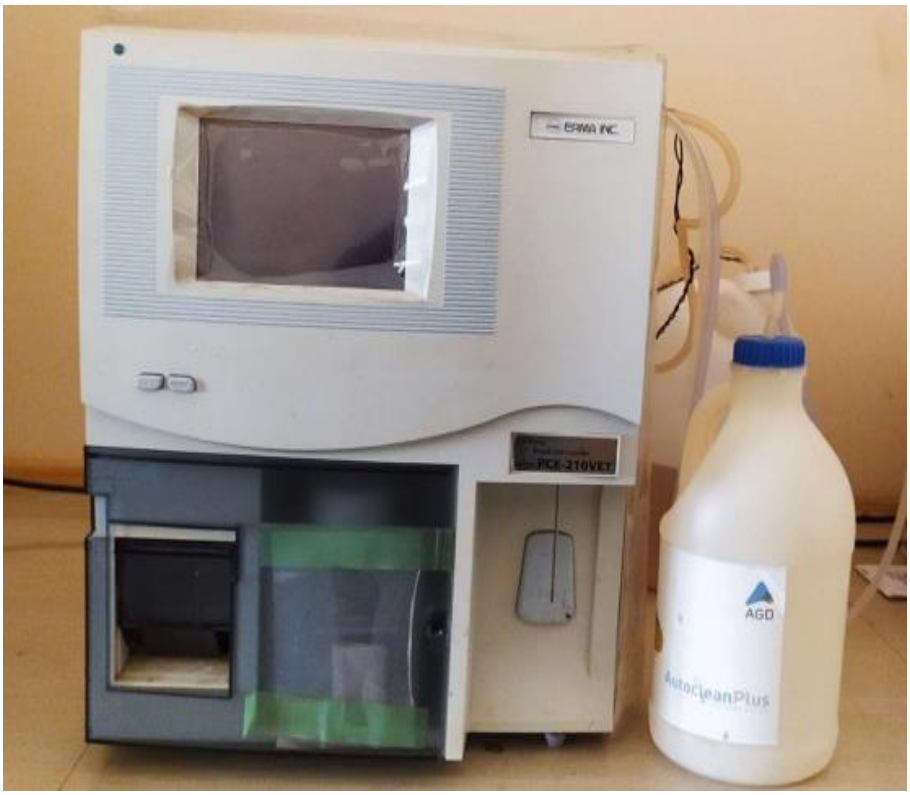


Plate 01: Automatic haematology analyser (ERMA PCE 210®)

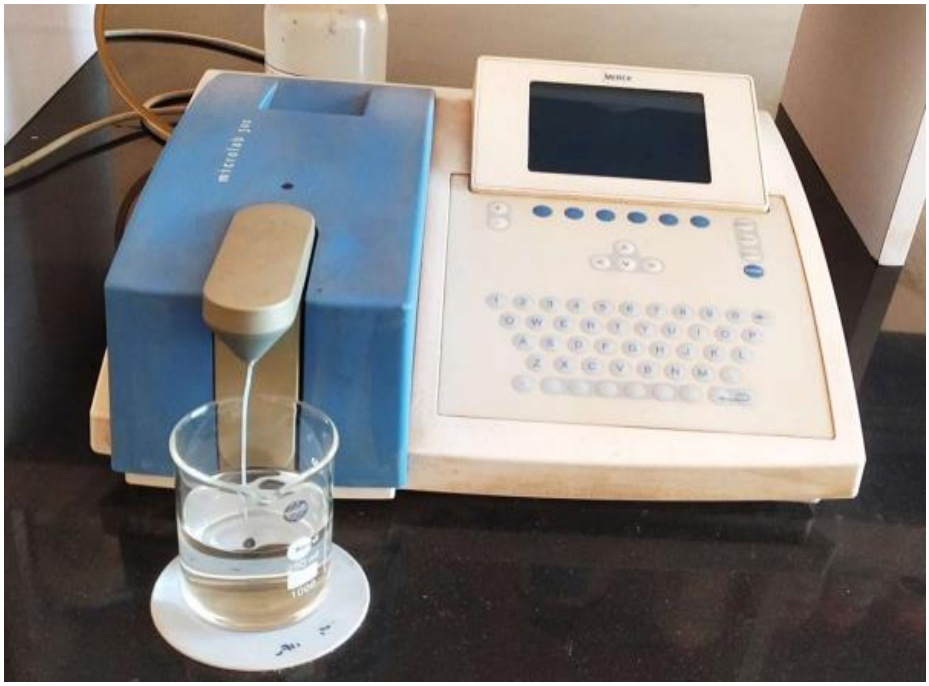


Plate 02: Semi-automatic biochemical analyser (MICROLAB-300®)

Plate 03: Thermocycler (S 1000)

Results

IV. RESULTS

In the present study, occurrence of contagious ecthyma (CE) in goats in and around Bidar was studied along with an attempt to determine the systemic infection and therapeutic efficacy in contagious ecthyma The results pertaining to them have been presented as here under.

4.1 Prevalence study

4.1.1 Overall prevalence of contagious ecthyma in suspected goats cases

A retrospective study of contagious ecthyma was carried out for a period of 1 year (January 2022 to December 2022) for which the records of the outpatient ward, Veterinary Clinical Complex, Veterinary College, Bidar were utilized to gather data on contagious ecthyma in goats. In the present investigation a total of 1642 goats presented during the study period, 195 goats were suspected for contagious ecthyma. Hence, the prevalence of contagious ecthyma was 11.87 per cent The details regarding the rate of occurrence have been shown in Table -3 and Fig-1

4.1.2 Prospective study

A total of 258 suspected cases of contagious ecthyma in goats in and around Bidar were utilized for prospective study. Various parameters like animal particulars, vital parameters and clinical signs which were suggestive of CE were collected.

4.1.2.1 Age-wise point prevalence of contagious ecthyma in goats (n=258)

The detail regarding age-wise point prevalence of contagious ecthyma in goats has been presented in Table-4 and Fig-4. Highest occurrence of contagious ecthyma in goats was observed in the age group of 0 to 6 month (42.25%) followed by the age group 6 month to 1 year (38.76%) and least in age group above 1 years (18.99%).

4.1.2.2 Gender- wise point prevalence of contagious ecthyma in goats (n= 258)

The details regarding the gender-wise point prevalence of contagious ecthyma in goats have been shown in Table-5 and Fig-5. In goats, females were found to be more affected with a percentage of 62.40% compared to the males with 37.60% of occurrence.

4.2 Clinical changes in contagious ecthyma in goats (n=258)

In the present study, the occurence of contagious ecthyma in goats, the clinical signs exhibited by the animals affected with contagious ecthyma were recorded. The affected animals appeared dull, weak and showed loss of appetite (83.33%), increase in body temperature (66.66%) which varied from 102-104°F, enlarged lymph node (27.77%), congested mucus membrane (50%), respiratory distress (50%) as shown in Table-6 and nasal discharge in some animals which varied from watery, mucoid to thick purulent in appearance. Some animals especially, the young ones showed debilitation.

The affected goats exhibited characteristic scabby lesions on oral commissures, lip, muzzle, gum, nostril, ear and teat.

4.2.1 Scabby lesions

Scabby lesions in CE affected goats varied in distribution on the body parts. All 258 goats showed scabby lesions (100.00 %). Among them 212 cases had lesions on oral commissures (82.17 %), 178 cases showed lesions on lip (68.99 %), 134 cases with lesion on muzzle (51.93 %), 68 cases had lesions on gums (26.35 %), 126 cases had lesions nostrils (48.83 %), 26 cases had lesions on ear (10.07 %), 1 case had lesion on udder (0.03 %) and 1 case on teat (0.03 %) as depicted in Table-7.

4.3 Hematological and Biochemical alterations in contagious ecthyma in goats (n=18)

4.3.1 Hematological alterations in contagious ecthyma in goats (n=18)

In the present study, blood sample were collected from 18 positive cases and subjected for various hematological parameters. The parameters were compared with the apparently healthy goats values. The details regarding the hematological alterations have been shown in Table-8.

4.3.1.1 Haemoglobin (g/dL)

The mean ± SE values of haemoglobin (Hb) in healthy control animal (group I) and CE affected goat (group II) were depicted in Table-8 and Fig.5. The average values of haemoglobin in group I and II were 12.53 ± 0. 11 and 12.33 ± 0.27 respectively. Comparison between group I and II revealed no significant difference in Hb values.

4.3.1.2 Total erythrocyte count (TEC x 10^6 /µL)

The mean ± SE values of total erythrocyte count (TEC) in healthy control animal (group I) and CE affected goat (group II) were depicted in Table-8 and Fig.6. The

average values of TEC in group I and II were 14.36 ± 0.61 and 14.65 ± 0.39 respectively. Comparison between group I and II revealed no significant difference in TEC values.

4.3.1.3 Packed cell volume (%)

The mean ± SE values of PCV (%) in healthy control animal (group I) and CE affected goat (group II) were depicted in Table-8 and Fig.7. The PCV (%) values in group I and II were 30.38 ± 0.39 and 32.63 ± 0.91 respectively. Comparison between group I and II revealed no significant difference in PCV values.

4.3.1.4 Total Leucocyte Count (TLC x 10^3 /µL)

The mean ± SE values of total leucocyte count (TLC) in healthy control animal (group I) and CE affected goat (group II) were depicted in Table-8 and Fig.8. The TLC values in group I and II were 12.13 ± 0.12 and 13.95 ± 0.88 respectively. There was a significant increase ($p \leq 0.05$) in animals of group II compared to group I.

4.3.1.5 Total Platelet count (x10^3 /µL)

The mean ± SE values of total platelet count in healthy control animal (group I) and CE affected goat (group II) were depicted in Table-8. The average values of PLT in group I and II were 179.4 ± 1.69 and 139.77 ± 9.07 respectively. Significant decrease ($p \leq 0.05$) in mean platelet count was observed in group II animals compared to group I animals.

4.3.1.6 Differential Leucocyte Count (%)

4.3.1.6.1 Lymphocyte count (%)

The mean ± SE values of lymphocyte count in healthy control animal (group I) and CE affected goat (group II) were depicted in Table-8. The lymphocyte count (%) values in group I and II were 62.66 ± 2.55 and 70.88 ± 3.66 respectively. There was no significant difference in lymphocyte count between the groups.

4.3.1.6.2 Neutrophil count (%)

The mean ± SE values of neutrophil count in healthy control animal (group I) and CE affected goat (group II) were depicted in Table-8. The average values of neutrophil count (%) in group I and II were 29.66 ± 2.80 and 22.27 ± 3.54 respectively. No significant difference observed in group I and II animals.

4.3.1.6.3 Eosinophil count (%)

The mean ± SE values of eosinophil count in healthy control animal (group I) and CE affected goat (group II) were depicted in Table-8. The average values of eosinophil count (%) in group I and II were 5.00 ± 0.57 and 4.27 ± 0.39 respectively. Comparison between group I and II revealed no significant difference.

4.3.1.6.4 Monocyte count (%)

The mean ± SE values of monocyte count in control animal (group I) and CE affected goat (group II) were depicted in Table-8. The average values of monocyte count (%) in group I and II were 2.16 ± 0.30 and 2.00 ± 0.25 respectively. Comparison between group I and II revealed no significant difference.

4.3.1.6.5 Basophil count (%)

The mean ± SE values of basophil count in control animal (group I) and CE affected goat (group II) were depicted in Table-8. The average values of basophil count (%) in group I and II were 0.50 ± 0.22 and 0.55 ± 0.18. Comparison between group I and II revealed that there is no significant difference.

4.3.2 Biochemical alterations in contagious ecthyma in goats

4.3.2.1 Aspartate aminotransferase (IU/L)

The mean ± SE values of serum aspartate aminotransferase (AST) levels in healthy control animal (group I) and CE affected goat (group II) were depicted in Table-9 and Fig.11. The AST (IU/L) values in group I and II were 104.58 ± 3.49 and 38.69 ± 0.94 respectively. There was significant ($p \leq 0.05$) decrease in the mean AST values in CE affected goat (group II) compared to group I.

4.3.2.2 Alanine transaminase (IU/L)

The mean ± SE values of serum alanine transaminase (ALT) levels in healthy control animal (group I) and CE affected goats (group II) were depicted in Table-9 and Fig.12. The average values of ALT (IU/L) recorded in animals of group I and II were 20.08 ± 1.00 and 37.67 ± 1.37 respectively. There was significant ($p \leq 0.05$) increase in the mean ALT values in CE affected goat (group II) compared to group I.

4.3.2.3 Total protein (g/dL)

The mean ± SE values of serum total protein (TP) levels in healthy control animals (group I) and CE affected goat (group II) were depicted in Table-9 and Fig. 9.

The TP values (g/dL) in group I and II were 6.55 ±0.23 and 5.75 ± 0.14 respectively. Comparison between group I and II revealed that serum TP levels did not vary significantly between the groups.

4.3.2.4 Serum albumin (g/dL)

The mean ± SE values of serum albumin levels in healthy control animal (group I) and CE affected goat (group II) are depicted in Table-9 and Fig.10. The average values of serum albumin (g/dL) in group I and II were 3.85 ± 0.09 and 3.26 ± 0.08 respectively. Comparison between group I and II revealed that serum TP levels did not vary significantly among the groups.

4.4 Diagnosis

4.4.1 Diagnosis of contagious ecthyma in goats by polymerase chain reaction

In the present study, occurrence of contagious ecthyma was confirmed by polymerase chain reaction for B2L gene of parapox Orf virus. The details regarding results of polymerase chain reaction have been shown in Table-10 and Plate -4.

A total of 25 scab and skin biopsy samples were subjected for PCR. Out of 25 CE suspected field outbreak samples 24 were positive for B2L gene and only one was negative to B2L gene. The total percentage positivity was 96.00 percent.

4.4.2 DNA sequencing and Phylogenetic analysis

B2L gene (1206 bp) sequences from the four CE positive samples by PCR were collected from the goats in and around Bidar for phylogenetic analysis. To determine the

genetic relationship between ORFV strains from India and those circulating in other countries, the sequences of nucleotides of B2L gene were aligned with other sequences registered with Genebank and Phylogenetic analysis showed that Orf virus Bhalki-1 KA and Orf virus Bidar-1 KA, Orf virus Bhalki-2 KA, and Orf virus Bidar-2 KA were similar and shared homologous sequence identity with JQ040300, KU128538 (India), KU597730 (India), KX129982 (India), KX377974 (India), MF462354 (India) and also revealed close sequence identity with KF666565, KF666560, KC485343 Chinese isolates followed by other countries (Plate 5).

In Bidar region, no previous study on phylogenetic analysis of CE sample was reported so, the sequences were compared with other known sequences in phylogenetic tree. Phylogenetic tree construction revealed that the current study isolate was having 98.7 to 99.8 per cent sequence identity with ORFV isolated from state (Plate 6). The phylogenetic analysis confirmed the presence of ORFV in the clinical samples.

4.4.3 Pathology

4.4.3.1 Histopathology of scab/skin lesions of contagious ecthyma

In the present study, the scab and skin biopsy samples collected from contagious ecthyma positive cases were subjected for detailed pathological examination.

The changes observed in epidermal layers were thick keritinized crust composed mainly of necrotic cell debris seen overlying the epidermis plate. Hyperkeratosis, parakeratosis and varying degree of degenerative changes were noticed in the epidermis showing ballooning degeneration, vacuolation, pyknosis, loss of nucleus with

inflammatory cell infiltration between the epidermal cells (Plate 7, 8, 9). In some of skin biopsy the microscopic lesions were advanced with vesicle formation (Plate 10, 11), pustular formation (Plate 12), and some showing loss of layers of epidermis with ulcer formation, necrosis and severe inflammation of inflammatory cells (Plate 13) and rarely some of the epidermal cells revealed presence of eosinophilic intracytoplasmic inclusion bodies (Plate 14) Microscopic lesions in some of the skin biopsy are characterized hyperkeratotic and acanthotic changes leading to deep downward growth (rete pegs) of the hyperplastic epithelial layers towards dermis with papillae projections (Plate 15, 16).

Other changes comprises of hyperplasia of hair follicle, sebaceous glands and hair follicle with thickening of wall and deep dermis also revealed severe inflammation with infiltration of mononuclear and polymorphonuclear cell infiltration (plate 17). Some of the skin biopsies revealed presence of necrosis of muscle fibers with disintegration and loss of striations with infiltration of inflammatory cells (Plate 18).

4.5 Therapeutic efficacy of certain ayurvedic and homeopathic drugs

In group 1 four goats (66.00 %) out of six goats showed complete recovery (Plate 20) and all goats in group 3 (100.00%) showed complete recovery on 7^{th} day post treatment (Plate 22). None of the goats in group 2 showed recovery on 7^{th} day post treatment (Plate 21).

The results obtained during the clinical study revealed that goats which were treated with oral preparation of Thuja occedentalis mother tincture @ 10 drops per os twice a day (Group 3) and goats treated with Thuja ointment (Group 1, Plate 19) showed better results compared to goats treated with application of Aloe-vera gel and turmeric in

1:1 ratio (Group 2) considering complete resolution of clinical signs and scabby lesions on 7th day post treatment (Along with parenteral antibiotic and anti-inflammatory to all groups) (Table 13).

Table 3: Retrospective study of contagious ecthyma in goats during the year 2022

Month	Total number of goats	Suspected cases of contagious ecthyma	Prevalence (%)
January	112	10	8.92
February	123	16	13
March	87	8	9.19
April	102	21	20.58
May	114	12	10.52
June	126	20	15.87
July	156	38	24.35
August	172	26	15.11
September	185	24	12.97
October	154	18	11.68
November	143	0	0
December	168	2	1.19
Total	**1642**	**195**	**11.87**

Table 4: Age-wise point prevalence of contagious ecthyma in goats (n=258)

Age group	No. Of animals	Point prevalence (%)
0-6 months	109	42.24
6m-1yr	100	38.76
Above 1yr	49	18.99
Total	**258**	**100.00**

Table 5: Gender-wise point prevalence of contagious ecthyma in goats (n=258)

Gender	No. Of animals	Point prevalence (%)
Male	97	37.59
Female	161	62.40
Total	**258**	**100.00**

Table 6: Frequency of clinical signs in contagious ecthyma affected goats

Sl. No.	Clinical signs	Number of goats affected (n=18)	Percent
1	**Fever**	12	66.66
2	**Mucus membrane**		
	Congested	10	55.55
	Pink	8	44.44
3	**Prescapular lymph nodes**		
	Normal	13	72.22
	Enlarged	5	27.77
4	**Inappatence and anorexia**	15	83.33
5	**Scabby lesions**	18	100.00
6	**Respiratory distress**	9	50.00

Table 7: Distribution of scabby lesions in contagious ecthyma affected goats (n=258)

Site of lesion	No. of animals	Per cent
Oral commissures	212	82.17
Lip	178	68.99
Muzzle	134	51.93
Nostril	126	48.83
Gums	68	26.35
Ear	26	10.07
Udder	1	0.03
Teat	1	0.03

Table 8: Mean ± SE of haematological values in the healthy and contagious ecthyma affected goats

Parameter	Healthy Control(n=6)	CE affected goat (n=18)
Hb (g/dL)	12.53 ± 0.11	12.33 ± 0.27
TEC (x 10^6 /µL)	14.36 ± 0.61	14.65 ± 0.39
PCV (%)	30.38 ± 0.39	32.63 ± 0.91
TLC (x 10^3 /µL)	12.13 ± 0.12^a	13.95 ± 0.88^b
N (%)	29.66 ± 2.80	22.27 ± 3.54
L (%)	62.66 ± 2.55	70.88 ± 3.66
E (%)	5.00 ± 0.57	4.27 ± 0.39
M (%)	2.16 ± 0.30	2 ± 0.25
B (%)	0.50 ± 0.22	0.55 ± 0.18
Platelets (x 10^3 /µL)	179.4 ± 1.69^a	139.77 ± 9.07^b

Mean±SE bearing different superscripts differ significantly ($P \leq 0.05$)

Table 9: Mean ± SE of Serum biochemical values in the healthy and contagious ecthyma affected goats

Parameter	Healthy Control (n=6)	CE affected goats (n=18)
Total protein (g/dL)	6.55 ± 0.23	5.75 ± 0.14
Albumin (g/dL)	3.85 ± 0.09	3.26 ± 0.08
AST (IU/L)	104.58 ± 3.49^{a}	38.69 ± 0.94^{b}
ALT (IU/L)	20.08 ± 1.00^{a}	37.67 ± 1.37^{b}

Mean±SE bearing different superscripts differ significantly between the group($P \leq 0.05$)

Table 10: Test results of ORFV infection in goat by gel based parapox virus PCR

Sample	Number of samples	PCR positive	Per cent
Scab	25	24	96.00

Table 11: Therapeutic efficacy of various preparation for resolution of scabby lesions on 7th day post treatment

Number of animals treated in each group	Number of animals cured		
	Group 1	**Group 2**	**Group 3**
06	4 (66.66%)	0 (0%)	6 (100%)

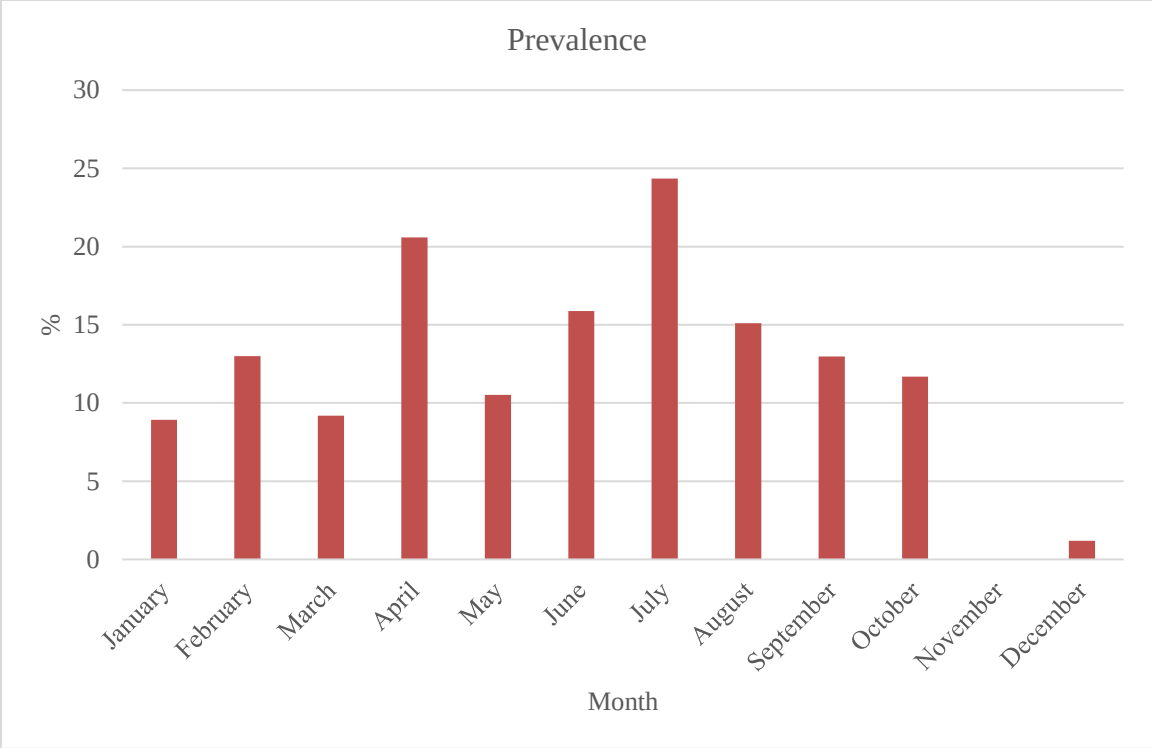


Fig. 1: Prevalence of contagious ecthyma in goats during year 2022

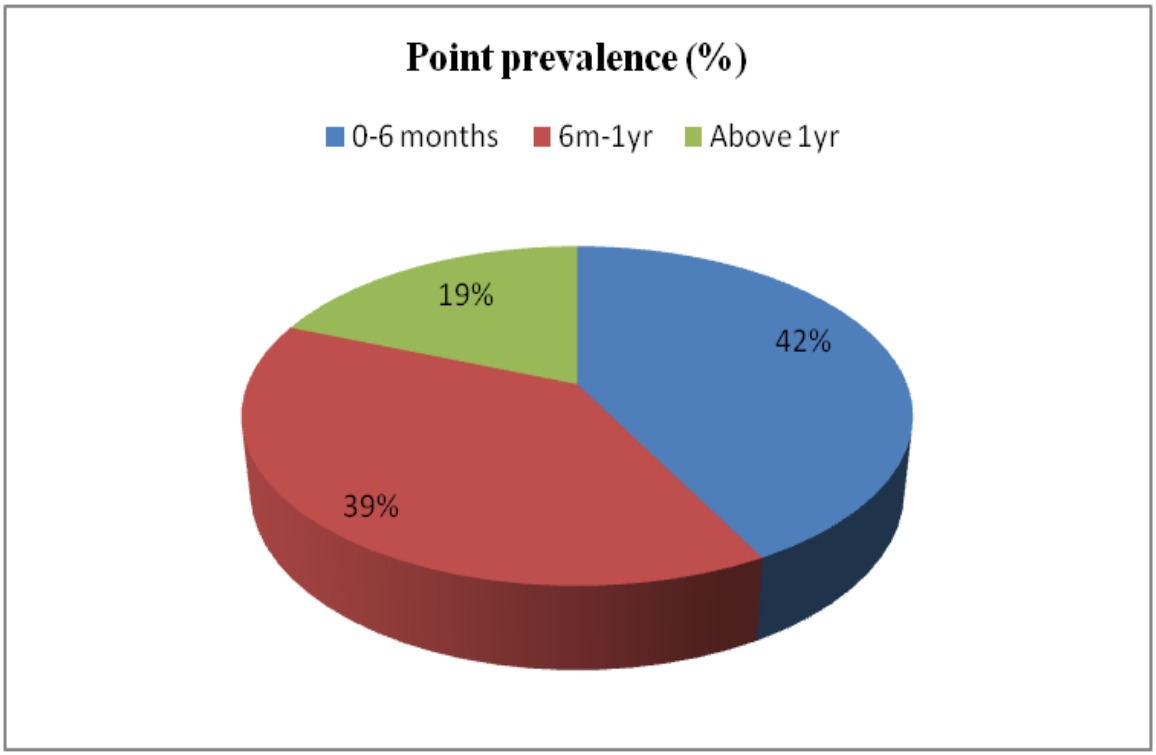


Fig. 2: Age-wise occurrence of contagious ecthyma in goats

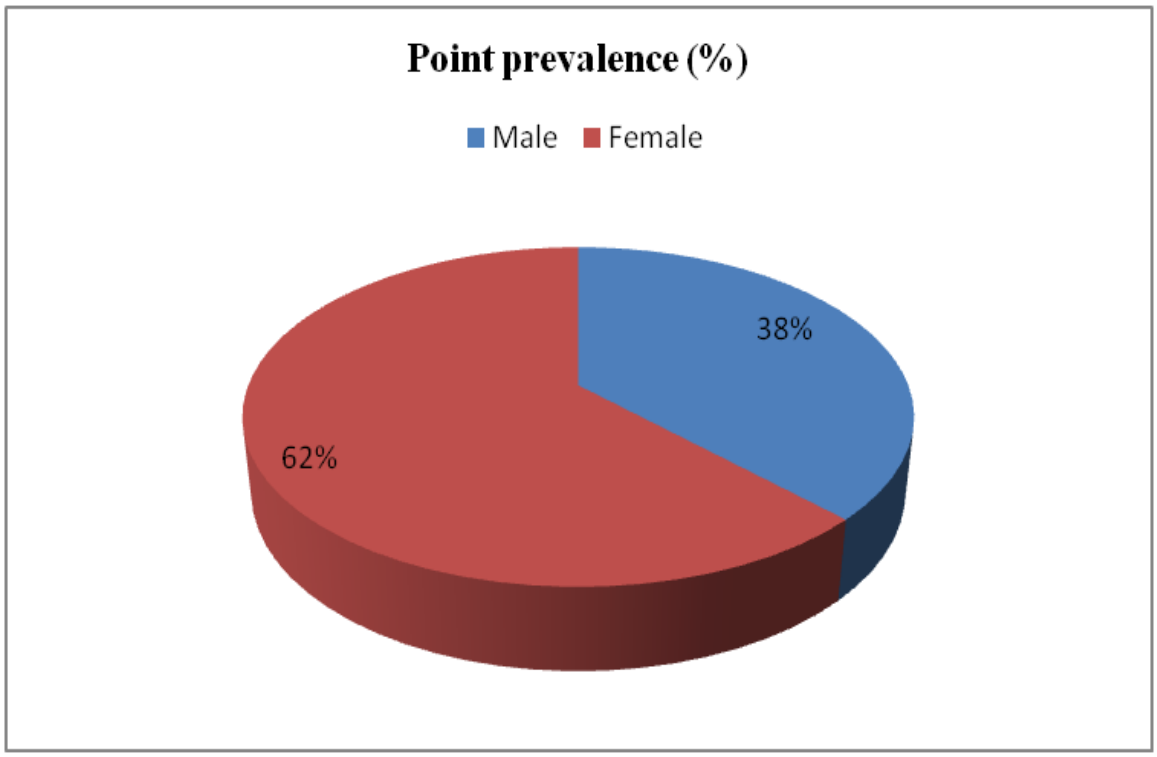


Fig. 3: Gender-wise occurrence of contagious ecthyma in goats

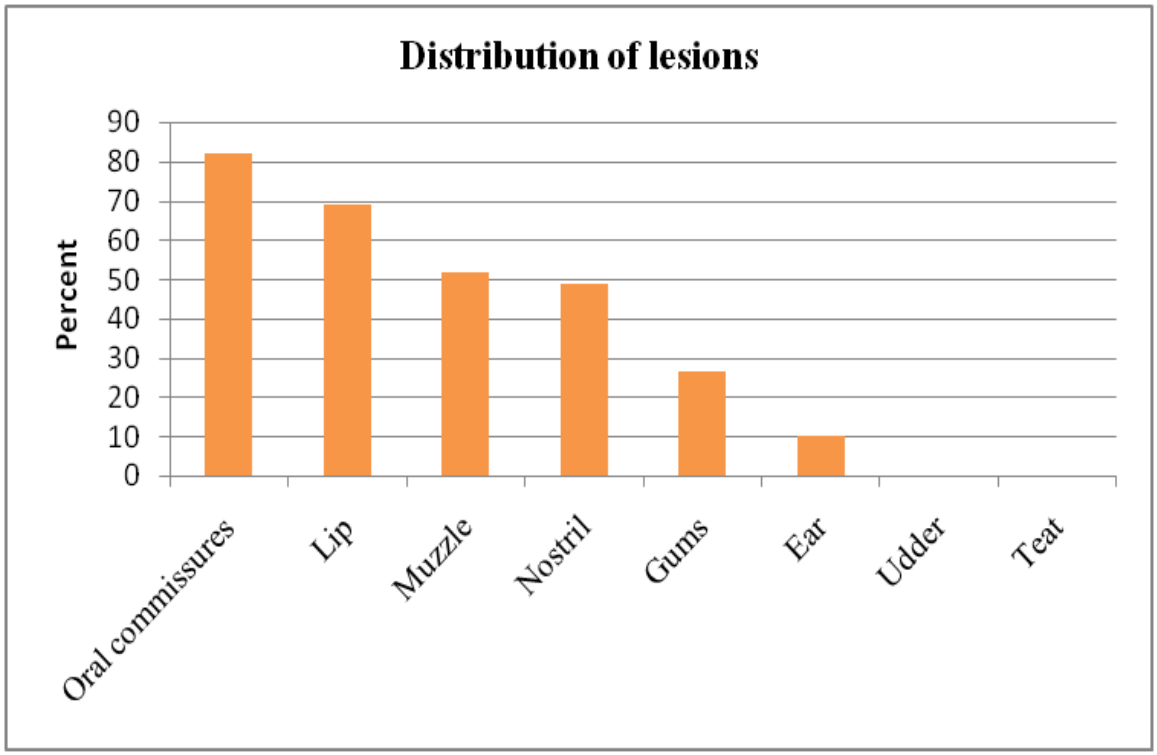


Fig. 4: Distribution of scabby lesions in contagious ecthyma affected goats

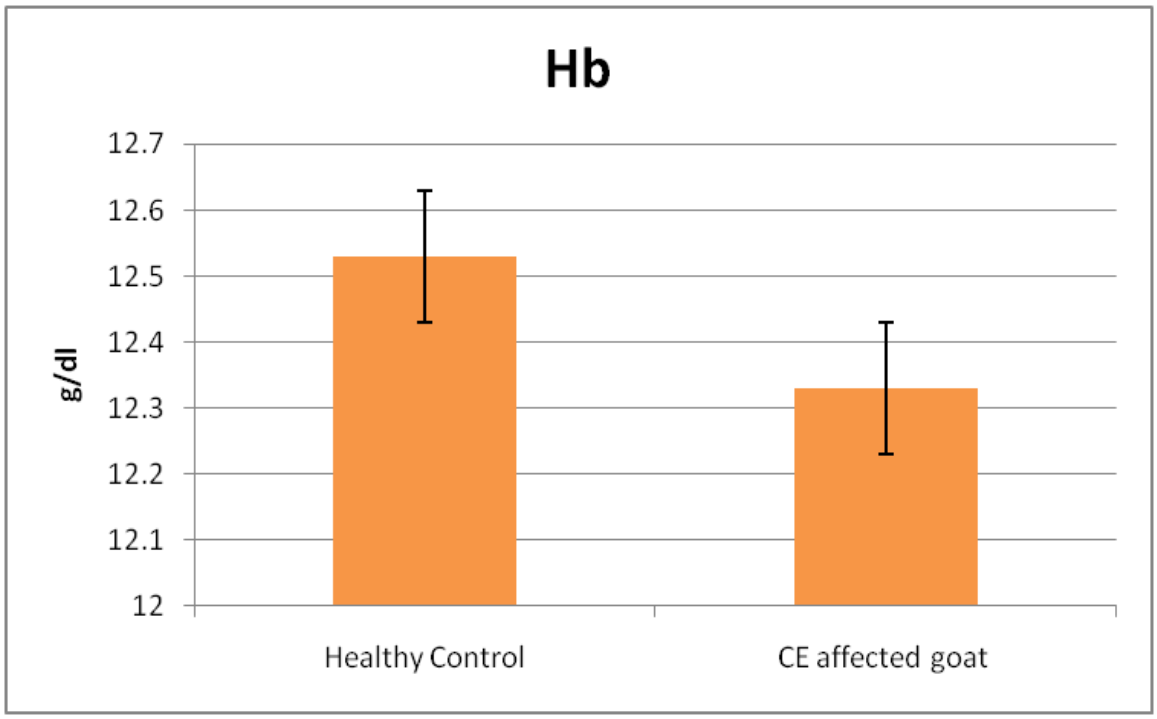


Fig. 5: Mean ± SE values of Haemoglobin in healthy control and contagious ecthyma affected groups

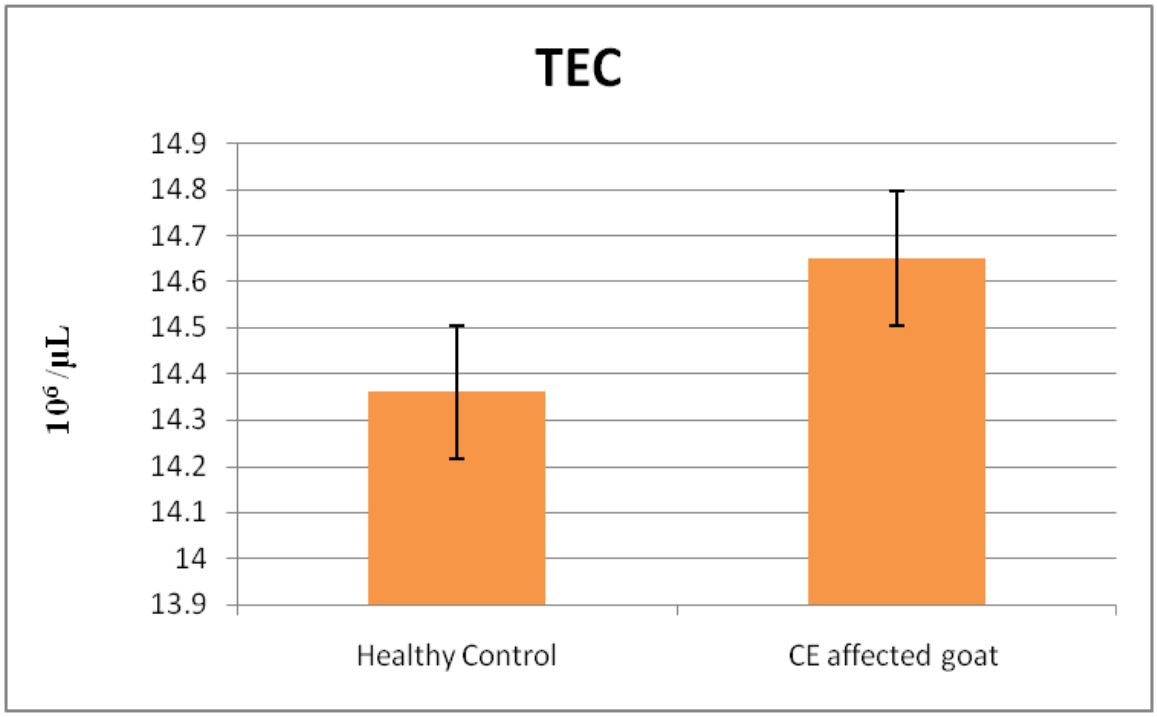


Fig. 6: Mean ± SE values of total erythrocyte count in healthy control and contagious ecthyma affected groups

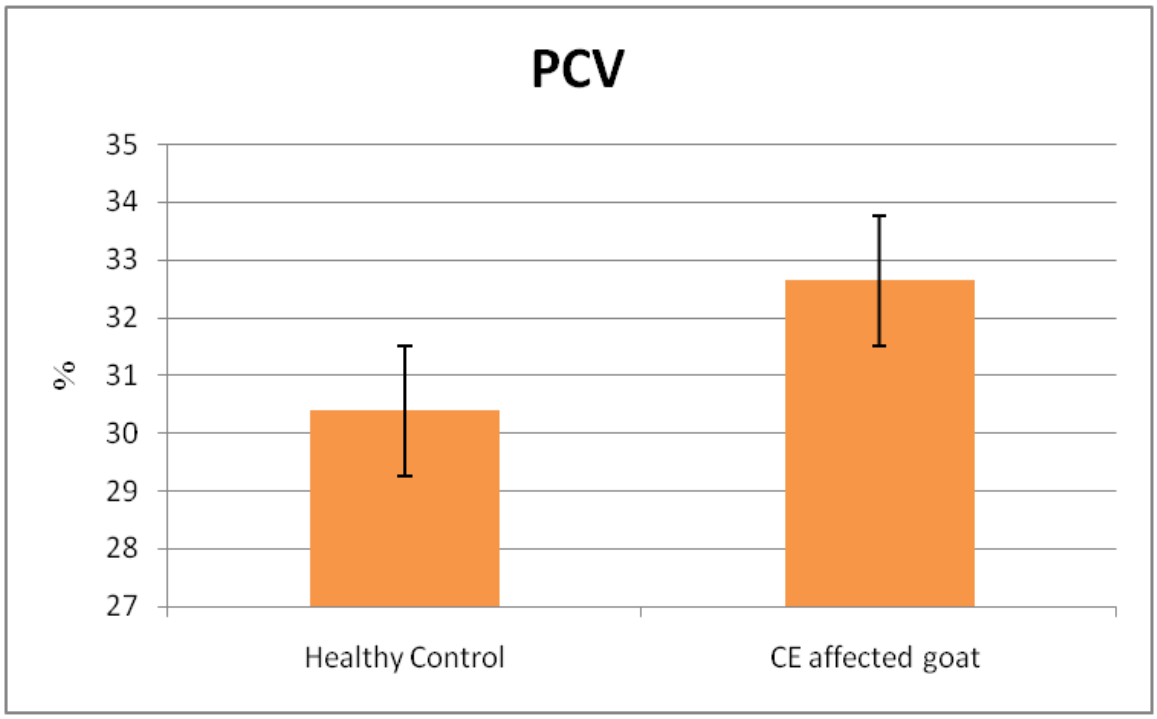


Fig. 7: Mean ± SE values of packed cell volume in healthy control and contagious ecthyma affected group

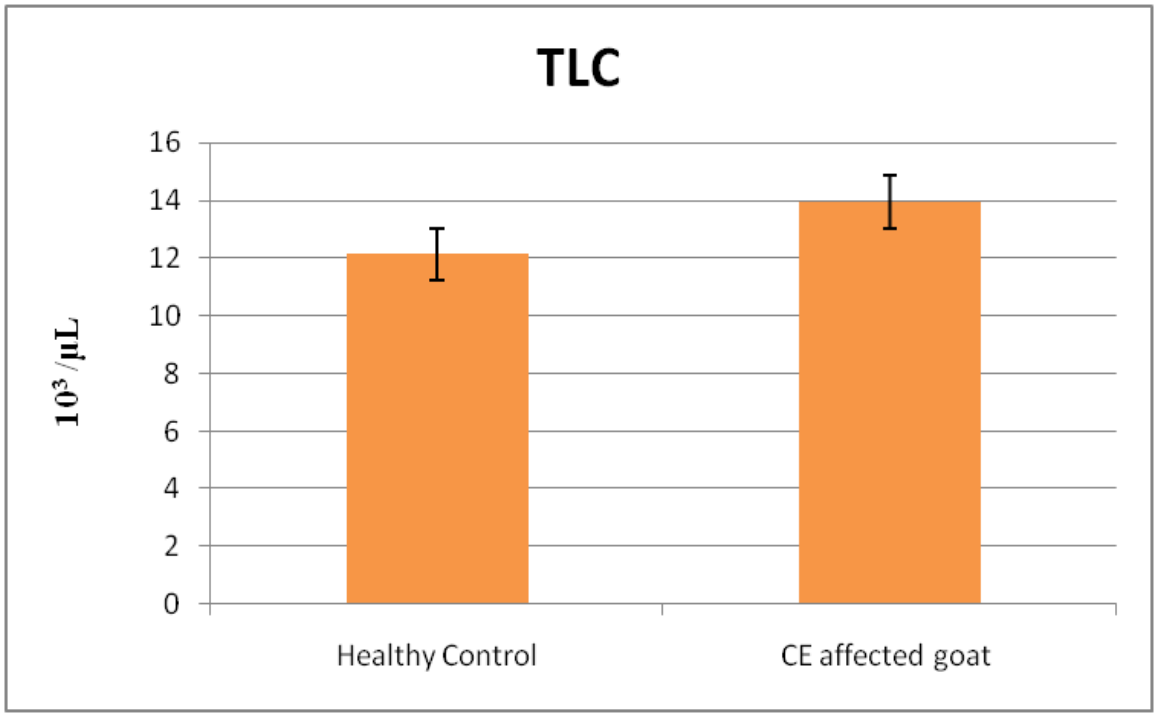


Fig. 8: Mean ± SE values of total leucocyte count in healthy control and contagious ecthyma affected groups

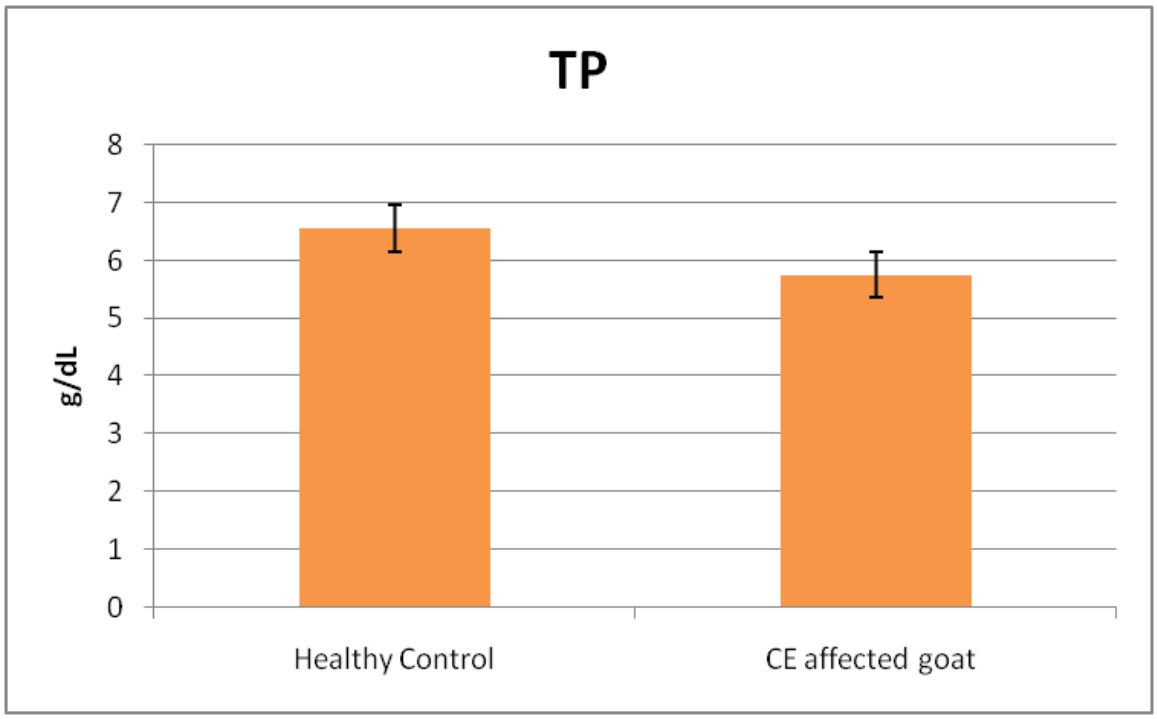


Fig. 9: Mean ± SE values of serum total protein in the healthy control and contagious ecthyma affected group

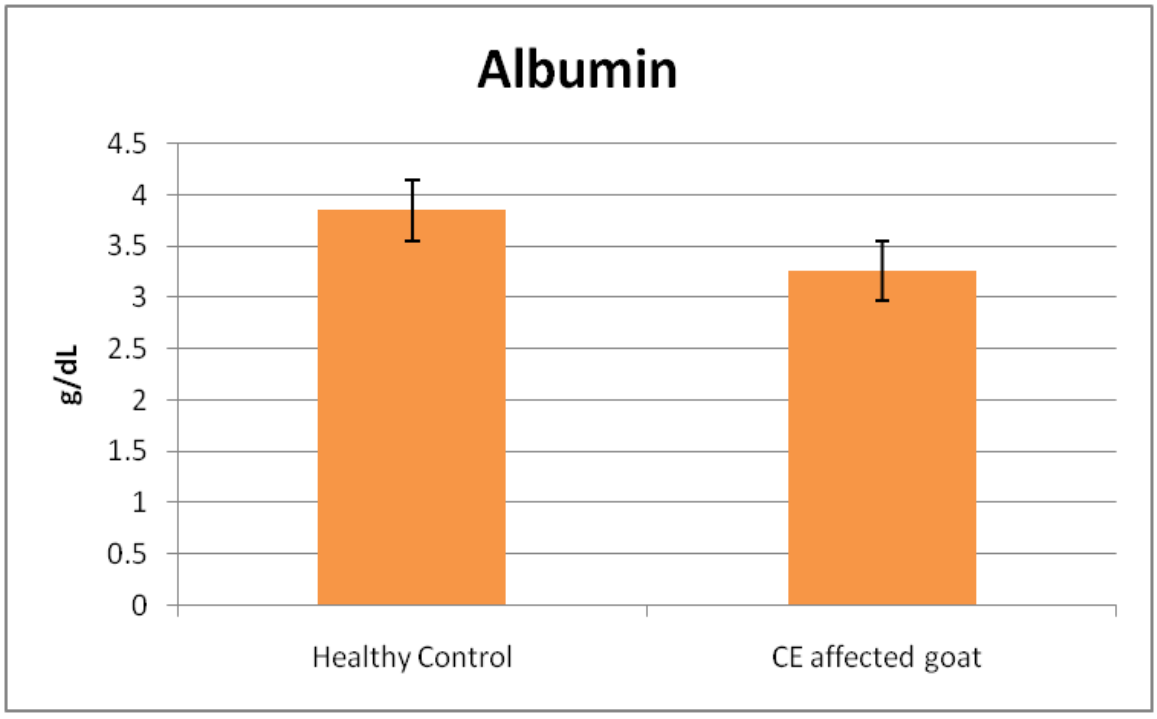


Fig. 10: Mean ± SE values of serum albumin in the healthy control and contagious ecthyma affected group

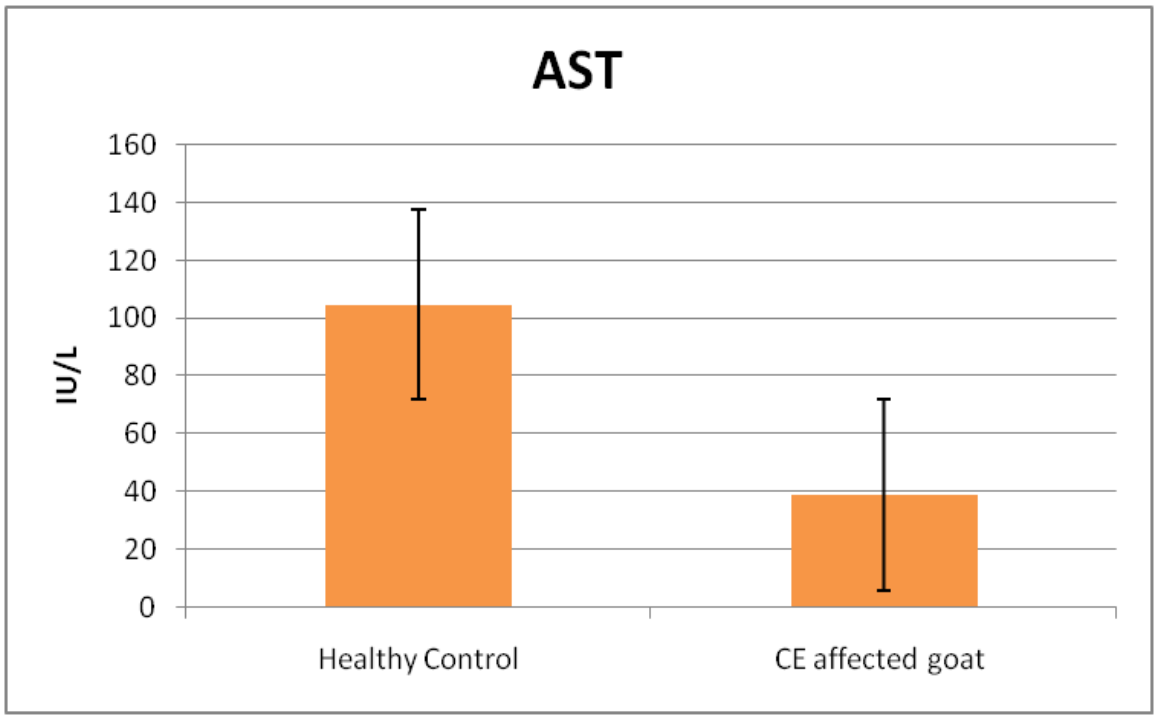


Fig. 11: Mean ± SE values of serum AST in the healthy control and contagious ecthyma affected group

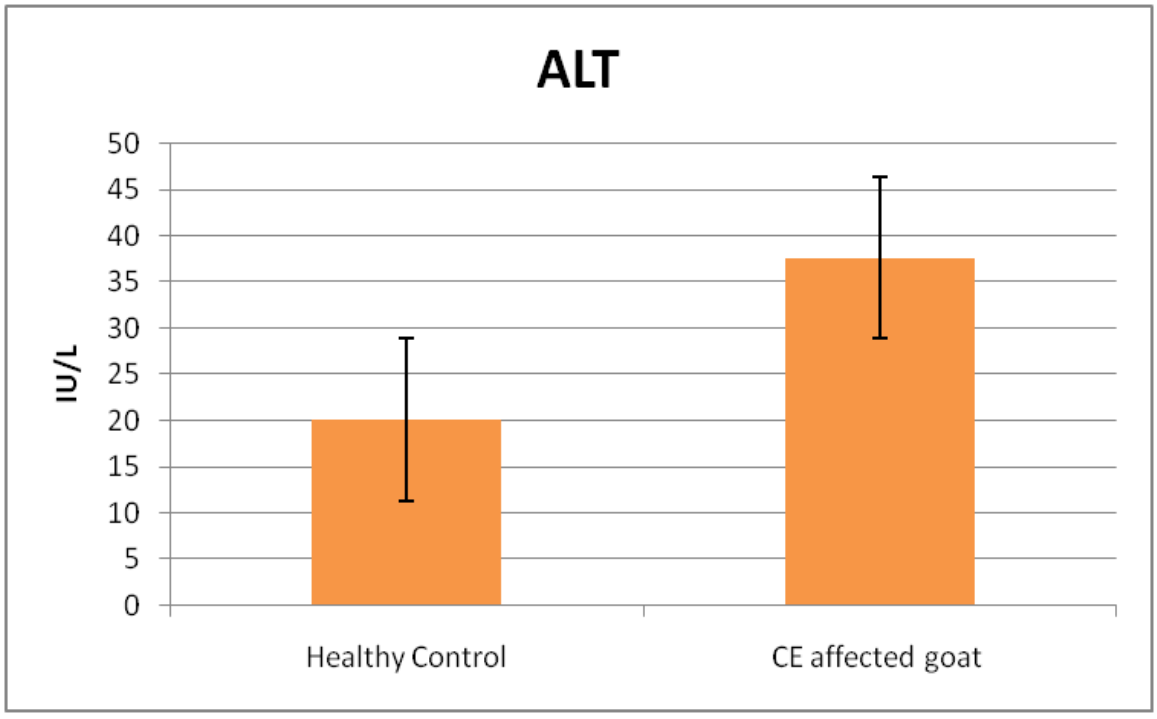


Fig. 12: Mean ± SE values of serum ALT in the healthy control and contagious ecthyma affected groups

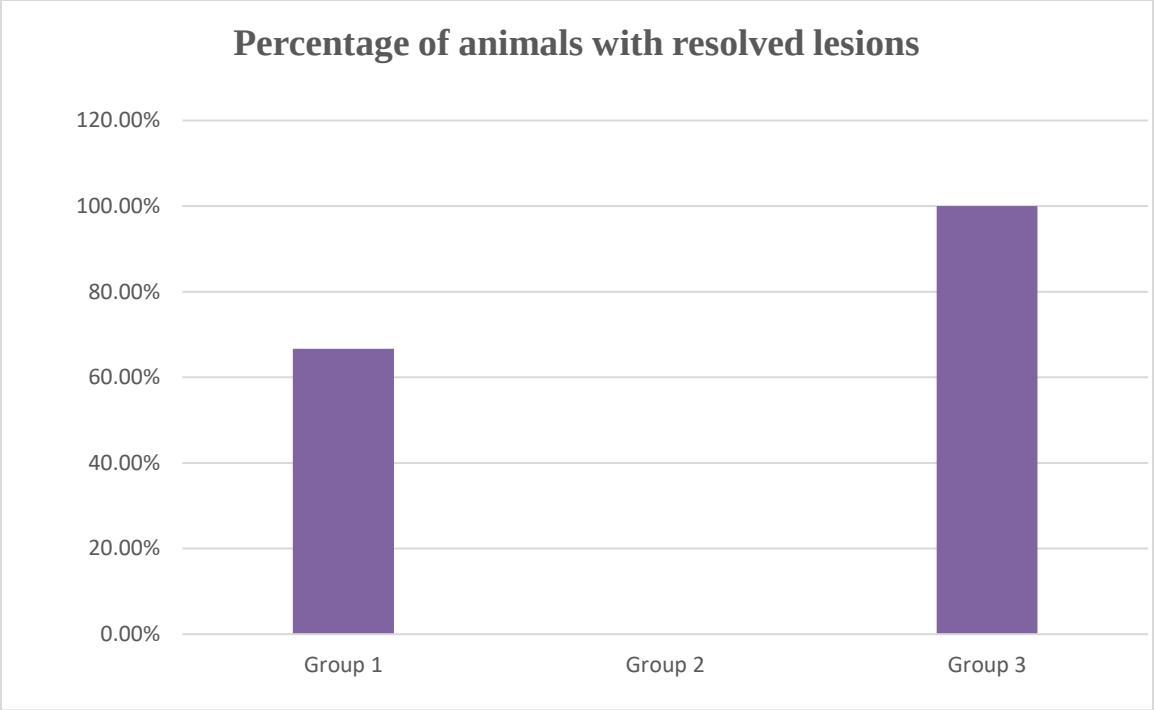


Fig. 13: Therapeutic efficacy of various preparation for resolution of scabby lesions on 7th day post treatment

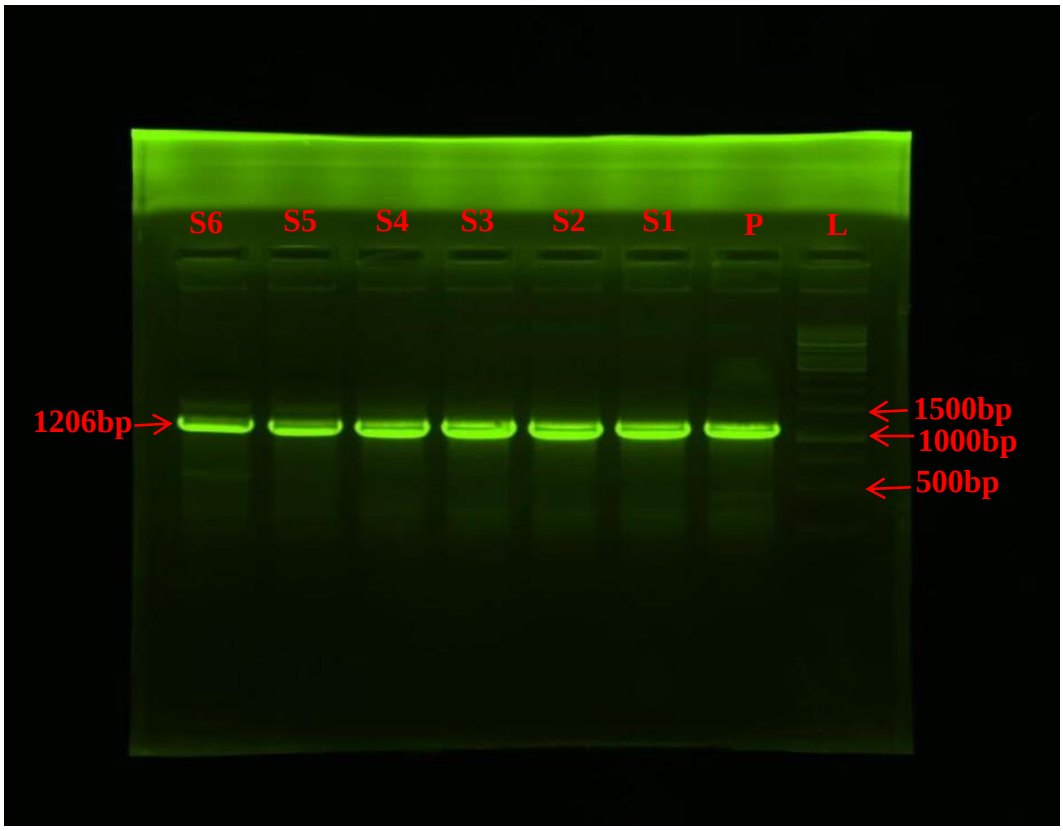


Plate 4: Results of PCR showing bands of 1206 bp encoding B2L gene of ORFV

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12		
1		99.3	100.0	100.0	99.6	99.5	99.6	99.6	99.5	99.5	99.1	99.6	**1**	Balki 1FE
2	0.7		99.3	99.6	98.9	98.7	98.9	98.9	98.7	98.7	98.7	98.9	**2**	Balki 2FE
3	0.0	0.7		100.0	99.6	99.5	99.6	99.6	99.5	99.5	99.1	99.6	**3**	Bidar 1FE
4	0.0	0.7	0.0		99.6	99.5	99.6	99.6	99.5	99.5	99.1	99.6	**4**	Bidar 2FE
5	0.4	1.1	0.4	0.4		99.8	99.6	99.6	99.5	99.8	99.1	99.6	**5**	JN846834
6	0.5	1.3	0.5	0.5	0.2		99.5	99.5	99.3	99.6	98.9	99.5	**6**	KU128538
7	0.4	1.1	0.4	0.4	0.4	0.5		99.6	99.5	99.5	99.1	99.6	**7**	MG334559
8	0.4	1.1	0.4	0.4	0.4	0.5	0.4		99.8	99.5	99.1	99.6	**8**	MH370052
9	0.5	1.3	0.5	0.5	0.5	0.7	0.5	0.2		99.3	98.9	99.5	**9**	MH370053
10	0.5	1.3	0.5	0.5	0.2	0.4	0.5	0.5	0.7		99.3	99.5	**10**	MH756172 EDITED
11	0.9	1.3	0.9	0.9	0.9	1.1	0.9	0.9	1.1	0.7		99.5	**11**	MT671192
12	0.4	1.1	0.4	0.4	0.4	0.5	0.4	0.4	0.5	0.5	0.5		**12**	OK169621
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**		

Plate 5: Phylogenetic analysis of ORF virus isolates from outbreak based on the complete nucleotide sequence of B2L gene. The percentages of bootstrap scores (100 replicates) are indicated on the branches. The scale bar represents genetic distance. The table represents Percent identity and divergence between the isolates from Indian subcontinent.

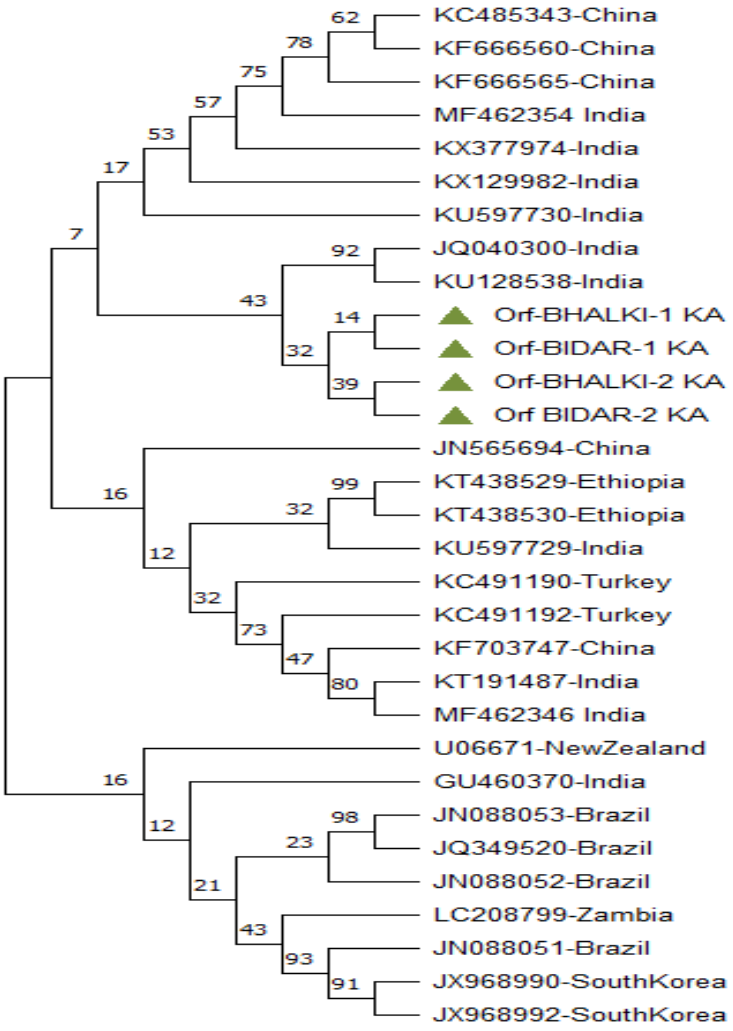


Plate 6: Phylogenetic analysis of B2L gene of ORFV. The sequences obtained from this study are labeled in filled triangles.

Evolutionary relationships of taxa:

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches.

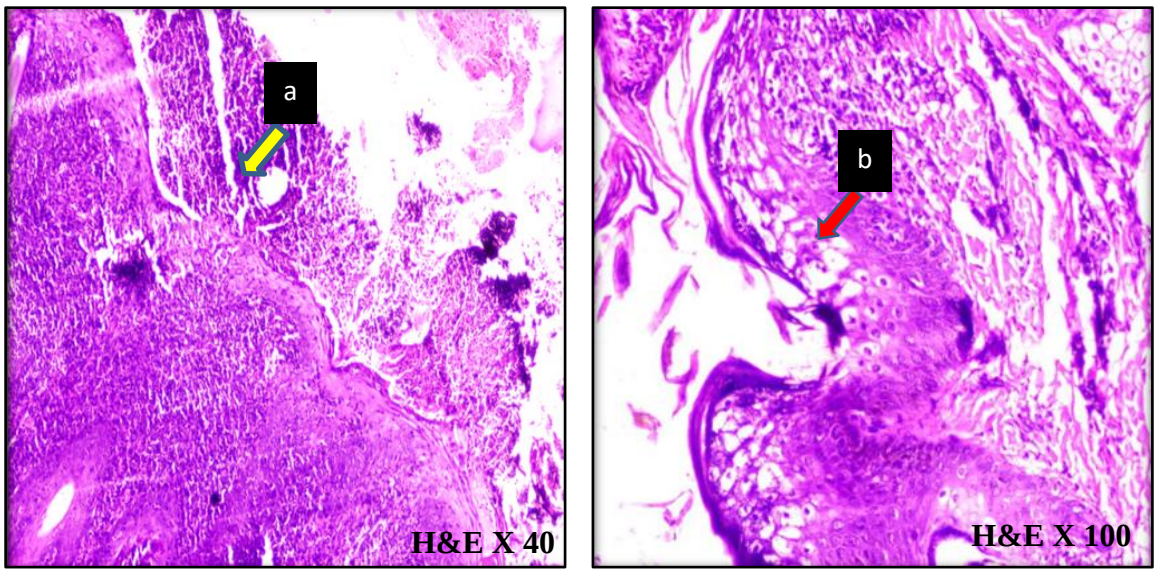


Plate 7, 8: Photomicrograph of contagious ecthyma mouth lesion from a goat showing a) Thick keratinized crust mainly composed of necrotic cell debris overlying the epidermis. b) Hyperkeratosis with balloning degeneration.

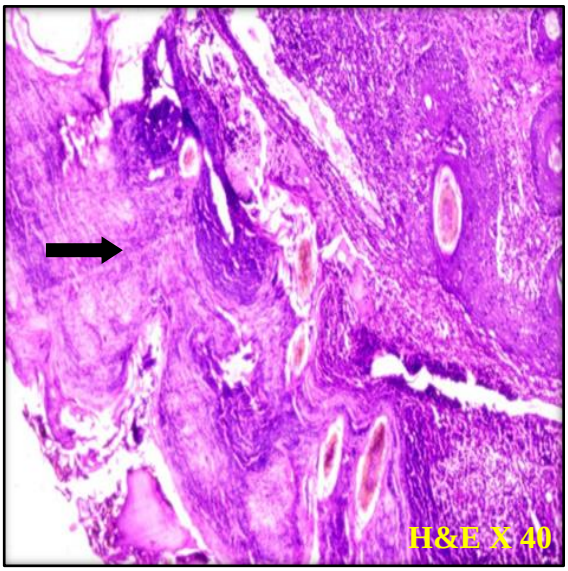


Plate 9: Plate Photomicrograph of contagious ecthyma mouth lesion from a goat showing severe hyperkeratosis of epidermis.

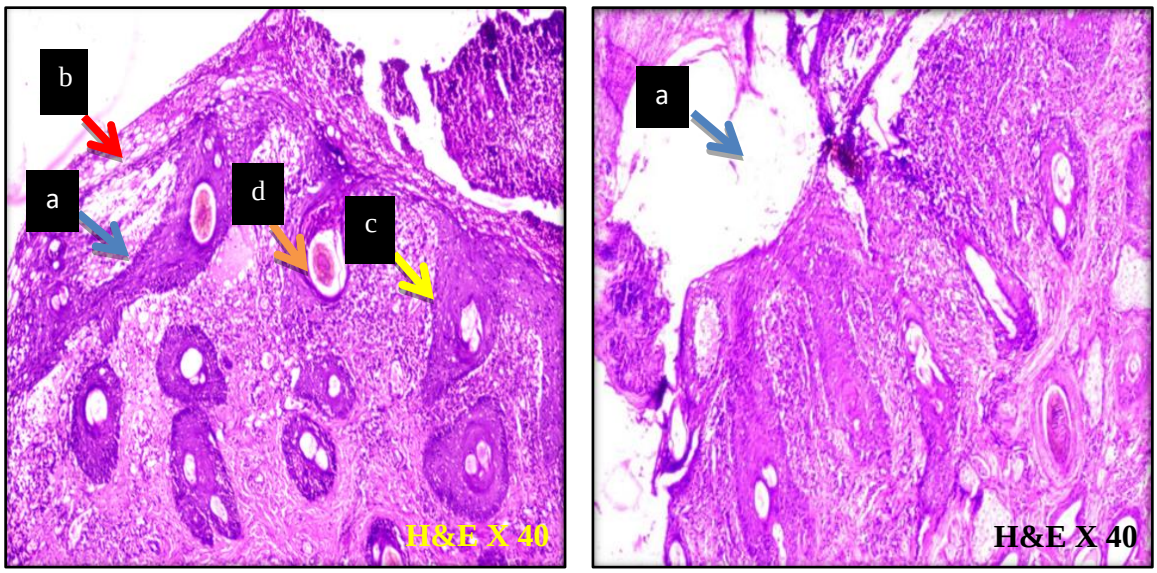


Plate 10, 11: Photomicrograph of contagious ecthyma mouth lesion showing a) Vesicle formation, b) Balloning degeneration, c) Hyperplastic epithelial cells with downwards growth, d) Hyperplasia of hair follicles.

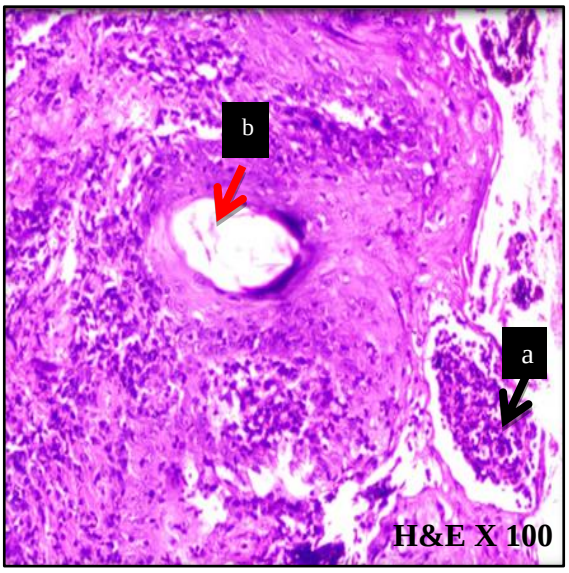


Plate 12: Photomicrograph of contagious ecthyma mouth lesion showing a) Pustule formation b) Severe hyperplasia of hair follicular layer with thickening of the wall.

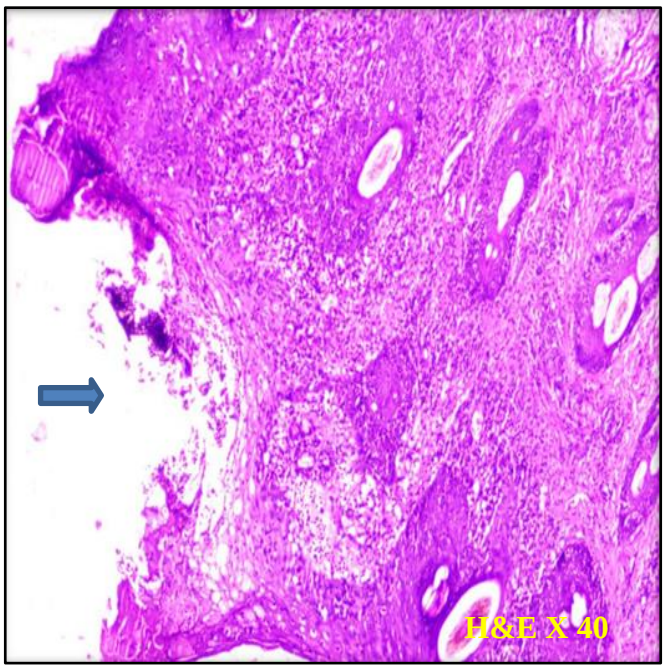


Plate 13: Photomicrograph of contagious ecthyma mouth lesion showing loss of layers of epidermis with ulcer formation and severe infiltration of inflammatory cells.

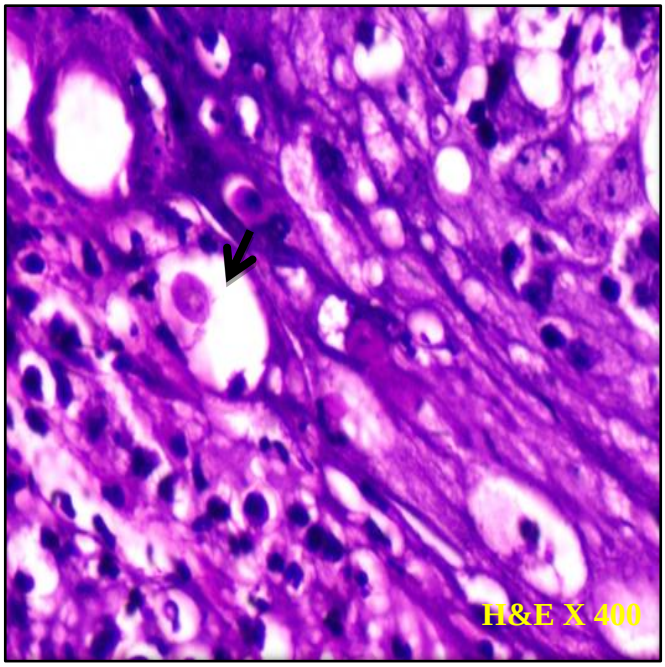


Plate 14: Photomicrograph of contagious ecthyma mouth lesion showing intra cytoplasmic eosinophilic inclusions (arrow) in the affected stratum spinosum cells.

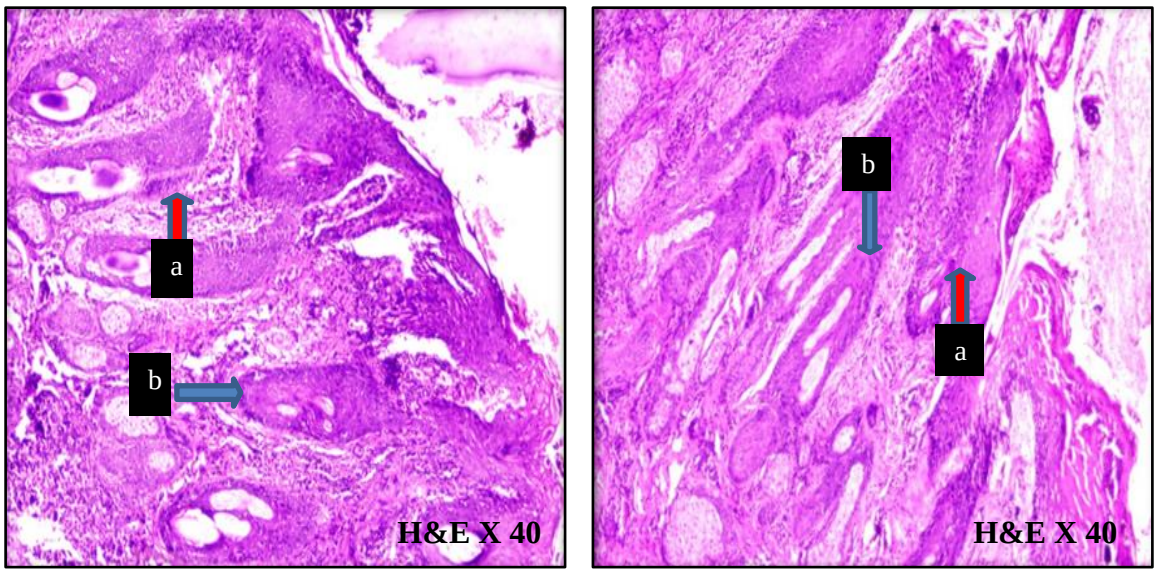


Plate 15, 16: Photomicrograph of contagious ecthyma mouth lesion showing a) acanthosis and b) formation of rete pegs extending deep into the dermis along with infiltration of inflammatory cells in the dermis.

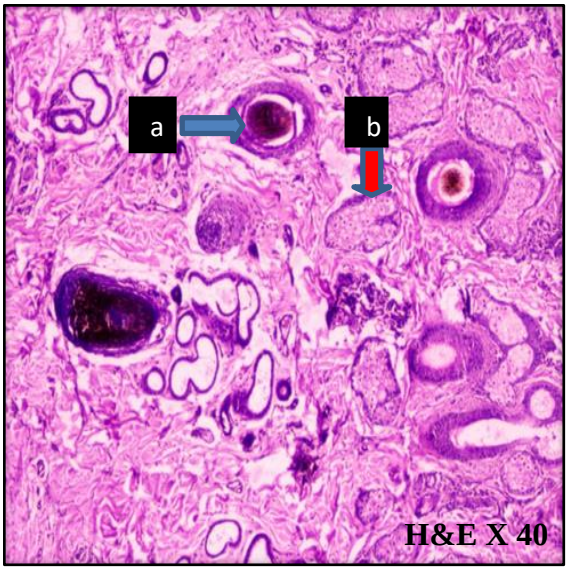


Plate 17: Photomicrograph of contagious ecthyma mouth lesion showing a) Hyperplasia of the hair follicle with thickening of the wall and b) Hyperplasia of sebaceous glands.

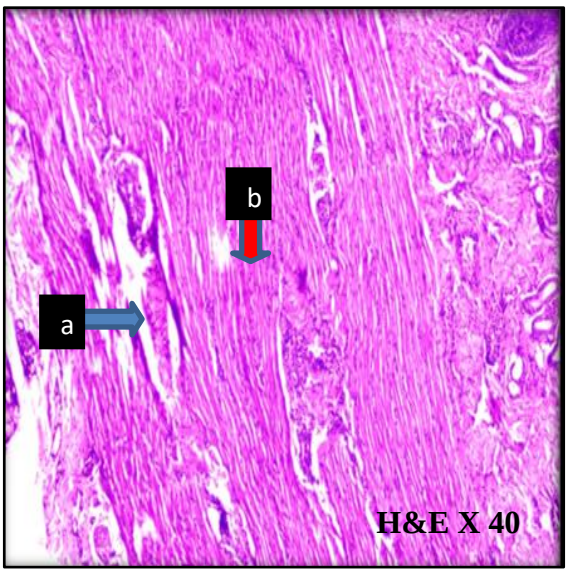
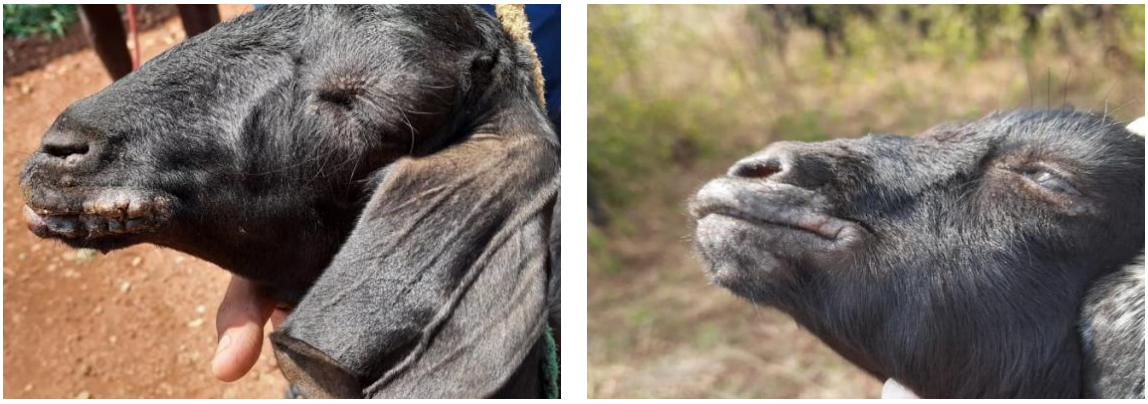


Plate 18: Photomicrograph of contagious ecthyma mouth lesion showing a) Necrosis of muscle fibers with infiltration of inflammatory cells, b) Disintegration and loss of striations.

Plate 19: Application of Thuja ointment externally on the lesions

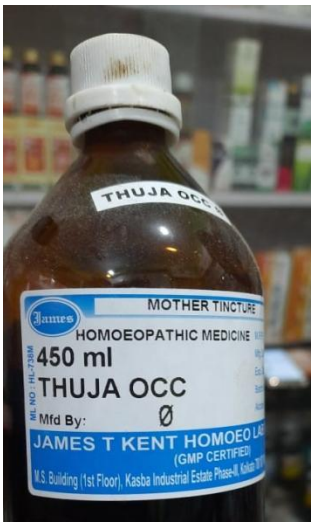


Pre-treatment **Post-treatment**

Plate 20: Case of contagious ecthyma: Pre-treatment and post treatment in group 1

Pre-treatment **Post-treatment**

Plate 21: Case of contagious ecthyma: Pre-treatment and post treatment in group 2



Thuja occidentalis oral drops

Drenching oral thuja drops

Pre-treatment

Post-treatment

Plate 22: A case of contagious ecthyma: Pre-treatment, and post treatment in group 3

Discussion

V. DISCUSSION

The results of prevalence, haematobiochemical alterations, diagnosis and therapeutic management of contagious ecthyma in goats are discussed.

5.1 Prevalence of contagious ecthyma in goats

In the present study a total of 1642 goats were presented to Veterinary Clinical Complex, Veterinary College Bidar, out of which a total of 195 animals were suspected for contagious ecthyma considering the characteristic scabby lesions accounting to 11.87 per cent of prevalence of contagious ecthyma in goats. Similar results were found by Bala *et al*. (2018) who reported prevalence of 14.4% in goats from 5 randomly selected farms. However Tedla *et al*. (2018) reported that the prevalence of Orf virus showed prevalence of 8.5% in goats and a high prevalence rate of 22.8% was reported by Bala *et al*. (2019).

Variation in the percentage prevalence of CE in goats per observed per previous workers could be due to difference in the managemental conditions, immune response of animals and studies conducted in different geographical areas.

5.1.1 Age wise point prevalence of contagious ecthyma in goats

In the present study on age-wise point prevalence of CE in goats, the animals aged between 0-6 months were found to be more affected in goats with a percentage occurrence of 42.24%. Followed by goats aged from 6 month to 1 year (38.76%) and least occurrence in goats more than 1year of age (18.99%). The percentage of occurrence was observed to be lesser in older age group. The results of present study were similar to the results reported by Begum *et al.* (2016). Yeruham *et al*. (2000), Venkatesan *et al*.

(2011), Billinis *et al*. (2012) and Balakrishnan *et al.* (2017) reported that CE can affect goats of any age. However, Nandi *et al*. (2011) stated that the kids are more susceptible to the disease than the adults.

In the present study high occurrence of contagious ecthyma was seen in young animals, it may be due to poor immune status of the young animals which was not competent enough to combat the virus pathogenesis.

5.1.2 Gender wise point prevalence of contagious ecthyma in goats

In the present study gender wise occurrence of CE infection was more in females compared to male goats. The prevalence of CE in male and female animal groups was similar as reported by Begum *et al.* (2016).

The incidence of CE has been reported to occur irrespective of sex in goats as reported by Yeruham *et al.* (1991), Nolikuwera (1992), Wu and Sun (1992) and Balakrishnan *et al.* (2017). In the present study the higher occurrence of disease in females could be related to their over presentation in the affected herds.

5.2 Clinical changes in contagious ecthyma infected goats

In the present study, the affected animals on the day of presentation were dull, depressed, inappatent (83.33%), pyrexia of 102-104°F (66.66%) with congested mucus membrane (50%), enlarged lymph node (27.77%), respiratory distress (50%) and watery, mucoid to thick purulent nasal discharge. Most of the animals showed difficulty in eating and some animals were debilitated. The typical scabby lesions observed were around the mouth involving oral commissures lips, muzzle. In some animals the lesions were also

exhibited in the oral mucosa, gums, ears, mucosa of nostrils and on mammary gland and teats. Similar observations were also reported by several earlier workers (Housawi *et al*., 1991; Hussain *et al*., 1992; McElroy and Bassett, 2007 and Balakrishnan *et al*., 2017). The CE lesions were moist, hemorrhagic, exudative or crusty and dry. They appeared as proliferative growth or papillomatous type with scabby surface. On sloughing of scabs ulcerative hemorrhagic areas were observed. In the oral mucosa involving gum the lesions appeared as thick proliferative growths and on mammary gland and teat as nodular, dry and scabby. Similar description of CE skin lesions have also been reported by several workers (Coates and Hoff, 1990; Venkatesan *et al*., 2011 and Nandi *et al*., 2011).

5.2.1 Distribution of scabby lesions

Among 258 goats, 212 cases had lesions on oral commissures (82.17 %), 178 cases showed lesions on lip (68.99 %), 134 cases with lesion on muzzle (51.93 %), 68 cases had lesions on gums (26.35 %), 126 cases had lesions nostrils (48.83 %), 26 cases had lesions on ear (10.07 %), 1 case had lesion on udder (0.03 %) and 1 case on teat (0.03 %). The findings were in agreement with the observations found by Guo *et al*. (2003) who reported that anatomical site-wise distribution of lesions revealed that in majority of the animals the lesions were noticed in oral commissures (69.1%), lips (56.1%), muzzle (50.8%), nostrils (42.6%) and gums (31.4%). Besides, the lesions were also observed on tongue, hard palate, coronary band, scrotum and udder. The lesion distribution pattern observed could be due to predilection of ORF virus on these anatomical sites. In Orf affected lambs and kids, the lesions usually develop at the area of

mouth, lips and nose but can also be seen in the buccal cavity (tongue, gums, hard palate) and occasionally in esophagous and abomassum of affected animals.

5.3.1 Haematological alterations in contagious ecthyma in goats

In the present study, hematological alterations in goats affected with contagious ecthyma were subjected for various hematological parameters. It was observed that there was a significant increase in the mean leucocyte count and significant decrease in mean platelet count compared to that of healthy control values in goat. The mean ± SE values of other parameters did not differ from those of control goats. In contagious ecthyma, majority of animals showed secondary bacterial infection of the lesions in and around mouth and could have account for increase in the total leucocyte count **(**Saravanan *et al*., 2007; Chu *et al*., 2011, Venkatesan *et al*., 2012 and Tomaselli *et al*., 2016**).** In the present study, in contagious ecthyma affected goats no variation in hematological parameters like Hb, TEC, PCV and DLC were observed in comparison with those of healthy control. Kazemi *et al*. (2018) reported significantly higher value of TLC, Lymphocytes, neutrophils and serum iron concentration of goats with Orf than the healthy goats.

5.3.2 Biochemical alterations in CE in goats

In the current study, the mean ± SE values of total protein, albumin, ALT and AST in contagious ecthyma cases were recorded. Among these parameters, the mean value of AST was significantly less and ALT was significantly higher in affected group when compared to healthy group. A similar report with an increase in the level of ALT has been reported in camels affected with contagious ecthyma by Narnaware *et al.* (2015). An increase in the level of ALT being a leakage enzyme was generally observed

in liver injury. In the present study a mild increase in ALT could be attributed to the hepatocytic degeneration and necrosis.

There was no significant difference in the mean values of total protein and serum albumin between CE affected and healthy goats in the present study. Similar results were found by Kazemi Asl *et al*. (2018) who reportd that there was no significant difference in serum total protien and albumin, between CE affected and healthy goats

5.4 Diagnosis

5.4.1 Polymerase chain reaction for B2L gene of ORFV

The occurrence of contagious ecthyma was confirmed by polymerase chain reaction on scab/ skin biopsy material for B2L gene of Orf virus. Out of 25 animals suspected for CE, 24 animals were confirmed with occurrence of 96 per cent.

ORFV encodes a range of molecules that play vital roles in immune evasion by way of production of anti -inflammatory proteins. These proteins which were mainly involved in the interaction with host immune defence mechanism include the following; ORFV interferon resistence protein (OVIFNR), GM-CSF/IL-2-inhibitory factor (GIF), virus IL-10 protein (vIL-10) and VEGF (Haig and McInnes., 2002 and Deane *et al*., 2000).

The B2L gene has been routinely chosen as the detection target of ORFV worldwide (Hosamani, *et al*., 2009). The B2L region was chosen in the present PCR assay in order to ensure adequate fidelity of the assay and to reduce interference due to

genetic mutation. Kottaridi *et al.* (2006) and Hoasamani *et al.* (2009) have found that B2L gene is highly specific and is the most important gene for the ORFV detection.

5.4.2 Sequencing of the polymerase chain reaction and phylogenetic analysis

Genetic characterization of circulating ORFV strains provides useful information on molecular epidemiology, tracing of origin of outbreaks, vaccine design and selection of appropriate vaccine for control of contagious ecthyma.

Out of 24 positive samples, four samples representing different regions of Bidar district were subjected for B2L gene (1206bp) nucleotide sequencing at commercial firm (Eurofins, India) for sequencing using both forward and reverse primers. The nucleotide sequence obtained from the B2L gene PCR products of 1206 base pair of ORFV were aligned with the ORFV nucleotide sequences published in GenBank using NCBI blast. The nucleotide sequence obtained showed 99.7 to 100% genetic similarity among the four Orf virus isolates confirming the presence of ORFV in the samples. A phylogenetic tree was constructed by using MEGA version 11.0 software.

The sequencing and phylogenetic analysis revealed 98.7 to 99.8% similarity with other published sequences from Indian isolates. The Indian isolates were closely related to China isolates followed by other countries. The results were in concurrence with findings of researcher Bora *et al.* (2015) who reported 97.6-100% similarities among study isolates and 98.4% similarity with CEV isolate 82/04 reported from Shahjahanpur, India. Venkatesan *et al.* (2011) also performed phylogenetic analyses of sheep isolates based on the B2L gene which revealed that the isolates were closely related to goat

isolates retrieved from an outbreak at the same geographic location. It also showed close genetic similarities with other Indian isolates reported earlier.

5.4.3 Pathology

5.4.3.1 Histopathology of scab/skin lesion of contagious ecthyma

In the present study, the scab and skin biopsy samples collected from contagious ecthyma positive cases were subjected for detailed histopathological examination.

The lesions in the contagious ecthyma mainly involve skin epithelium as the Orf virus is epitheliotropic. The virus replicates in the epidermal epithelial cells and leads to formation of acanthosis, ballooning degeneration of stratum spinosum cells and granulomatous inflammation of dermis. The virus induces the characteristic lesions in a sequence of papules, vesicles, pustules, scabs and resolution (Nandi *et al*., 2011). Intra cytoplasmic eosinophilic inclusions were observed in affected cells. In addition, sero cellular crust formation, pustular vesicle formation intra epidermally and in keratin layer, mild to massive infiltration of inflammatory cells at the junction of epidermis and dermis.

Ballooning degeneration, vacuolation of granulocytes, hyperkeratosis of stratum corneum, and parakeratosis and downward growth of epithelium were also observed.

In goats the contagious ecthyma (CE) lesions around the mouth were predominantly papillomatous type. These lesions were characterized by finger like papillomatous growths lined by multiple layers of epithelial cells covered by several layers of keratin or sero-cellular crust encompassing conspicuous keratin layers. In addition sabeceous glands were distended and contained eosinophilic fluid in the lumen

and thickening of the wall was also observed. Necrosis of muscle fibers with infiltration of cells, disintegration and loss of striations was observed.

A very similar microscopical description of contagious ecthyma lesions have been reported by many researchers **(**Guo *et al*., 2004; Chan *et al.,* 2009; Trylanda *et al*., 2013 and Tomaselli *et al*., 2016**).**

5.5 Therapeutic efficacy

The findings obtained with respect to the clinical study conducted revealed better results with topical application of thuja ointment and oral Thuja occ. mother tincture dilution compared to application of aloevera and turmeric combination (1:1 ratio) tested in terms of number of days taken for resolution of scabby lesions. Similar findings were observed by Shuttleworth (1988) who reported that the scabs dropped off in about eight days, accompanied by an observable improvement in condition. Thuja occidentalis presents a varied range of pharmacological activities, such as antioxidant activity, anti-inflammatory, antibacterial, antifungal, antiviral, and immunostimulent (Caruntu *et al.,* 2020). Kumar *et al*. (2012) reported that Thujone rich fraction of Thuja occidentalis demonstrated major anti-cancer potentials evidences from in vitro studies. Crude ethanolic extract of Thuja occidentalis was used as homeopathic mother tincture (TOΦ) to treat various ailments, particularly moles and tumors, and also used in various other systems of traditional medicine.Hansel *et al*. (2013) have described the drug to contain 1.4-4% essential oil (critical factor as medicinal herb), 60% of which is thujone, which corresponds to 2.4% thujone in the whole drug. The pharmacological potential of T. occidentalis, antiviral action and immunopharmacological action of Thuja as stimulatory

effects on cytokine and antibody production and also activate macrophages cells has been investigated in various in vitro and in vivo studies. It showed significant increase in interleukin 1, interleukin 6, and tumour necrosis factor alpha and caused local activation of cytokine producing cells for priming without a systemic rise. Thuja occidentalis is widely used in homeopathy and evidence based phytotherapy. Its antiviral action and immunopharmocological potential such as stimulatory and co-stimulatory effects on cytokine and antibody production and activation of macrophages and other immunocompetant cells, have been evaluated in numerous invitro and invivo investigations (Nazer *et al*., 2005)

Shakoor *et al*. (2012) reported that teat warts treated by using the thuja occidentalis (thuja-30, a homeopathic medicine) @ 10 drops per orally twice a day for a span of 3 weeks lead to complete recovery. Contagious ecthyma affected goats which were treated with application of Aloe vera gel and turmeric preparation in 1:1 ratio took more than 7 days to completely resolve scabby lesions. However Brahma *et al*. (2020) in his study reported that lesions treated with external application of herbal paste prepared from turmeric powder and aloe vera gel in 1:1 ratio, showed complete recovery within one week of treatment. Although ORF is a self-limiting disease, symptomatic treatment with local antiseptics is very helpful (Nandi *et al*., 2011). Topical and systemic antimicrobials can be used to prevent secondary bacterial complications.

Summary

VI. SUMMARY

In the present study, the occurrence of contagious ecthyma (CE) in goats was studied along with an attempt to determine the systemic infections in contagious ecthyma. In the present study outbreaks of contagious ecthyma in and around Bidar during 2022 were utilized for prospective study. A total of 258 animals were screened for typical contagious ecthyma lesions and were diagnosed tentatively as contagious ecthyma based on the scabby lesion in and around the mouth region. Among 258 CE suspected animals, the various samples were collected from 25 animals for molecular study.

In the present study, the prevalence of CE in retrospective study was found to be 11.87 per cent.

In the prospective study the highest occurrence of contagious ecthyma was observed in the age group of 0-6 months (45.64%) followed by the age group 6 months-1 year (33.33%) and least in the age group above 1 year (19.48 %). However, females goats were found to be more affected (63.58%) than males goats (36.41%).

Clinically CE affected animals appeared dull, and weak with rough hair coat and showed loss of appetite, increase in body temperature which varied from 102-104°F and nasal discharge in some animals which varied from watery, mucoid to thick purulent in appearance. The animals evinced pain and showed difficulty in eating. Some animals empirically, young ones showed debilitation. Grossly contagious ecthyma lesions were observed around the mouth involving lips, oral commissures and muzzle. In some animals, the lesions were observed on the oral mucosa, gums, and dental pad, skin of the

head, ears, mucosa of nostrils, and on mammary glands including teats. The CE lesions were moist, hemorrhagic, and exudative in some animals whereas, they were crusty and dry in some goats.

Hematological evaluation in goats revealed a significant increase in total leukocyte count and a significant reduction in platelet count compared to that of healthy control animals. Serum biochemical analysis in goats revealed significantly reduced mean value of AST in CE-affected goats compared to that of control animals and a significant increase in mean ALT value compared to control animals. However, the mean values of other parameters did not differ much from those of the healthy control goats.

The occurrence of contagious ecthyma was confirmed by polymerase chain reaction on scab/skin biopsy material for the B2L gene of CE. PCR for a total of 25 scabs and skin biopsy samples collected from suspected goat cases and gave a total percentage positivity of 96.00% for the B2L gene.

Phylogenetic analysis based on nucleotide sequences of the B2L gene revealed that the study ORFV isolates in present study had homology with other Indian ORFV isolates and also with ORFV isolates of Chinese confirming the virus in clinical samples.

Histopathologically the scab/skin biopsy of contagious ecthyma lesions included hyperkeratosis, parakeratosis, and acanthosis with a varying number of short or deep rete pegs extending into the dermis. There was an affection of prickle cell layer of the hyperplastic epidermis which showed severe cell swelling, vacuolar degeneration with pyknotic nuclei, or loss of nuclei. In addition intra cytoplasmic eosinophilic inclusions

were observed in the affected as well in cells adjacent to the affected area. In addition, serocellular crust formation, pustular vesicle formation intra-epidermally and in the keratin layer, and mild to massive infiltration of inflammatory cells predominantly with lymphoid cells at the junction of the epidermis and dermis were also observed. The epithelial cells appeared swollen, detached or separated with an increase in intercellular space and loss of intercellular bridges. Hyperkeratosis and in some cases parakeratosis were also observed.

The response shown to different therapy indicated that the oral drops of Thuja mother tincture dilution and topical application of Thuja ointment showed better results compared to herbal mixture of aloe-vera and turmeric paste application considering complete resolution of scabby lesions on 7^{th} day post-treatment. The results also indicated potential role of Thuja in clinical management of ORF, and offers a viable and a non-antimicrobial alternative in addressing animal diseases like contagious ecthyma.

Conclusions

- The study revealed an prevalence of 11.87 per cent of contagious ecthyma in goats in retrospective study. The highest occurrence of CE occurred in goats, in the age group of 0-6 months, and in females in prospective study.
- Hematology revealed leucocytosis and biochemically indicated decrease in AST and slight increase in ALT in contagious ecthyma-affected goats.
- Grossly contagious ecthyma lesions occur around the mouth involving lips, oral commissures, muzzle, in the oral mucosa, gums, dental pad, on the skin of the head, ears, mucosa of nostrils, and on mammary gland and teats.

- PCR for B2L genes of Orf virus detected contagious ecthyma cases effectively.
- Phylogenetic analysis based on nucleotide sequences of B2L gene showed homology of ORFV isolates with other Indian ORFV isolates and China isolates.
- Histopathology revealed varying degree of degenerative changes in epidermis showing hyperkeratosis, parakeratosis, ballooning degeneration, vescicle, pustule and ulcer formation. Some of the epidermal cells showed presence of intracyoplasmic eoisinophilic inclusion bodies.
- Therapeutic management with Thuja mother tincture dilution per orally and application of thuja ointment showed better results compared to application of aloe-vera gel and turmeric mixture to the scabby lesions along with parenteral antibiotic and anti-inflammatory.

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VII. BIBLIOGRAPHY

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Abstract

VIII. ABSTRACT

Diagnosis and therapeutic management of Contagious Ecthyma in Goats

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Contagious ecthyma is an infectious, emerging viral disease of goats caused by Orf virus (ORFV). The present study reports prevalence of CE in goats in and around Bidar was 11.87 per cent. Clinical signs, hemato-biochemical alterations, gross pathology, PCR, sequencing and phylogenetic analysis and histopathology were recorded. Occurrence of CE in goats was more in the age group between 0-6 months and occurrence was more in females than in males. Clinical signs observed in goats with CE in the present study were pyrexia, anorexia, depression, painful scabby lesions around mouth leading to inappatence and debilitation. Grossly contagious ecthyma lesions were observed around the mouth involving lips, oral commissures, muzzle and mouth. There was a significant increase in the TLC values and significant decrease in platelet value. There was significant decrease in the AST values and significant increase in ALT values in goats. PCR for B2L gene of ORFV was carried out on scab/skin biopsy samples to confirm the disease. Detection of ORFV infection using polymerase chain reaction revealed, out of 25 tissue samples 24 (96%) were found positive for B2L gene with 1206 bp. Sequencing and phylogenetic analysis revealed ORFV isolates were close to one another followed by other Indian isolates and more closely related to China compared to other global isolates from other countries. Histopathology revealed hyperkeratosis, parakeratosis, acanthosis, ballooning degeneration, degeneration of epithelial cells, presence of intracytoplasmic eosinophilic inclusions in affected stratum spinosum cells, vesicle, pustule and ulcer formation. A clinical study was done on CE affected goats evaluating the efficacy of topical application of Thuja oint, aloevera gel and turmeric (1:1 ratio) and Thuja occidentalis mother tincture per orally for reducing the clinical severity along with parenteral antibiotic and anti-inflammatory. The response shown to different treatment protocols indicated that the topical application of Thuja oint and oral Thuja occ. mother tincture showed better results compared to herbal mixture of aloe-vera gel and turmeric powder paste, considering the number of days taken for complete resolution of scabby lesions.

Appendices

APPENDIX-A

ESTIMATION OF SERUM AST

International Federation of Clinical Chemistry (IFCC) Method with kits from ERBA Diagnostic, Manufactured by Transasia Bio-medicals Ltd, Nalagarh Road, Village Malpur, Baddi, Dist, Solan, (HP)-173205.

Principle: AST (SGOT) catalyses the transfer of amino group between L-Aspartate and 2-Oxoglutarate to form oxaloacetate and L-Glutamate. The oxaloacetate formed reacts with NADH in the presence of Malate dehydrogenase (MDH) to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the SGOT (AST) activity in the sample.

Reagent composition:

Reagent 1: AST Reagent

2-Oxoqiutarate	12 mmol/L
L- Aspartate	200mmol/L
MDH	≥545 U/L
LDH	909U/L
NADH (yeast)	≥0.18 mmol/L
Tris Buffer	80mmol/L
EDTA	5.0mmol/L

Preparation of working reagent: Allowed the reagent bottle and Aqua-4 (supplied in the kit) to attain room temperature (15-30ºC) added the amount of Aqua-4 indicated on the label to contents of each vial and swirled to dissolve.

Procedure: Pipette in to tubes marked as

Pipette	Volume
Working reagent	1000 µl
Test	100 µl

Allowed the working reagent to attain 37ºC before performing the test, contents of test tube were mixed well and aspirated through semi-automatic clinical chemistry analyser microlab 300 and read the results.

APPENDIX-B

ESTIMATION OF SERUM ALT

International Federation of Clinical Chemistry (IFCC) Method with kits from ERBA Diagnostic, Manufactured by Transasia Bio-medicals Ltd, Nalagarh Road, Village Malpur, Baddi, Dist, Solan, (HP)-173205.

Principle: ALT (SGPT) catalyses the transfer of amino group between L-Aspartate and 2-Oxoglutarate to form Pyruvate and L-Glutamate. The Pyruvate formed reacts with NADH in the presence of Lactate dehydrogenase (LDH) to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance which is proportional to the ALT (SGPT) activity in the sample.

Reagent composition:

Reagent 1: ALT Reagent

Tris/L-alanin	100 mmol/L
Hydrochloric acid buffer	80 mmol/L
Pyridoxal phosphate	167 mg
NADH	113 mmol/L
Lactate dehydrogenase	252 U/L
2-oxoglutarat	180 mmol/L
Sodium chloride	154 mmol/L

Preparation of working reagent: Allowed the reagent bottle and Aqua-4 (supplied in the kit) to attain room temperature (15-30ºC) added the amount of Aqua-4 indicated on the label to contents of each vial and swirled to dissolve.

Procedure: Pipette in to tubes marked as

Pipette	Volume
Working reagent	1000 µl
Test	100 µl

Allowed the working reagent to attain 37ºC before performing the test, contents of test tube were mixed well and aspirated through semi-automatic clinical chemistry analyser microlab 300 and read the results.

APPENDIX-C

ESTIMATION OF SERUM TOTAL PROTEIN

Method: Biuret method with kits from ERBA Diagnostic Manufactured by Transasia Bio-medicals Ltd, Nalagarh Road, Village Malpur, Baddi, Dist, Solan, (HP)-173205.

Principle: Colorimetric determination of the total protein based on the principle of biuret reaction (copper salts in an alkaline medium). Protein in plasma or serum forms a blueviolet coloured complex when treated with cupric ions in alkaline solution. The intensity of the colour is proportional to the protein concentration and is measured at 546nm (520560nm).

Reagent composition:

Total protein reagent

Copper II sulphate	19mmol/L
Potassium sodium Tartarate	43mmol/L
Potassium Iodide	30mmol/L
Sodium Hydroxide	600mmol/L

Standard

Protein standard	6.0g/dL

Procedure: Sterile test tubes labelled as blank, standard and test were taken

Pipette into tubes marked	Blank	Standard	Test
Working reagent	1000 µl	1000 µl	1000 µl
Distilled Water	20 µl	--	--
Standard	--	20 µl	--
Test	--	--	20 µl

The contents of test tube were mixed well and incubated for 10 minutes at 37ºC and then absorbance of the standard and test was read against reagent blank at 546 nm on semi-automatic clinical chemistry analyser microlab 300.

APPENDIX D

ESTIMATION OF SERUM ALBUMIN

Method: Bromocresol Green (BCG) dye method with kits from ERBA Diagnostic. Manufactured by Transasia Bio-medicals Ltd, Nalagarh Road, Village Malpur, Baddi, Dist, Solan, (HP)-173205.

Principle: The method is based on the specific binding of bromocresol green (BCG), an anionic dye and the protein at acidic pH 4.2 producing a colour change of the indicator from yellow to blue-green with the resulting shift in the absorption wavelength of the complex. The intensity of the colour formed is proportional to the concentration of albumin in the sample when measured photometrically.

REAGENT COMPOSITION

Reagent: Albumin reagent

Bromocresol green	0.08mmol/L
Succinate Buffer	50mmol/L
Sodium Azide	1gm/L
Surfactant	--

Albumin Standard

Albumin Standard	3.6g/dL

Preparation of working reagent: The reagent is ready for use.

Procedure: Sterile test tubes labelled as blank, standard and test were taken

Pipette into tubes marked	Blank	Standard	Test
Albumin reagent	1000 µl	1000 µl	1000 µl
Distilled Water	10 µl	--	--
Standard	--	10 µl	--
Test	--	--	10 µl

Contents of the test tube were mixed well and read the absorbance of standard and test at 630nm against reagent blank, after 1 minute incubation at 37ºC. on semiautomatic clinical chemistry analyser microlab 300.